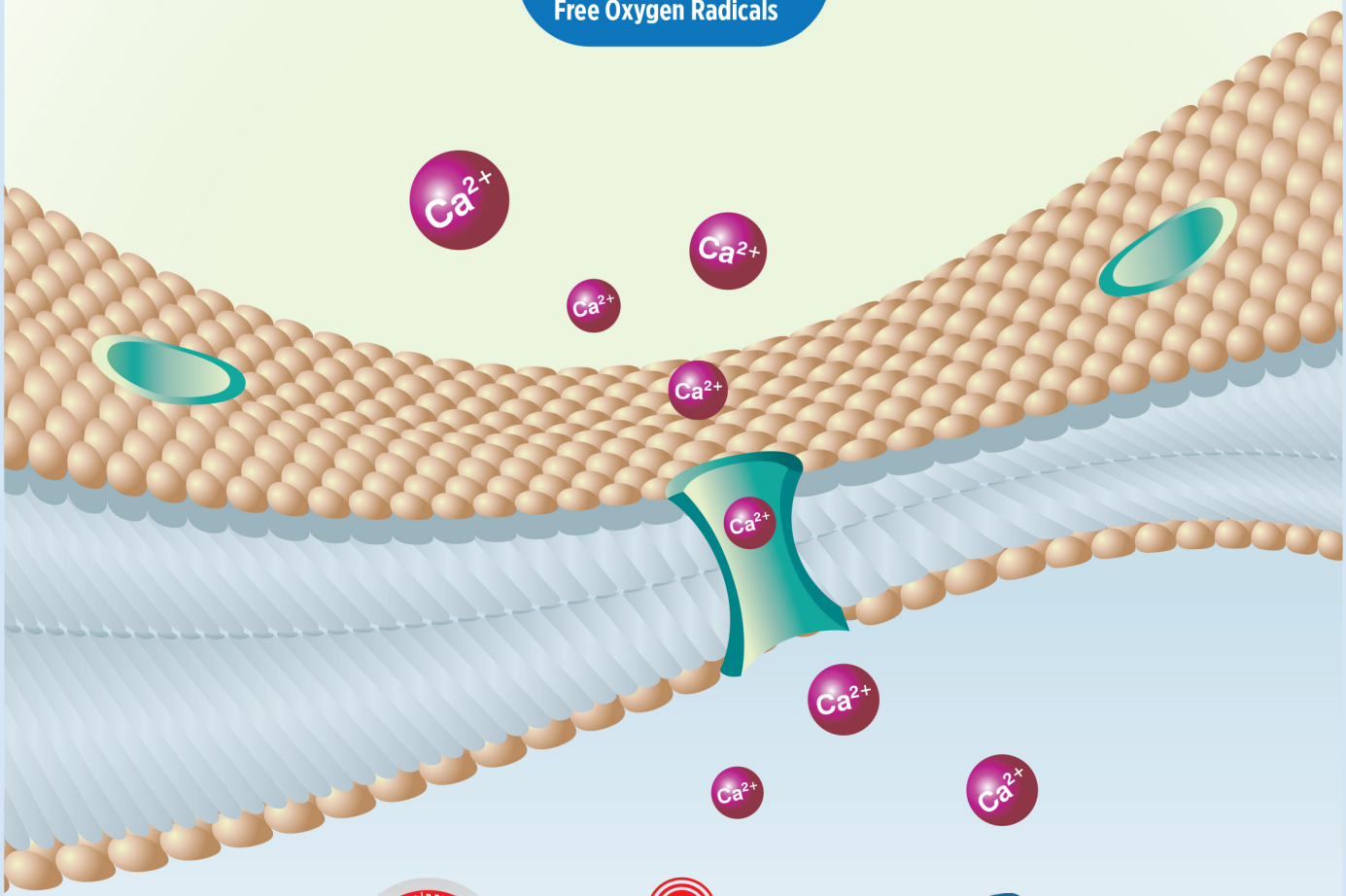
ISSN: 1308-416X

Cell Membranes and Free Radical Research

HSORD
Society of
Cell Membranes and
Free Oxygen Radicals



Süleyman Demirel Üniversitesi 1992

TÜBİTAK

european Calcium society

Editor in Chief
Prof.Dr. Mustafa NAZIROĞLU

Volume 4, Number 1, 2012

4th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels

Volume 4, Number 1, 2012

4th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels

Volume4, Number1, 2012
ISSN Numbers: 1308-4178 (On-line), 1308-416X
Indexing: Google Scholar, Index Copernicus, Chemical Abstracts, Scopus (Elsevier), EBSCOhost Research Database

AIM AND SCOPES

Cell Membranes and Free Radical Research is a print and online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+ - K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals

C- Interaction Between Oxidative Stress and Ion Channels (Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels)

D- Gene and Oxidative Stress (Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics
Biochemistry
Biology
Biomedical Engineering
Pharmacology
Physiology
Genetics
Cardiology
Neurology
Oncology
Psychiatry
Neuroscience

Keywords

Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide synthase, ageing, antioxidants, neuropathy.

Republic of Turkey
Promotion Fund of the Prime Ministry

TÜBİTAK

4th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels was supported by The Scientific and Technological Research Council of Turkey.

The Abstract book of the congress is published in this issue.

Abstract Book

of

4th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels

26 - 29 June 2012

Isparta, Turkey

by

Suleyman Demirel University Medical Faculty Department of Biophysics

4th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels

The congress organization committee wishes thanks to the sponsors below;

TÜBİTAK

THE SCIENTIFIC AND TECHNOLOGICAL RESEARCH COUNCIL OF TURKEY

SULEYMAN DEMIREL UNIVERSITY

EA TEKNOLOJİ

EA TECHNOLOGY

OPTRON ZEISS

OPTRONIK

HEKA

HEKA

KORO İLAÇ VE SAĞLIK ÜRÜNLERİ

KORO ILAÇ

Gülbirlik "güle dair herşey" | Rosense

GÜLBIRLIK

ikbal 1922 Şekerleme

İKBAL

ISPARTA PETROL TOURISM
BARAN MEDICAL
IRFANDO ICECREAM

MUNICIPALITY of Egirdir, ISPARTA
(Osman Nuri Özmeral)

4th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels

[Organization Committee]

Congress Honour Committee

Prof. Dr. Hasan İBİCİOĞLU
Rector of The Suleyman Demirel University

Prof. Dr. Huseyin YORGANCIGİL
Dean of The Faculty of Medicine
Suleyman Demirel University

Chairman

Prof. Dr. Mustafa NAZIROĞLU
Department of Biophysics, Faculty of Medicine
Suleyman Demirel University

Vice Chairman

Prof. Dr. James W. Jr. PUTNEY
NIEHS Calcium Regulation Group Leader

Prof. Dr. Süleyman KUTLUHAN
Headmaster of The Department of Neurology Faculty of Medicine
Suleyman Demirel University

Prof. Dr. Fatih GULTEKIN
Department of Biochemistry, Faculty of Medicine
Suleyman Demirel University

Prof. Dr. Osman GOKALP
Department of Pharmacology, Faculty of Medicine
Dicle University

Assist. Prof. Dr. Ahmet KOÇAK
Department of Histology and Embryology
Dumlupinar University School of Medicine

Congress Local Chairman

Assist Prof. Dr. A. Cihangir UĞUZ
Department of Biophysics, Faculty of Medicine
Suleyman Demirel University

Assist Prof. Dr. Ömer CELİK
Department of Biophysics, Faculty of Medicine
Suleyman Demirel University

Social Committee

Assoc. Prof. Dr. Recep Oktay PEKER
Faculty of Medicine
Department of Cardiovascular Surgery
Süleyman Demirel University

Assist. Prof. Dr. Abdullah Çim
Faculty of Medicine,
Department of Medical Genetics
Dicle University

Z.Zahit Çiftci
Department of Pedodontology, Faculty of Dentistry
Suleyman Demirel University

Mustafa Deryal
Pharmacist

Muhammet Şahin
Technician

Congress Secretariat

I. Suat ÖVEY & Bilal CİĞ & Mehmet AYKUR
Department of Biophysics, Faculty of Medicine
Suleyman Demirel University

Accountants

Mustafa KÜÇÜKAYAZ & Cemil ÖZGÜL & Ercan SÖZBİR (Webmaster)
Department of Biophysics, Faculty of Medicine
Suleyman Demirel University

Design

Serdar DURAN *(Graphic Designer)*
Faculty of Medicine Suleyman Demirel University

Yasemin DURAN *(Graphic Designer)*
Mehmet Akif Ersoy University

[CONTENTS]

Conference

Oral Presentations

Poster Presentations

CONFERENCE

Conference No. 1

The STIM1 domains that regulate Orai and TRPC channels

Shmuel Muallem

NIDCR, NIH, BETHESDA MD, USA

The receptor-evoked Ca^{2+} signal involves Ca^{2+} release from internal stores and subsequent activation of Ca^{2+} influx channels in the plasma membrane. Ca^{2+} influx is mediated by the TRPC and Orai channels and is regulated by the ER Ca^{2+} sensor STIM1. STIM1 ER Ca^{2+} sensing is conferred by the STIM1 EF hand, while channel activation is mediated by the cytoplasmic domain of STIM1. Several studies identified functional domains in the STIM1 cytoplasmic C terminus, the most important of which are the SOAR coiled-coil domain that is sufficient to fully activate the Orai channels and the two mist C terminus lysines (KK) that gate the TRPC channels. Additional regulatory domains where reported that regulate the activity of the SOAR domain or the accessibility of the KK to the TRPC channels. The functional role of these domains will be discussed as information is developed.

Conference No. 2

From Ca^{2+} signalling to bioenergetics and vice versa

Svetlana Voronina, Gyorgy Lur, Stephanie Barrow, Lee Haynes, Ole Petersen, Robert Sutton, David Criddle and Alexei Tepikin

Department of Cellular and Molecular Physiology, Institute of Translational Medicine, The University of Liverpool, Crown Street, Liverpool, L69 3BX, UK.

Important secretagogues (e.g. ACh and CCK) utilise Ca^{2+} signalling to trigger and regulate secretion in pancreatic acinar cells. Ca^{2+} release from the internal stores and Ca^{2+} influx via plasma membrane channels are involved in both physiological and pathological Ca^{2+} responses. The relationships between these two mechanisms are complicated - the Ca^{2+} release preferentially occurs in the apical region of the cell (the region occupied by secretory granules) whilst the influx is stimulated along the lateral and basal membrane. $InsP_3$ receptors in the apical region and STIM1/Orai1 complexes in the basolateral region play important roles in the regulation of release and influx (1, 2). Recently discovered ribosome-free ER-PM junctions serve as a platform for the store-operated Ca^{2+} influx. Mitochondria play an important role in restricting Ca^{2+} signals to the secretory part of the cell. The Ca^{2+} signals stimulate mitochondrial ATP production (3) whilst the loss of ATP suppresses both Ca^{2+} release (and consequently Ca^{2+} oscillations) and Ca^{2+} influx in this cell type.

References

1. György Lur, Mark W Sherwood, Etsuko Ebisui, Lee P Haynes, Stefan Feske, Robert Sutton, Robert Burgoyne, Katsuhiko Mikoshiba, Ole H Petersen and Alexei V Tepikin. IP_3 receptors and Orai channels in pancreatic acinar cells: co-localisation and its consequences. Biochemical Journal 2011; 436(2): 231-239.
2. Lur G, Haynes LP, Prior IA, Gerasimenko OV, Feske S, Petersen OH, Burgoyne RD and Tepikin AV. Ribosome-free terminals of rough ER allow formation of STIM1 puncta and segregation of STIM1 from IP3 receptors. Current Biology 2009; 19(19):1648-53.
3. Voronina SG, Barrow SL, Simpson AW, Gerasimenko OV, da Silva Xavier G, Rutter GA, Petersen OH, Tepikin AV. Dynamic changes in cytosolic and mitochondrial ATP levels in pancreatic acinar cells. Gastroenterology 2010; 138: 1978-1987

Conference No. 3

Modulation of SERCA function through redox processes, Bcl-2 and Hsp70

Christian Schöneich

Department of Pharmaceutical Chemistry, The University of Kansas, 2095 Constant Avenue, Lawrence, KS 66047, USA.

The sarco/endoplasmic reticulum Ca-ATPase (SERCA) serves a key function in cellular Ca^{2+} homeostasis transporting cyctosolic Ca^{2+} into the SR/ER. Its activity is regulated/modulated via interactions with proteins and lipids, and via reaction with reactive oxygen species generated, for example, as a result of inflammatory processes. This presentation will focus on the characterization of free radical mechanisms, catalyzed by Cys residues, which can ultimately lead to irreversible covalent SERCA modifications, including fragmentation and aggregation, i.e. modifications which have been observed *in vivo* during various pathologic conditions and aging.

Key to the regulation/modulation of SERCA function is the reversible/ irreversible modification

of Cys through S-glutathiolation and/or oxidation. To date, most experiments on SERCA redox regulation have focused on two-electron oxidation/reduction pathways. However, one-electron oxidation/reduction pathways may be equally important, proceeding via intermediary Cys thiyl radicals. These thiyl radicals easily form through reaction of thiol/thiolate with one-electron oxidants generated under biologic conditions of oxidative stress, for example nitrogen dioxide (NO_2). In the presence of excess glutathione and oxygen, thiyl radicals may convert into mixed disulfides, i.e. S-glutathiolated proteins. However, frequently protein thiols are located in the more hydrophobic interior of proteins, preventing facile access to glutathione unless the protein is (partially) unfolded. Thiyl radicals generated from such thiols will have access to a number of amino acids, and involve in reversible hydrogen transfer reactions with amino acid C-H bonds. In addition, such hydrogen transfer reactions also occur within the Cys residues themselves, generating precursors for elimination reactions and the formation of reactive electrophiles. In SERCA, these processes occur site-specifically, and can be mapped by HPLC-MS/MS analysis. Experimental data obtained during the exposure of SERCA to conditions of oxidative stress are corroborated by analogous experiments with other proteins and model peptides leading to a paradigm according to which protein Cys residues may rather selectively catalyze protein oxidation/modification by reactive oxygen species/free radicals.

Conference No. 4

Store-operated Calcium Channels.

J.W. Putney[1], G.S. Bird[1], J.T. Smyth[2], N. Russan[2], M. Fukushima[3], T. Tomita[1], A. Janoshaz[1]

1 Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA.
2 Cell Biology and Physiology Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA.
3 Department of Physiology, Nihon University School of Dentistry at Matsudo, 2-870-1, Sakaecho-Nishi, Matsudo, Chiba, JAPAN.

Activation of phospholipase C results in release of intracellular Ca^{2+} and activation of Ca^{2+} entry. Plasma membrane Ca^{2+} entry most commonly is signaled by the depletion of intracellular Ca^{2+} stores, a mechanism referred to as *capacitative calcium entry* or *store-operated calcium entry* (SOCE). Recent work from a number of laboratories has highlighted the role of a Ca^{2+} sensor protein, STIM1, and a channel subunit, Orai1. STIM1 activates Orai channels by a mechanism that depends upon its co-localization with Orai at endoplasmic reticulum – plasma membrane junctions.

Signaling proteins often occur in multiple forms with distinct properties and/or functions. Such forms arrise by a number of mechanisms, including covalent modification, alternative splicing, and alternative translation initiation. STIM1 is organized within the endoplasmic reticulum through interactions with a microtubular +end binding protein, EB1. However, during mitosis, STIM1 dissociates from microtubules and moves to the cell periphery, while microtubules form the mitotic spindle. The dissociation of STIM1 from microtubules occurs because of multiple phosphorylations in the vicinity of the EB1 interacting site. In the absence of these phorphorylations, STIM1 fails to dissociate, and STIM1 and much of the endoplasmic reticulum is carried along microtubules during cytokinesis.

The SOCE channel subunit, Orai1, occurs in two forms in mammalian cells, Orai1α and Orai1ß, due to alternative translation initiation. Proteins formed from the second start site (Orai1ß) lack a number of previously described regulatory domains, including PKC phosphorylation sites, a PIP2 binding site, a caveolin interacting site, and a binding site for Ca^{2+}-stimulatable adenylyl cyclase 8. The loss of one or all of these interacting domains in Orai1ß results in significantly enhanced plasma membrane mobility, as evidenced by rates of fluorescence recovery after photobleaching.

Conference No. 5

Resolving the contribution of TRPC1 and Orai1 to cytosolic Ca^{2+} signals and regulation of cell function

Indu S. Ambudkar

Secretory Physiology Section, Molecular Physiology and Therapeutics Branch, NIDCR, NIH, BETHESDA MD 20892. USA.

Agonist stimulation of cells leads to the generation of intracellular Ca^{2+} signals that are decoded for the regulation of various cellular functions, including Ca^{2+}-dependent gene regulation. How cells decode individual Ca^{2+} signals, especially in cases where more than one channel contributes to the $[Ca^{2+}]_i$ signal in a single cell, is not well understood. Two STIM1-regulated Ca^{2+} entry channels, Orai1 and TRPC1, are activated in response to agonist-induced depletion of salivary gland cells and contribute to sustained $[Ca^{2+}]_i$ elevation by mediating Ca^{2+} influx into cells. We have previously reported that TRPC1

is a critical component of SOCE in these cells and that salivary acinar cells from TRPC1-/- mice display reduced SOCE which accounts for loss of sustained KCa activation and, consequently, salivary fluid secretion in the animals. While Orai1 controls the activation of TRPC1 in response to store depletion, the individual Ca^{2+} signals generated by the two channels and their impact on cell function is not yet known. We have studied the contributions of TRPC1 and Orai1 to agonist stimulated $[Ca^{2+}]_i$ signals and Ca^{2+}-dependent regulation of gene expression by examining agonist-induced $[Ca^{2+}]_i$ signals and Ca^{2+}-dependent gene expression in a single cell as well as Ca^{2+}-dependent cell function in TRPC1-/- mice. Together, our findings suggest that Orai1 and TRPC1 mediate distinct local and global Ca^{2+} signals that determine the channel specificity in the regulation of cell function. These recent studies will be discussed.

Conference No. 6

Role of IP_3-dependent Ca^{2+} signaling in apoptosis and autophagy

Jan B. Parys, Giovanni Monaco, Haidar Akl, Jean-Paul Decuypere, Tim Vervliet, Hristina Ivanova, Santeri Kiviluoto, Tomas Luyten, Kirsten Welkenhuyzen, Ludwig Missiaen, Humbert De Smedt, Geert Bultynck

Laboratory of Molecular and Cellular Signalling, Faculty of Medicine, KU Leuven, Leuven, BELGIUM.

Ca^{2+} release by the IP_3 receptor (IP_3R) controls many cytosolic processes, but also mitochondrial energy production and structure. Regulatory proteins acting on the IP_3-dependent Ca^{2+} flux from the ER to the mitochondria can therefore modulate apoptosis and autophagy (1). We especially investigated the role herein of Bcl-2, Bcl-2-family members and proteins interacting with them. Anti-apoptotic Bcl-2 inhibits IP3-induced Ca^{2+} release by binding through its BH4 domain to IP_3R a.a. 1389-1408 (regulatory domain). Interestingly, the anti-apoptotic protein Bcl-Xl, despite its functional and structural homology with Bcl-2, does not interact with the IP3R through its BH4 domain and therefore does not inhibit IP_3-induced Ca^{2+} release. A critical lysine residue (K^{17}) only present in the BH4 domain of Bcl-2 is mainly responsible for this. IP_3-dependent Ca^{2+} signaling also participates in autophagy. During the first 3 hours of nutrient starvation, autophagic flux is upregulated without induction of cell death or ER stress. This is due to a sensitization of the IP_3R, combined with an increased ER Ca^{2+}-store content. IP_3R sensitization correlates with enhanced binding of the essential autophagy protein Beclin-1 to it. Beclin-1 contains a BH3-domain that interacts with Bcl-2, but the observed IP_3R sensitization is independent of a Beclin-1 / Bcl-2 interaction. Purified recombinantly expressed full-length Beclin-1 as well as its N-terminal moiety directly sensitize IP_3-induced Ca^{2+} release in permeabilized cells. Chelating intracellular Ca^{2+} or inhibiting the IP_3R blunts autophagy initiation, indicating that sensitization of IP_3-dependent Ca^{2+} signaling is essential for proper autophagy initiation. Our results further indicate that modulation of the interaction between the IP_3R and Bcl-2-family members or the Beclin-1 protein may offer new possibilities e.g. in the treatment of cancer or neurodegenerative diseases.

References

1. Decuypere J.P., Monaco G., Bultynck G., Missiaen L., De Smedt H., Parys J.B. The IP3 receptor-mitochondria connection in apoptosis and autophagy. Biochim. Biophys. Acta 2011; 1813:1003-1013.

Conference No. 7

Redox regulation of Ca-signaling

Volker Ullrich

Department of Biology, University of Konstanz, Konstanz, GERMANY.

Intracellular Ca-levels are tightly controlled by redox reactions. Likewise the entry of Ca from the extracellular space or release from intracellular stores depends on oxidative events at the corresponding channels and proteins whereas the uptake in such compartments is simultaneously inhibited in order to avoid futile circling. This guarantees rapid changes of Ca levels and hence an efficient cellular response. The underlying redox chemistry involving oxidants and their protein targets has been increasingly studied and has revealed a surprising complexity. To the previously known reactive oxygen species (ROS= superoxide,hydrogen peroxide,hydroxyl radicals) those derived from nitric oxide (NO) have been added as reactants (nitrogen dioxide, dinitrogen trioxide, peroxynitrite) responsible for posttranslational protein modification like the formation of disulfides, methionine sulfoxides, nitrotyrosines or S-nitroso-cysteines. Focus will be put on the latter since S-nitrosation is a preferred modification of Ca-channels and Ca-pumps. The chemical nature of the nitrosating species is still under debate but evidence exists for the involvement of NO and superoxide in a flux ratio of 3:1 (1,2). If the ratio approaches 1:1 peroxynitrite is formed which reacts with dithiols and zinc fingers under disulfide

formation. Thus only by changing the flux ratios of both radicals different protein modifications are achieved (3,4) allowing that within one protein thiol groups can be modified by S-nitrosation as well as disulfide formation at different flux rates of NO and superoxide. If NO formation becomes exhausted, eg by oxidative NO-synthase inhibition, superoxide remains as a messenger. Its chemical reactivity is very limited and therefore its specificity can be high. It was found to selectively block calcineurin leading to hyperphosphorylation in case of some substrates. Interestingly, superoxide can reduce S-nitroso groups and thus restore the thiolate status of proteins.

References

1. Daiber A, Frein D,Namgaladze D,Ullrich V Oxidation and nitrosation in the nitrogen monoxide/ superoxide system J Biol Chem 2002,277,11882-88
2. Daiber A,Schildknecht S, Müller J,Kamuf J, Bachschmid MM, Ullrich,V, Chemical model systems for cellular nitros(yl)ation reactions, Free Radic Biol Med 2009, 47,458-67
3. Frein D.,Schildknecht S.,Bachschmid M, Ullrich V. Redox regulation: a new challenge for pharmacology. Biochem Pharmacol. 2005, 70 , 811-2
4. UllrichV.,Kissner,R. Redox signaling: bioinorganic chemistry at its best. J Inorg Biochem 2006, 100,2079-86
5. Namgaladze D, Hofer HW, Ullrich V Redox control of calcineurin by targeting the binuclear Fe2+ Zn2+ center at the enzyme active site, J Biol Chem 2002, 277,5962-69.

Conference No. 8

Calcium signalling and apoptosis: Role of melatonin

Javier Espino, Ignacio Bejarano, José A. Pariente

Department of Physiology, Neuroimmunophysiology and Chrononutrition Research Group, Faculty of Science, University of Extremadura, 06006 Badajoz, SPAIN.

Apoptosis is a gene-regulated form of cell death that is critical for normal development and tissue homeostasis. A major component of the apoptotic machinery involves a family of aspartic acid-directed cysteine proteases, called caspases, which cleave multiple protein substrates en masse, leading the loss of cellular structure and function, and ultimately resulting in cell death. In addition, numerous reports suggest that aging is accompanied by alterations in the apoptotic behaviour of a variety of cell types and tissues. On the other hand, the calcium ion is one of the cellular signalling mechanisms most widely used by different cell types, and with the greatest number of physiological and pathological implications. Alterations of calcium homeostasis, particularly excessive and prolonged increases in cytosolic free calcium concentration ($[Ca^{2+}]_c$), are early signs that precede other morphological and functional alterations responsible for the development of irreversible damage in various tissues. In fact, it has been reported that sustained elevation of intracellular calcium plays a role in cell death. The proapoptotic effects of calcium are mediated by a diverse range of calcium-sensitive factors that are compartmentalised in various intracellular organelles, including endoplasmic reticulum and mitochondria.

The pineal gland hormone melatonin regulates seasonal and circadian rhythms of mammals and functions as a powerful free radical scavenger, but emerging evidence suggests that it may be involved in other important processes such as the protection of human leukocytes and other cell types against damage-induced apoptosis. Recent convincing evidence suggests that the so-called intrinsic pathway might represent the main target of melatonin to antagonize apoptosis in human leukocytes and in other tumor cell lines and *in vivo* models.

Here, we have evaluated the effect of melatonin on apoptosis evoked by increases in $[Ca^{2+}]_c$ in human leukocytes. Our results show that treatment of neutrophils with the calcium mobilising agonist FMLP or the specific inhibitor of calcium reuptake thapsigargin induced a transient increase in $[Ca^{2+}]_c$. FMLP and thapsigargin increased the caspase-9 and -3 activities and the active forms of both caspases. The effect of FMLP and thapsigargin on caspase activation was time-dependent. Similar results were obtained when lymphocytes were stimulated with thapsigargin. This stimulatory effect was accompanied by activation of the proapoptotic protein Bax and release of cytochrome c. However, when leukocytes were pre-treated with melatonin, all apoptotic features indicated above were significantly reversed, suggesting that melatonin reduces caspase-9 and -3 activities induced by increases in $[Ca^{2+}]_c$ in human leukocytes, which is produced through modulation of Bax activation. The protective effects on leukocyte apoptosis resulting from melatonin administration depends on melatonin's antioxidant action, as melatonin treatment substantially prevented intracellular reactive oxygen species (ROS) production induced by thapsigargin and FMLP. Finally, melatonin was able to delay endoplasmic reticulum stress-induced apoptosis in aged leukocytes and may counteract, at the cellular level, age-related degenerative phenomena linked to oxidative stress.

Supported by Ministerio de Ciencia e Innovación-Fondo Social Europeo de Desarrollo Regional (FEDER) grant BFU2010-15049.

References

1. Espino J, Bejarano I, Paredes SD, Barriga C, Reiter RJ, Pariente JA, Rodríguez AB. 2011. Melatonin is able to delay endoplasmic reticulum stress-induced apoptosis in leukocytes from elderly humans. Age 33:497-507.

2. Espino J, Bejarano I, Paredes SD, Barriga C, Rodríguez AB, Pariente JA. 2011. Protective effect of melatonin against human leukocyte apoptosis induced by intracellular calcium overload: relation with its antioxidant actions. Journal of Pineal Research 51:195-206.
3. Espino J, Bejarano I, Redondo PC, Rosado JA, Barriga C, Reiter RJ, Rodriguez AB, Pariente JA. 2010. Melatonin reduces apoptosis induced by calcium signalling in human leukocytes: Evidence for the involvement of mitochondria and Bax activation. Journal of Membrane Biology 233:105-118.
4. Espino J, Bejarano I, Paredes SD, Gonzalez D, Barriga C, Reiter RJ, Pariente JA, Rodríguez AB. 2010. Melatonin counteracts alterations in oxidative metabolism and cell viability induced by intracellular calcium overload in human leucocytes: changes with age. Basic & Clinical Pharmacology & Toxicology 107:590-597.

Conference No. 9

Roles of calcium and ROS in acute pancreatitis

David N. Criddle

Department of Molecular and Cellular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool L69 3BX, UK.

Mitochondrial dysfunction has been implicated as a core feature in the development of acute pancreatitis (AP)[1], a severe and sometimes fatal inflammatory disease caused primarily by excessive alcohol consumption and gallstones. Precipitants of AP such as bile acids and non-oxidative ethanol metabolites have been shown to disrupt normal calcium signalling and induce sustained cytosolic calcium elevations which lead to mitochondrial depolarisation, loss of ATP production and pancreatic acinar cell death. Recent evidence suggests that formation of the mitochondrial permeability transition pore (MPTP), modulated by cyclophilin D, may be integral to necrotic cell death pathway activation. The precise roles of calcium and reactive oxygen species (ROS) in modulating mitochondrial dynamics and cell death modalities are currently unclear[2], although we have recently demonstrated that ROS generation may exert an important protective role in pancreatic acinar cell death by promotion of apoptosis rather than necrosis[3, 4]. Using a combination of experimental techniques including confocal imaging, mitochondrial bioenergetics measurement and in vivo models of AP, we have investigated the detrimental actions of precipitants of AP on mitochondrial function, cell fate and inflammation to address potential therapeutic avenues for disease prevention.

References

1. Criddle DN, Gerasimenko JV, Baumgartner HK, Jaffar M, Voronina S, Sutton R, Petersen OH, Gerasimenko OV. Calcium signalling and pancreatic cell death: apoptosis or necrosis? Cell Death Differ 2007;14:1285-94.
2. Booth DM, Mukherjee R, Sutton R, Criddle DN. Calcium and reactive oxygen species in acute pancreatitis: friend or foe? Antioxid Redox Signal 2011;15:2683-98.
3. Criddle DN, Gillies S, Baumgartner-Wilson HK, Jaffar M, Chinje EC, Passmore S, Chvanov M, Barrow S, Gerasimenko OV, Tepikin AV, Sutton R, Petersen OH. Menadione-induced reactive oxygen species generation via redox cycling promotes apoptosis of murine pancreatic acinar cells. J Biol Chem 2006;281:40485-92.
4. Booth DM, Murphy JA, Mukherjee R, Awais M, Neoptolemos JP, Gerasimenko OV, Tepikin AV, Petersen OH, Sutton R, Criddle DN. Reactive oxygen species induced by bile acid induce apoptosis and protect against necrosis in pancreatic acinar cells. Gastroenterology 2011;140:2116-25.

Conference No. 10

Tyrosine nitration is involved in the pathogenesis of insulin resistance

Serge P. Bottari

Fundamental and Applied Bioenergetics Laboratory, INSERM U1055 and Grenoble University and Radioimmunology Laboratory, University Hospital, Grenoble, FRANCE.

Numerous clinical trials have shown that inhibition of the renin-angiotensin system not only slows down the pro-gression of cardiovascular morbidity and mortality in insulin resistance and NIDDM but also reduces the risk of developing diabetes in hypertensive patients. Even more interesting are the observations showing that this inhibi-tion appears to increase insulin sensitivity, indicating that angiotensin II (Ang II) interferes with insulin-dependent metabolic pathways and is therefore probably involved in the etiology of NIDDM.

Several reports indicate that Ang II reduces insulin-mediated glucose uptake and GLUT4 translocation in skeletal muscle, but the mechanisms involved in the insulin-desensitizing effects of Ang II remain ill defined.

In order to identify upstream targets of Ang II involved in the insulin-dependent glucose uptake pathway and to investigate the molecular mechanisms through which these proteins' function is affected, we examined the effects of Ang II on GLUT4 and Akt, an upstream regulator of its translocation. We found Ang II to block insulin-dependent GLUT4 translocation in L6 myotubes in an .NO- and $O_2^{\cdot -}$-dependent fashion suggesting the involve-ment of peroxynitrite. This hypothesis was confirmed by the ability of Ang II to induce tyrosine nitration of the MAP kinases ERK1/2 and of Akt. Whereas tyrosine nitration of ERK1/2 was required for their activating phosphoryla-tion, it completely inhibited Akt phosphorylation and activation. The inhibitory effect of nitration on Akt was con-

firmed by the ability of the peroxynitrite donor SIN-1 to completely block Akt-dependent GSK3α phosphorylation in vitro. Inhibition of nitric oxide synthase 2 and NAD(P)H-oxidase and scavenging of reactive species restored insu-lin-stimulated Akt phosphorylation and GLUT4 translocation in the presence of Ang II.

Similar restoration of Akt activity was obtained by inhibiting the ERK activating kinase MEK, indicating that these kinases regulate Akt activation. We mapped the nitration sites of ERK1/2 and Akt and found some to be located in their kinase domains close to their active sites in agreement with a regulatory function of their activity. Taken together, our data show that Ang II inhibits insulin-mediated GLUT4 translocation in a skeletal muscle model through at least two pathways: 1° through the transient activation of ERK1/2 which may inhibit IRS-1/2 and/or activate iNOS and 2° through nitration of Akt. These observations indicate that nitro-oxidative stress plays a key role in the pathogenesis of insulin resistance. They underline the role of protein nitration as a major mechanism in the regulation of Ang II and insulin signaling pathways and more generally as a key regulator of protein kinase activity.

Conference No. 11

Nitro-oxidative stress in neonatal emergencies: Asphyxia and Hypoglycemia

J.-L. Wayenberg

Department of Pediatrics Hôpital Universitaire des Enfants – Reine Fabiola Université Libre de Bruxelles, Brussels, BELGIUM.

Nitro-oxidative stress, i.e. peroxynitrite generation and subsequent nitration and/or oxidation of proteins, lipids and DNA, is implicated in neuronal death as a common pathway leading from cerebral injury to apoptosis. Two causes of cerebral injury occur frequently in newborns: hypoxo-ischemia or hypoglycemia. In order to investigate the association of hypoxo-ischemia and hypoglycaemia with peroxynitrite generation, we measured plasma protein nitration as a marker of neonatal nitro-oxidative stress.

Using a sensitive double-sandwich ELISA, plasma nitroalbumin (PNA) concentration was measured in cord blood and at days 0, 1 and/or 4 of life in 323 newborns. We investigated differences in PNA concentrations according to the occurrence and the severity of hypoxo-ischemic or hypoglycemic events.

In asphyxiated infants, PNA concentration at day 1 of life increased with the severity of post-asphyxia encephalopathy. In contrast, PNA concentration did not change with neurological course when measured at days 0 and 4 of life and was not correlated with systemic complications of perinatal asphyxia. PNA is a specific marker of neurological injury after perinatal asphyxia and may serve as a secondary end-point in neuroprotective clinical trials.

In unasphyxiated preterm and small for gestational age term infants, PNA concentrations at days 0, 1 and 4 of life were significantly higher in patients who developed at least one hypoglycemic event than in normoglycemic patients. PNA further increased with repeated hypoglycemic episodes and in patients treated with oral versus IV glucose. In hypoglycemic infants, PNA was inversely correlated with lactate. We were not able to demonstrate any confounding factor that may interfere with the interpretation of these results. Our findings plead in favor of a causal relationship between hypoglycemia and plasma albumin nitration.

Thus perinatal asphyxia and postnatal hypoglycemia are associated with increased albumin nitration in newborns. This suggests the occurrence of nitro-oxidative stress implying a risk of end-organ damage due to protein nitration, lipid peroxidation and DNA damage. Evidence of nitrating stress in these conditions may open new and exciting perspectives in neuroprotection.

Conference No. 12

Cysteine-mediated oxidation activates TRP channels

Tomohiro Numata[1,2], Nobuaki Takahashi[1,3], Shigeki Kiyonaka[1,2,4], Daisuke Kozai[1,2], Yasuo Mori[1,2,4]

[1] Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, JAPAN.
[2] Department of Technology and Ecology, Hall of Global Environmental Studies, Kyoto University, Kyoto, JAPAN.
[3] Advanced Biomedical Engineering Research Unit, Kyoto University, Kyoto, JAPAN.
[4] Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Tokyo, JAPAN.

Proteins are capable of sensing the redox status of cells. Cysteine residues, which react ith oxidants, reductants, and electrophiles, have been increasingly recognized as the mediators of this redox sensitivity. Cation channels encoded by the *transient receptor potential (trp)* gene superfamily are characterized by a wide variety of activation triggers that act from

outside and inside the cell. Recent studies have revealed that a class of TRP channels is sensitive to changes in redox status and is notably susceptible to modifications of cysteine residues, such as oxidation, electrophilic reaction, and S-nitrosylation of sulfhydryls. Here we focus on TRP channels, which directly sense redox status, and discuss the biological significance of cysteine modifications and the consequences of this chemical reaction for physiological responses.

Conference No. 13

Oxidant-sensitive calcium gating via TRPM2 channels induces apoptosis

Asrar B. Malik

Department of Pharmacology University of Illinois College of Medicine, Chicago, IL. USA.

We addressed the role of oxidant-activated transient receptor potential melastatin 2 (TRPM2) channel in the gating of Ca^{2+} and how it mediates apoptosis of lung endothelial cells (LECs). We showed that PKCα-induced phosphorylation of the TRPM2-associated short splice variant (TRPM2-S) blocked its association with TRPM2, and thereby activated Ca^{2+} gating. Exposure of cells to H_2O_2 induced the association of PKCα with TRPM2-S and PKCα phosphorylation of TRPM2-2. TRPM2 phosphorylation at S39A in turn induced the dissociation of TRPM2-S from TRPM2. Dissociation of TRPM2-S induced Ca^{2+} gating via TRPM2 and enabled Ca^{2+} entry. This mechanism of TRPM2 channel activation was required for oxidant mediated apoptosis. Thus, oxidant-induced apoptosis is critically dependent of PKCα phosphorylation of TRPM2-S and resultant TRPM2 gating of Ca^{2+}.

Conference No. 14

Regulation of SOCE during the cell cycle

Fang Yu, Lu Sun, Raphael Courjaret, Senthil Selvaraj, Satanay Hubrak and Khaled Machaca

Department of Physiology, Weill Cornell Medical College in Qatar, QATAR

Store-operated Ca^{2+} entry (SOCE) is a ubiquitous Ca^{2+} influx pathway that is important for several physiological functions including immune response and skeletal muscle development. Ca^{2+} influx through SOCE contributes to shaping the dynamics of the Ca^{2+} signal and as such the ensuing cellular response. Interestingly during the division phase of the cell cycle SOCE inactivates. This inactivation in meiosis is due to internalization of the Orai1 channel into an endosomal compartment and to the inability of STIM1 to cluster in response to Ca^{2+} store depletion. At steady state Orai1 recycles between the cell membrane of an endosomal compartment with enrichment at the cell membrane representing ~80% of the total Orai1 protein pool. Following store depletion the intracellular Orai1 pool translocates to the cell membrane. We will present an update of the regulation of Orai1 trafficking in mammalian cells and its involvement in SOCE inactivation during cell division.

Conference No. 15

TRP channels and the primary cilium

Leonidas Tsiokas

Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA.

The primary cilium is an antennae-like organelle projecting from the plasma membrane of most quiescent mammalian cells. Primarily, it consists of the basal body (a differentiated form of the centrosome) and the axoneme. It is widely believed that the primary cilium has a sensory role in detecting and decoding signals from the surrounding environment. In addition to this important sensory role, the primary cilium has an equally important role in controlling the cell cycle by regulating the time by which the centrosome (in the form of basal body) resides at the plasma membrane. Several TRP channels are localized within the primary cilium. One of them, the TRPP2 is of particular interest because it is associated with the Autosomal Dominant form of Polycystic Kidney Disease, a disease belonging to a large number of pathologies associated with the primary cilium. My laboratory has shown that TRPP2 functions as a receptor-operated channel regulated by the phosphoinositide pathway and small GTPases. Some of the essential components controlling TRPP2 gating have been localized at the cilium suggesting that activation of TRPP2 channel can occur at the cilium and may mediate some of the sensory functions of the cilium. Potential cilium-based functions mediated by TRPP2 include modulation of the Wnt pathway and regulation of ciliary disassembly in response to mitogenic stimuli.

Conference No. 16

Special electrophysiological characteristics of the nucleus Locus Coeruleus

Saeed Semnanian, Hossein Azizi, Javad Mirnajafizadeh

Department of Physiology, School of Medical Sciences, Tarbiat Modares University Tehran, IRAN.

The Locus Coeruleus (LC) nucleus is a well-delineated cluster of NE-containing neurons, located adjacent to the fourth ventricle in the pontine brainstem. The tiny nucleus LC, comprised of only 1,500 neurons in the rat, sends projections to most brain regions.

There is recent evidence that the LC neurons and the noradrenergic system are involved in both the rewarding properties of opiates and the physical signs of withdrawal syndrome. Also it is shown that the neurotransmitter orexin is effective in morphine dependence. LC neurons have the most dense orexin inputs and are rich in Orexin-1 receptors. So we tried to study whether Orexin-A have any effect on the stimulatory synaptic activity of LC neurons in morphine dependent rats using electrophysiological whole cell clamp technique.

Morphine dependence was induced in young rats (20 mg/kg, 0.005 ml/g, s.c., BID, for 6 days). Thereafter passive properties of the LC neurons, spontaneous excitatory postsynaptic current (sEPSC), paired-pulse ratio (PPR) and evoked excitatory postsynaptic current (eEPSC), NMDA and AMPA mediated excitatory postsynaptic current (eEpsc) in LC neurons of control and dependent rats were studied before and after induction of orexin-A (100 nM, 5 min) and the orexin type 1 receptor antagonist SB-334867.

The results obtained show that in vitro application of orexin-A increases the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) of LC neurons, but does not change the sEPSCs frequency. Also, Orexin-A application did not modify the paired-pulse facilitation (PPF) in LC neurons. The bath application of orexin-A increases NMDA receptor mediated eEPSCs responses in LC neurons, but it did not modify AMPA receptor mediated eEPSCs in these neurons.

The data presented here show that orexin induces a postsynaptic potentiation of excitatory synaptic transmission to the LC neurons synapses.

Conference No. 17

Functional Genomic Analysis of TRPC1 Gene Silencing in Liver Cancer

Metiner Tosun[1], Cigdem Selli[1], Yasemin Erac[1], Buket Kosova[2], Esra Erdal[3]

Departments of Pharmacology, Faculty of Pharmacy[1], Medical Biology[2], Ege University, and Medical Biology and Genetics[3], Dokuz Eylul University, Izmir, TURKEY.

Altered activity or expression of some calcium ion channels and pumps appears to signify the type of certain cancers. Some calcium signaling pathways involve in tumorigenesis including proliferation, apoptosis, gene transcription and angiogenesis. Spatio-temporal changes in calcium homeostasis may either trigger apoptosis or survival of the cancer cells depending on the cancer type. Expression pattern as well as the mislocation of the channel of interest may modulate the fate of the cancer cell. Within this scope, this talk will only cover functional consequences as well as the genome-wide affects of TRPC1 ion channel downregulation on HuH-7 human hepatocellular carcinoma cell line transfected with shRNA-expressing vector. Briefly, our preliminary results suggest a regulatory role for TRPC1 in store-operated Ca^{2+} entry and proliferation of hepatocellular carcinoma cells. Furthermore, transcriptome analysis showing drastic up- and down-regulation of 40 transcripts suggests a possible epigenetic control over TRPC1 expression. This study was supported in part by The Scientific and Technological Research Council of Turkey (TUBITAK, 108S072 to MT; BIDEB-2211 to CS) and Ege University (BAP 08ECZ009 to MT).

References

1. Erac Y, Selli C, Kosova B, Akcali KC, Tosun M. Expression levels of TRPC1 and TRPC6 ion channels are reciprocally altered in aging rat aorta: implications for age-related vasospastic disorders. Age 2010; 32:223-230.
2. Selli C, Erac Y, Kosova B, Tosun M. Post-transcriptional silencing of TRPC1 ion channel gene by RNA interference upregulates TRPC6 expression and store-operated Ca^{2+} entry in A7r5 vascular smooth muscle cells. Vascul Pharmacol 2009; 51(2-3):96-100.

Conference No. 18

Role of Bcl-2 family members in irradiation-stimulated activation of TRPM2 channels in lymphoma cells

Kalina Szteyn[1], Milan Misovic[2], Dominik Klumpp[2], Ekaterina Shumilina[1], Justine Rudner[2] and Stephan M. Huber[2]

[1]Departments of Physiology and [2]Radiation Oncology, University of Tübingen, GERMANY.

The TRPM2 Ca^{2+}-permeable cation channels have been ascribed both, a tumor promoting as well as a tumor suppressing function. For the latter function, TRPM2 has been proposed to trigger the oxidative stress-induced cell death by overloading the cells with Ca^{2+}. Oxidative stress may activate TRPM2 indirectly through mitochondrial formation of the TRPM2 activator ADP-ribose suggesting a

close interaction between the mitochondria and the plasmalemmal TRPM2 channels. Bcl-2 is an anti-apoptotic protein that preserves the mitochondrial integrity during cellular stress. Overexpression of Bcl-2 occurs in various tumor entities and confers resistance against anti-cancer treatments such as radiation therapy. The present study aimed to analyze a potential crosstalk between Bcl-2 and TRPM2 channels in human lymphoma cells during cellular stress as conferred by ionizing radiation. To this end, the plasmalemmal cation currents, the Ca^{2+} signaling, and the cell cycle progression were compared between Bcl-2 overexpressing and empty vector-transfected Jurkat cells by patch-clamp recording, Fura-2 ratiometric Ca^{2+} imaging, and flow cytometry, respectively. Experiments were performed in irradiated cells (0 or 5 Gy X-ray) in the presence or absence of the TRPM2 inhibitors clotrimazole and N-(p-amylcinnamoyl)anthranilic acid. As a result, the Bcl-2 mediated radioresistance was associated with higher cytosolic free Ca^{2+} concentrations. In part, this was due to an ionizing radiation- and TRPM2-dependent Ca^{2+} entry which was higher in Bcl-2 overexpressing than in control cells. Moreover, whole-cell recordings indicated an ionizing radiation-stimulated and TRPM2-dependent increase in K^+ conductance which again was higher in Bcl-2 overexpressing than in control cells. Finally, inhibition of TRPM2 induced a release from G2/M cell cycle arrest in both cell types. Unexpectedly, this release resulted in pronounced cell death only in the vector-transfected Jurkat cells. Collectively, this data suggest a pivotal function of TRPM2 in the response to genotoxic stress and cell cycle control. Bcl-2 overexpressing cells demonstrate, on the one hand, higher TRPM2 activities. On the other hand, they seem to be less dependent on the TRPM2 channels.

Conference No. 19

Functional regulation and pharmacology of TRPC channels

Michael X. Zhu and Jin-Bin Tian

Department of Integrative Biology and Pharmacology, The University of Texas Health Science Center at Houston, Houston, Texas, USA.

Transient receptor potential canonical (TRPC) channels play diverse physiological functions. This subgroup of TRP channels are generally activated following stimulation of phospholipase C; however, the precise mechanisms underlying the activation of different TRPC channel subtypes remain elusive. Recent studies have revealed that TRPC channel activation is under a polymodal control, in which a single factor alone typically induces only partial activation whereas two or three factors in combinations are often required to fully activate the channel. Thus, the ability to sense and distinguish simultaneous activations of multiple signaling events/pathways suggests that TRPC channels may serve as coincidence detectors of extra- and intracellular signals. Consistent with this idea, TRPC4 channels, which mediate the major portion of the muscarinic agonist-elicited cation currents in intestinal smooth muscle cells [1], are activated more efficiently with the co-stimulation of both $G_{q/11}$ and $G_{i/o}$-coupled receptors than the stimulation of a single receptor type alone [2,3]. In lateral septal neurons, the TRPC1/C4 channels are involved in the pronounced depolarization plateau potential underlying the epileptiform burst firing following stimulation by agonists of metabotropic glutamate receptors and critical for seizure-induced neuronal death [4]. Because the lateral septum plays an important role in modulating mode and motivation, the ability of TRPC4 to detect coincident activations of $G_{q/11}$ and $G_{i/o}$ proteins at the lateral septal neurons should have important functional implications in integrating and sorting inputs from such broad brain areas as hippocampus, hypothalamus, amygdale, raphe nuclei, ventral tegmental area, nucleus accumbens, and bed nucleus of the stria terminalis. As a result, the outputs from the lateral septum reflect coordinated instructive signals to multiple brain areas with diverse functional effects, some of which also contribute to neuropsychiatric behaviors including depression, drug abuse, fear, anxiety, and social recognition. The TRPC4-like activities in lateral septal neurons are evoked via a brief co-stimulation of $G_{q/11}$-coupled (e.g. metabotropic glutamate, acetylcholine, substance P and vasopressin) receptors, $G_{i/o}$-coupled (e.g. GABAB, somatostatin, dopamine, and serotonin) receptors, and a depolarization pulse. The resultant depolarization plateau potential lasts much longer than the stimulation, indicative of a sustained effect of TRPC4 on neuronal activity, likely due to the unique Ca^{2+}-dependent dual regulation on the TRPC4 channel. Therefore, the spatial and temporal patterns of various inputs into the lateral septum can affect neuronal activities quite differently depending on the activity levels of TRPC4 channels. With the development of novel small molecular probes for TRPC channels, the critical roles of these channels in integrating multitudes of extra- and intracellular signals to guide neuronal processes and the function of other organ systems will be gradually unveiled.

References

1. Tsvilovskyy VV, Zholos AV, Aberle T, Philipp SE, Dietrich A, Zhu MX, Birnbaumer L, Freichel M, Flockerzi V. (2009) Deletion of TRPC4 and TRPC6 in mice impairs smooth muscle contraction and intestinal motility in vivo. Gastroenterology 137(4):1415-24.
2. Zholos AV, Bolton TB. (1997) Muscarinic receptor subtypes controlling the cationic current in guinea-pig ileal smooth muscle. Br J Pharmacol. 122(5):885-93.
3. Otsuguro K, Tang J, Tang Y, Xiao R, Freichel M, Tsvilovskyy V, Ito S, Flockerzi V, Zhu MX, Zholos AV. (2008) Isoform-specific inhibition of TRPC4 channel by phosphatidylinositol 4,5-bisphosphate. J Biol Chem. 283(15):10026-36.
4. Phelan KD, Mock MM, Kretz O, Shwe UT, Kozhemyakin M, Greenfield LJ, Dietrich A, Birnbaumer L, Freichel M, Flockerzi V, Zheng F. (2012) Heteromeric canonical transient receptor potential 1 and 4 channels play a critical role in epileptiform burst firing and seizure-induced neurodegeneration. Mol Pharmacol. 81(3):384-92.

Conference No. 20

TRPM4 is regulator of Ca^{2+} dependent cell functions

Rudi Vennekens[1], Andrei Segal[1], Ilka Mathar[3], Bernd Nilius[1], Marc Freichel[3], Paul Herijgers[2]

[1]Laboratory of Ion Channel Research and [2]Experimental Cardiac Surgery, KU Leuven, BELGIUM, [3]Institut fur Experimentele und Klinische Pharmakologie und Toxikologie, Universitat des Saarlandes, GERMANY.

TRPM4 is a Ca^{2+} activated non-selective cation channel. Together with TRPM5 it's the only molecular candidate for this class of ion channels to date. Both genes are members of the Transient Receptor Potential (TRP) family of ion channels, which constitutes 28 mammalian genes subdivided into six subfamilies: the TRPC ('Canonical'), TRPV ('Vanilloid'), TRPM ('Melastatin'), TRPP ('Polycystin'), TRPML ('Mucolipin') and the TRPA ('Ankyrin') groups. We have shown previously, using *Trpm4-/-* mice, that this channel plays a critical role in setting the membrane potential, and consequently the driving force for Ca^{2+} entry in mast cells, which determines ultimately the strength of an allergic reaction. The role of CAN channels in excitable cells however is unclear. In this study we tested whether TRPM4 plays a role in cardiac muscle. We can show that TRPM4 is present in ventricular myocytes from human and mouse heart. Analysis of pressure-volume loops in living mice shows in basal conditions no difference between WT and *Trpm4-/-* mice. However, upon stimulation with beta-adrenergic agonists, e.g. isoprenaline, *Trpm4-/-* mice show an increased inotropic effect. The chronotropic effect of beta-adrenergic stimulation is not different between WT and *Trpm4-/-* mice. These results could be reproduced in isolated left ventricular papillary muscle preparations, indicating that the effect is indeed cardiac muscle specific. We present data illustrating the consequences of TRPM4 ablation for cellular signaling in isolated cardiomyocytes.

Conference No. 21

TRP Channels in noxious temperature sensing

Thomas Voets

Laboratory of Ion Channel Research, KU Leuven, BELGIUM.

The ability to sense environmental temperatures and to avoid noxious heat or cold is crucial for the survival of all organisms. In mammals, sensory neurons from dorsal root and trigeminal ganglia convey thermal information from the skin, mouth and nose to the central nervous system. Recent evidence has established that thermoTRPs, a subset of the TRP superfamily of cation channels, act as primary temperature sensors in cold- and heat-sensitive neurons. The gating of these thermoTRPs exhibits strong temperature dependence, leading to steep changes in inward current upon heating or cooling. Moreover, thermoTRPs can also be activated by chemical ligands, evoking a thermal sensation and/or pain. In this presentation, new findings will be presented related to the molecular mechanisms of thermo- and chemosensing by TRP channels, and their contribution to pain sensing *in vivo*.

Conference No. 22

NCLX, the mitochondrial Na/Ca exchanger

Israel Sekler

Department of Physiology, Faculty of Health Sciences, Ben Gurion University of the Negev, ISRAEL.

Mitochondria are not providing ATP to cells but are also a major hub in cellular Ca^{2+} signaling. Powered by the steep mitochondrial membrane potential Ca^{2+} enters into the mitochondria via the Ca uniporter and is pumped out by a mitochondrial Na^{+} /Ca^{2+} exchanger. Mitochondrial Ca^{2+} shuttling is shaping local Cai^{2+} and activity of Ca^{2+} channels in these regions. Moreover disturbances in mitochondrial Ca shuttling triggers mitochondrial Ca overload, a major cause for brain and cardiac

damage during neurodegenerative diseases or ischemia. The molecular identity of the mitochondrial Ca uniporter and the Na^+/Ca^{2+} exchanger remained however elusive. In the first part of my talk I will describe the molecular identification of NCLX as the mitochondrial exchanger The second part of the presentation will focus on the physiological role of NCLX describing : 1) The regulation of Ca^{2+} signaling in β cells and its role in regulating insulin secretions. 2) How Na^{2+} and Ca^{2+} signaling are linked to mitochondrial Ca^{2+} shuttling in regulation of the store operated Ca^{2+} pathway and 3) How a toxic Ca^{2+} surge in neuron leads to degradation of NCLX thereby affecting neuronal survival. Our results indicate that by linking Na^+ and Ca^{2+} transport mitochondrial NCLX plays a role in global cellular Ca^{2+} and Na^+ signaling in health and diseases.

Conference No. 23

TRiP to Automation

David R. Guinot[1], Ilka Rinke[1], Alison Obergrussberger[1], Sonja Stölzle[1], Michael George[1], Andrea Brüggemann[1], Niels Fertig[1]

[1] Nanion Technologies GmbH, Gabrielenstr. 9, 80636 München, GERMANY.

Transient receptor potential (TRP) channels are an important class of receptors found widely distributed throughout the mammalian central and peripheral nervous systems. They have been shown to be activated by many stimuli including temperature, mechano-stimulation, divalent cations and pH. TRP channels are receiving much attention as potential targets for the treatment of, for example, chronic pain, asthma, and diabetes isipidus.

Patch clamp electrophysiology remains the gold standard for studying ion channels. We have employed a planar patch clamp workstation to study TRPV channels using a variety of stimuli. High quality data could be achieved with a high success rate for obtaining giga-seals (typically 60-80%). TRPV1 or TRPV3 stably expressed in CHO or HEK cells were activated by ligands such as capsaicin (TRPV1) or 2-APB (TRPV1 & TRPV3) and inhibited by ruthenium red (TRPV1 & TRPV3). Data will be presented showing activation and inhibition of TRPV channels by a variety of agonists and antagonists. TRPV channels are also activated by temperatures. Using a heated pipette, the temperature of the added solution was increased and then rapidly applied to the cell. Very rapid changes in temperature can be achieved at the cell (within ms), from room temperature up to ~65˚C, whilst continuously recording. Whole-cell currents will be presented showing heat activation of TRPV1 and TRPV3 using a range of temperatures. Pharmacology can also be performed using elevated temperature as the activator of TRPV1 and a direct comparison can be made between the ability of a compound to block the ligand response, e.g., by capsaicin, and/or the temperature response of TRPV1 channels.

The ability to distinguish between the different modes of action of TRP channels may have important implications for drug design and may give further clues about the physiological and pathophysiological roles of these channels.

Conference No. 24

Orai1 channels and vascular smooth muscle remodeling

Mohamed Trebak

Center for Cardiovascular Sciences, Albany Medical College, Mail Code 8, 47 New Scotland Ave, Albany, NY 12208, USA.

The store-operated Ca^{2+} entry (SOCE) pathway and its molecular correlates: STIM1/Orai1 channels are upregulated in proliferative vascular smooth muscle cells (VSMC). We used lentiviral particles encoding short-hairpin RNA (shRNA) targeting either Orai1 or STIM1 for specific knockdown of their respective target mRNA and proteins *in vitro* and *in vivo*. Here, we show that this molecular knockdown abrogates SOCE in primary VSMC *in vitro*. We used balloon injury of rat carotid arteries as an *in vivo* model for vascular remodeling and show upregulated protein expression of Orai1 and STIM1; increased proliferation as assessed by Ki67 and PCNA and decreased expression of contractile proteins such as myosin heavy chain in medial and neointimal VSMC. Incubation of the injured vessel with shOrai1 prevented Orai1, but also STIM1upregulation in the media and neointima; inhibited cell proliferation and markedly reduced neointima formation 14 days post injury; similar results were obtained with shSTIM1. VSMC Orai1 and STIM1 knockdown inhibited nuclear factor for activated T-cell (NFAT) nuclear translocation and activity. Furthermore, Orai1 and STIM1 were upregulated in mice carotid arteries subjected to ligation. We conclude that Orai1 and STIM1 are upregulated in VSMC during vascular injury and are required for NFAT activity, VSMC proliferation, and neointima formation following balloon injury of rat carotids. Orai1 and STIM1 may represent novel targets for control of VSMC remodeling during vascular injury or disease.

Conference No. 25

Crosstalk between mitochondrial and NADPH oxidase derived reactive oxygen and nitrogen species - implications for vascular function

Matthias Oelze, Swenja Schuhmacher, Philip Wenzel, Alexander Scholz, Steffen Daub, Sebastian Steven, Ning Xia, Eberhard Schulz, Huige Li, Karl J. Lackner, Thomas Münzel, and Andreas Daiber

From the 2nd Medical Clinic, Pharmacology Department and Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center of the Johannes Gutenberg University, Mainz, GERMANY.

Many diseases and drug-induced complications are associated or even based on an imbalance between the formation of reactive oxygen and nitrogen species (ROS/RNS) and antioxidant enzymes catalyzing the break-down of these harmful oxidants (1). Previously we and others have reported on a crosstalk between different sources of oxidative stress (2). With the present study we sought to determine the underlying mechanism for this crosstalk.

Human neutrophils were used to study the induction of Nox-dependent oxidative burst by mitochondrial ROS (mtROS) in response to antimycin A or myxothiazol. Activation of Nox2 was measured by extracellular detection of ROS by a peroxidase-coupled chemiluminescence assay. We used different inhibitors for the involved signaling cascade such as cyclosporine A (inhibits the mitochondrial permeability transition pore [mPTP]), chelerythine (inhibitor of protein kinase C) and apocynin (inhibitor of NADPH oxidase). All of these inhibitors suppressed the antimycin A/myxothiazol-triggered extracellular ROS signal, whereas exogenous hydrogen peroxide mimicked the effect of mitochondrial ROS formation and caused extracellular superoxide formation (lucigenin ECL). According to preliminary data, this crosstalk between mtROS and NADPH oxidase was also suppressed in white blood cells from $p47^{phox}$ deficient mice (an essential subunit for activation of Nox2) or from cyclophilin D knockout mice (a regulatory subunit of the mPTP), whereas this crosstalk was amplified in white blood cells from GPx-1 deficient mice or from $MnSOD^{+/-}$ mice. We also observed that increases in blood pressure, in endothelial dysfunction as well as in NADPH oxidase activity were more pronounced in $MnSOD^{+/-}$ mice as compared to their wild type littermates. Finally, we have evidence for mtROS-induced dysfunction/uncoupling of eNOS via adverse phosphorylation and S-glutathionylation (3).

Previous data by Dikalov and coworkers have demonstrated that NADPH oxidase derived ROS/RNS stimulate mitochondrial oxidative stress contributing to overall angiotensin-II dependent cellular dysfunction (4,5). Our data now shows that mitochondrial ROS trigger the activation of NADPH oxidase, which may have severe effects on progression of cardiovascular diseases since it represents a feedback loop creating a vicious circle.

References

1. Chen AF, Chen DD, Daiber A, et al. Free radical biology of the cardiovascular system. Clin Sci (Lond) 2012;123:73-91.
2. Daiber A. Redox signaling (cross-talk) from and to mitochondria involves mitochondrial pores and reactive oxygen species. Biochim Biophys Acta 2010;1797:897-906.
3. Knorr M, Hausding M, Kroller-Schuhmacher S, et al. Nitroglycerin-Induced Endothelial Dysfunction and Tolerance Involve Adverse Phosphorylation and S-Glutathionylation of Endothelial Nitric Oxide Synthase: Beneficial Effects of Therapy With the AT1 Receptor Blocker Telmisartan. Arterioscler Thromb Vasc Biol 2011.
4. Doughan AK, Harrison DG, Dikalov SI. Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction. Circ Res 2008;102:488-96.
5. Dikalova AE, Bikineyeva AT, Budzyn K, et al. Therapeutic targeting of mitochondrial superoxide in hypertension. Circ Res 2010;107:106-16.

Conference No. 26

Calcium, mitochondria and cognition in normal ageing

Emil C. Toescu

Neuronal Networks Group, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Vincent Drive, Edgbaston, Birmingham B15 2TT, UNITED KINGDOM.

Oscillations in hippocampal neuronal networks in the gamma frequency band have been implicated in various cognitive tasks and we showed previously that aging reduces the power of such oscillations. In a series of experiments, using submerged hippocampal slices allowing simultaneous electrophysiological recordings and imaging, we studied whether this age-dependent reduction in gamma oscillations can be explained by the changes in intrinsic properties of hippocampal interneurons, and also monitored the correlation between the changes in kainate-evoked gamma oscillation and the changes in Ca^{2+} homeostasis and mitochondrial activity. One of the salient features of our experiments was that ageing affected the KA-activated neuronal

signaling. Thus, whereas the passive membrane properties, firing properties, medium- and slow-afterhyperpolarisation amplitudes, basal [$Ca^{(2+)}$] (i) and firing-induced [$Ca^{(2+)}$](i) transients were not different with ageing, kainate caused a larger depolarisation and increase in [$Ca^{(2+)}$](i) signal in aged interneurons than in young ones. In contrast to young interneurons, kainate increased the medium- and slow-afterhyperpolarisation in a Ca^{2+} dependet fashion, sensitive to the inhibition of the voltage-operated Ca channels. Separate experiments showed that kainate-evoked gamma oscillations induced mitochondrial depolarization, indicating a strong metabolic response. Aging had an opposite effect on these parameters: while depressing the gamma oscillation strength, it increases mitochondrial depolarization, related to the significant increase in the Ca^{2+} signals evoked by KA in the aged neurons. In younger slices, acute mitochondrial depolarization induced by low concentrations of mitochondrial protonophores strongly, but reversibly, inhibits gamma oscillations. These data indicating that the complex network activity required by the maintenance of gamma activity is susceptible to changes and modulations in mitochondrial status, which in turn will affect Ca^{2+} homeostasis. The overall fuctional effects are changes in the capacity of the interneurones to regulate the oscillatory activity, and thus underlie at least a part of the cognitive declines associated with ageing.

Conference No. 27

Neuroprotection induced by N-acetylcysteine against cytosolic glutathione depletion dependent oxidative stress and Ca^{2+} influx in DRG neuron: Involvement of TRPM2 and TRPV1 channels

Mustafa Nazıroğlu

Department of Biophysics, Faculty of Medicine, University of Suleyman Demirel, Isparta, TURKEY.

Glutathione (GSH) is the most abundant thiol antioxidant in mammalian cells and maintains thiol redox in the cells. N-acetylcysteine (NAC) is a thiol-containing antioxidant, which contributes to regeneration of GSH and also acts through a direct reaction with free radicals. Thiol depletion-induced neuropathy is associated with peripheral demyelination and degeneration of nerve fibers. The mechanism underlying dorsal root ganglion (DRG) injury through GSH depletion remains unclear. Immunohistochemical studies confirmed highly the presence of transient receptor potential melastatin 2 (TRPM2) and vanilloid 1 (TRPV1) in dorsal root ganlion (DRG) neurons. TRPM2 and TRPV1 channels are activated by oxidative stress and we reported recently a modulator role of NAC on TRPM2 channel current in rat DRG. NAC may also have a regulator role on TRPV1 channels in the neurons. Therefore, we tested the effects of NAC on TRPV1 channel currents in intracellular GSH depleted DRG in rats.

DRG neurons were freshly isolated from rats and the neurons were incubated for 24 hrs with buthionine sulfoximine (BSO). Cytosolic treatment of cultured DRG neurons with GSH and NAC, results in a protection against capsaicin (CAP) activation. This neuroprotection is associated with the attenuation of a Ca^{2+} influx triggered by CAP, oxidative stress and GSH depletion via BSO. Here, we evidence the contribution of thiol groups on activation of TRPV1 channels in this mechanism. TRPV1 channels are activated by various agents including capsaicin, the pungent component of hot chili peppers and blocked by capsazepine (CPZ) although TRPM2 channels were activated by BSO and H_2O_2 as an oxidant. CPZ, anthralic acid and 2-aminoethyl diphenylborinate TRPV1 inhibitors strongly reduced CAP-induced TRPV1 currents, oxidative stress-induced TRPM2 currents and Ca^{2+} influx, in the same way as to GSH and NAC treatments.

In conclusion, in our experimental model, TRPM2 and TRPV1 channels are involved in the CAP and oxidative stress-induced neuronal death, respectively. A negative modulation of this channel activities by GSH and NAC treatment may account, at least in part, for the neuroprotection against GSH depletion induced oxidative toxicity.

Conference No. 28

Neuroregenerative diseases, calcium and microglia

Nicole Mahy (Barcelona, Spain)

Conference No. 29

ATAD3 is a limiting factor in mitochondrial remodelling and lipogenesis in white adipocyte 3T3-L1 cells

Shui Li, Yiao Yiao, Rui Xu, Cécile Cottet, Uwe Schlattner, Kan Liao and Denis Rousseau

Laboratory for Fundamental and Applied Bioenergetic, Université Joseph Fourier, INSERM U1055, Grenoble, FRANCE.
Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, CHINA.

ATAD3 is a ubiquitous and vital ATPase located

in the inner membrane of mitochondria but whose ATPase-associated function is still unknown. However, invalidation of ATAD3 blocks the development of C. elegans and of D. melanogaster, at stages requiring a significant mitochondrial mass increase, by disturbing the mitochondrial network and its interactions with the endoplasmic reticulum. The first organs affected by ATAD3 depletion in C. elegans development (stage L1) are the white adipocyte-like intestinal fat tissue and the gonads. To go further into the understanding of ATAD3 mitochondrial function, we used therefore the mouse 3T3-L1 cell line as a homologous mammalian white adipose cellular model. These cells have been already well characterized as it is known that insulin-induced adipogenesis/lipogenesis is associated with a significant increase of mitochondrial mass coupled with mitochondrial remodelling, this preceding the step of triglycerides storages. By stable and transient modifications of ATAD3 level in these cells, we show that (i) ATAD3 is overexpressed early during insulin-induced 3T3-L1 differentiation at the onset of mitochondrial mass increase and remodelling processes (ii) ATAD3 is essential and limiting for in vitro adipocyte differentiation and lipogenesis (iii) ATAD3 down-regulation inhibits mitochondrial remodelling and mitochondrial metabolic mass increase without affecting insulin-transduction pathway neither down-stream increase of nuclear-encoded mitochondrial protein amount, but inhibits the accumulation of mitochondrial-encoded protein and of the activated form of Acetyl-CoA Carboxylase (ACC) and (iiii) these effects can be rescued by ATAD3 re-expression or complemented by Drp1 overexpression, but not by Mfn2, two main actors in fission/fusion processes.

These results show that, in white adipocyte-like 3T3-L1 cells, ATAD3 contributes mainly to mitochondrial biogenesis-linked remodelling and down-stream activation of mitochondrial metabolic processes involved in lipid droplet formation, specifying here its level of action.

References

1. Daniel F. BOGENHAGEN, Denis ROUSSEAU and Stéphanie BURKE (2008) The layered structure of human mtDNA nucleoids. *Journal of Biological Chemistry* 283, 3665-3675.
2. Arnaud HUBSTENBERGER, Gérard LABOURDETTE, Jacques BAUDIER and Denis ROUSSEAU (2008) ATAD 3A and ATAD 3B are distal 1p-located genes differentially expressed in human glioma cell lines and present in vitro anti-oncogenic and chemoresistant properties. *Experimental Cell Research* 314, 2870-2883.
3. Arnaud HUBSTENBERGER, Nicolas MERLE, Romain CHARTON, Gérard BRANDOLIN, and Denis ROUSSEAU (2010) Immuno-topological analysis of ATAD3A in human mitochondria. *Journal of Bioenergetic and Biomembrane* 42, 143-50.
4. Benoît GILQUIN, Brian R. CANNON, Arnaud HUBSTENBERGER, Boualem MOULOUEL, Elin FALK, Nicolas MERLE, Nicole ASSARD, Sylvie KIEFFER, Denis ROUSSEAU, Paul T. WILDER, David J. WEBER, and Jacques BAUDIER (2010) The calcium-dependent interaction between S100B and the mitochondrial AAA-ATPase ATAD3A and the role of this complex in the cytoplasmic processing of ATAD3A. *Molecular and Cellular Biology* 30, 2724-36.
5. Shuijie LI, Benjamin CLEMENCON, Patrice CATTY, Gérard BRANDOLIN, Uwe SCHLATTNER and Denis ROUSSEAU (2012) Human ATAD3A expression and purification in Saccharomyces cerevisiae. *Under publication at Protein Expression and Purification.*

Conference No. 30

Oxidative lipidomics of cell death signaling - specific involvement of anionic phospholipids

Valerian E Kagan

Center for Free radical and Antioxidant Health, Department of Environmental Health, University of Pittsburgh, Pittsburgh, USA.

There are two major doctrines dominating the field of free radical/antioxidant research: i) oxidative stress induced by reactive oxygen species (ROS) is involved in the pathogenesis of various diseases and ii) antioxidants are effective in the prevention and treatment of diseases. These concepts have received much attention of researchers, clinicians and general public. However, most of the clinical intervention trials of antioxidants and their meta-analysis did not reveal significant beneficial effects of antioxidants resulting in the emergence of more cautious or even pessimistic views on these concepts. “The nice part about being a pessimist is that you are constantly being either proven right or pleasantly surprised” (GF Will). It is possible that the dogma of non-enzymatic “free” radical reactions does not adequately describe the mechanisms and significance of ROS and other radical intermediates in disease pathogenesis. If non-enzymatic “free radical” reactions are the major mechanism of lipid peroxidation, this random process should mostly affect highly polyunsaturated fatty acid residues with six, five and four double bonds. We employed mass-spectrometry-based oxidative lipidomics and performed detailed analysis of lipid oxidation products accumulating *in vivo* in several tissues of mice exposed to a lethal dose of irradiation as well as in the brain of rats after brain trauma. Lipid peroxidation did not follow the profile predicted by stochastic involvement of lipids in the oxidation process but displayed highly selective patterns. Two anionic phospholipids – mitochondrial-specific cardiolipin (CL) and extra mitochondrial phosphatidylserine (PS) – were the major substrates of peroxidation reactions whereas more abundant and highly polyunsaturated phosphatidylcholine

and phosphatidylethanolamine molecular species remained non-oxidized. This pattern was associated with the execution of apoptotic program and subsequent clearance of apoptotic cells by professional phagocytes. Further, we designed and tested several novel small molecule inhibitors that specifically affect the specific redox pathways – rather than random "free radical" peroxidation – that are important for oxidative modifications of phospholipids involved in the disease process. Thus, selective inhibitors of specific peroxidation reactions, catalyzed by redox-enzymes, represent new targets for mechanism-based "antioxidant" interventions. Finally, interactions of phospholipid peroxidation reactions with other important mechanisms and pathways, particularly those dependent on the release and re-uptake of Ca, may be of primary importance for the understanding of complex synergistic interactions involved in the regulation of oxidative stress in the body.

Conference No. 31

Crononutriton Against Oxidative Stress

A.B. Rodríguez, M.Garrido, A.M. Marchena

Department of Animal Physiology, Faculty of Science, University of Extremadura, Badajoz, SPAIN.

Chrononutrition indicates that the effectiveness of diet is determined not only by the composition of food, but also by the time of day of food intake.

In other words, it is necessary consuming food when the bioactive compounds, which exert a biological activity in the organism producing benefits for health, are more useful for the organism (1). Oxygen is an essential element for the survival of aerobic organism. However, the use of oxygen during normal metabolism generates reactive oxygen species (ROS), some of them being highly toxic and deleterious to cells and tissues. To protect cells from the damage caused by ROS and related reactants, organisms have evolved several antioxidant defence mechanisms to rapidly and efficiently remove ROS from the environment. Antioxidant defence systems may be generally classified into indirect antioxidant enzymes and into low-molecular weight molecules which act as scavengers of free radicals (2). When the equilibrium between free radicals (oxidants) and antioxidant defense systems is imbalanced in favour of oxidants, the condition causes what is known as oxidative stress, which is involved in the development of inflammatory, neurodegenerative and autoimmune diseases (3). Antioxidants are found as dietary components (vitamins, phenolic compounds, flavonoids or carotenoids, among others) in fruits, vegetables and natural beverages like tea. It is known how a balanced diet that is naturally rich in antioxidants protects against oxidative stress and also induces protective effects against inflammatory pathologies, mood-related disorders, insomnia and immunosenescence, among others age-related disorders (4). Therefore, we have evaluated the physiological effect exerted by fruits such as cherries, grapes or plums, which are rich in melatonin and/or its precursors, the amino acid tryptophan and the neurotransmitter serotonin (5-8). These three natural compounds reportedly present well-known antioxidant properties, particularly on antioxidant status, mood or inflammation. Thus, we have reported that the consumption of cherries, grapes and plums contributed to elevating antioxidant capacity in humans as well as 6-sulfatoxymelatonin (aMT6-s) levels, urine metabolite of melatonin (7-9). Likewise, urine 5-hidroxyindoleacitc acid (5-HIAA), urine metabolite of serotonin, and aMT6-s, were measured after the ingestion of a Jerte Valley cherry-based product, thereby suggesting that this product augments both 5-HIAA and aMT6-s availability. In addition, psychological tests, such as STAI test and Visual Analogue Scales, were carried out to determine mood status in humans, while urine cortisol levels were assessed as stress index after the ingestion of the cherry-based product, thus proving that such product enhances mood and antioxidant defense systems associated with decreasing cortisol levels. We have also measured serum pro- and anti-inflammatory cytokines as well as acute-phase proteins as indicators of the systemic inflammatory load after the ingestion of the cherry-based product, herein demonstrating that its consumption diminishes systemic inflammatory conditions (10, 11). Currently, our studies are also focused on revealing the antioxidant effect of lycopene, a pigment (carotenoid) present in tomatoes. On the one hand, we have demonstrated that the consumption of a lycopene-enriched virgin olive oil increases the antioxidant status in humans. On the other hand, we have elaborated a lycopene-enriched cream that protects skin against symptoms of aging, such as pigmentation, loss of firmness and loss of elasticity, acting as a sun block. In conclusion, following a balanced, antioxidant-rich diet contributes to the proper functioning of our organism, but always bearing in mind the most appropriate time of day of food intake to obtain the best health benefits.

References

1. Escobar et al. 2006. Ed. Editec@red. Reiter et al. 2009.
2. Crit Rev Biochem Mol Biol 44: 175-200. Pham-Huy et al. 2008.

3. Int J Biomed Sci 4: 89-96. Visioli and Galli, 2001.
4. Lipids 36: 49-52. González-Gómez et al. 2009.
5. Eur Food Res Technol 229: 223-229. Cubero et al. 2010.
6. Food Anal Methods 3: 36-39. González-Flores et al. 2011.
7. J Food Nutr Res 50: 229-236. González-Flores et al. 2012.
8. Food Funct 3: 34-39. Garrido et al. 2010.
9. J Gerontol A Biol Sci med Sci 65A: 909-914. Delgado et al. 2012.
10. J Appl Biomed10: 41-50. Delgado et al. 2012. J Med Food (in press).

Conference No. 32

Clock genes in human tissues

Cem Ekmekcioglu

Institute of Physiology, Centre for Physiology and Pharmacology, Medical University of Vienna, AUSTRIA.

Circadian rhythms are controlled and generated by the biological clock located in the hypothalamic suprachiasmatic nucleus (SCN) (1). This "master clock" is synchronized to 24 h by various environmental factors, primarily the dark-light-cycle but also by regularly occurring social processes, motor activity and food intake. These synchronizers are called "Zeitgebers", a german term, meaning Zeit = time and gebers = giving. The SCN transmits the inputs of these Zeitgebers via nerval and endocrine pathways to the peripheries where these physical informations are translated into molecular changes via the expression of a set of clock genes that control the expression of up to 10 % of the genome (2).

Clock genes constitute auto-regulatory feedback loops with heterodimers formed between BMAL 1 (brain-muscle-Arnt-like 1) and CLOCK (circadian locomotor output cycles kaput), which then serve as positive transcription factors binding to the E-box cis-regulatory enhancer elements which are found within target gene promoters or enhancers. The most important downstream transcriptional targets for CLOCK/BMAL 1 are those which encode PER (Period 1 and 2) and CRY 1 (Cryptochrome 1 and 2). As cellular levels of PER and CRY proteins increase they accumulate in the nucleus forming a negative feedback loop by downregulating the expression of BMAL 1/CLOCK complex and therefore their own expression.

We provided evidence for the expression of clock genes in the human heart and showed that the clock genes display significant circadian rhythms (3). In a recent project we analyze the expression of clock genes in synovial tissues and fibroblasts from patients with rheumatoid arthritis (RA) and compare the data with those derived from patients with osteoarthritis (OA). All of the clock genes were found in the specimens with some differences in the expression profiles between RA and OA samples.

References

1. Van Someren EJ, Riemersma-Van Der Lek RF: Live to the rhythm, slave to the rhythm. *Sleep medicine reviews* 2007, 11(6):465-484.
2. Okamura H, Doi M, Fustin JM, Yamaguchi Y, Matsuo M: Mammalian circadian clock system: Molecular mechanisms for pharmaceutical and medical sciences. *Adv Drug Deliv Rev* 2010, 62(9-10):876-884.
3. Leibetseder V, Humpeler S, Svoboda M, Schmid D, Thalhammer T, Zuckermann A, Marktl W, Ekmekcioglu C: Clock genes display rhythmic expression in human hearts. *Chronobiol Int* 2009, 26(4):621-636.

Oral Presentations

Oral Presentation No. 1

STIM1-mediated PMCA inhibition during T-cell activation

Jonathan Soboloff

Department of Biochemistry, Temple University School of Medicine, 3440 North Broad Street, Philadelphia, PENNSYLVANIA 19140, USA.

T cell activation involves a complex signaling cascade uniquely dependent on elevated cytosolic Ca^{2+} levels. Further, the spatiotemporal characteristics of this Ca^{2+} signal play a critical role in this process via selective activation of transcription factors. In T cells, store-operated Ca2+ entry (SOCe) is the primary Ca^{2+} influx pathway, however, cytosolic Ca^{2+} concentration depends upon the balance between Ca^{2+} influx and extrusion. The Plasma Membrane Ca^{2+} ATPase (PMCA) has previously been identified as a critical player in Ca^{2+} clearance in T cells. Here we provide data revealing both functional and physical links between the activation of Stromal Interacting Molecule 1 (STIM1) and PMCA-mediated Ca^{2+} clearance. Due to the ubiquitous expression of both STIM1 and PMCA, these findings have wide-ranging implications for Ca^{2+} signaling in multiple cell types.

Oral Presentation No. 2

Menadione-induced Oxidative Stress Modulates Mitochondrial Bioenergetics in Pancreatic Acinar Cells

Armstrong J[1], Sutton R1 & Criddle DN[1,2]

[1]NIHR Pancreas Biomedical Research Unit, RLBUHT and [2]Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, UK.

Oxidative stress has been implicated in the pathogenesis of acute pancreatitis although the precise role(s) remain unclear. We have analysed the effects of reactive oxygen species (ROS) generation on the mitochondrial bioenergetics of murine pancreatic acinar cells.

Mitochondrial respiration (oxygen consumption rate: OCR) and glycolysis (extracellular acidification rate: ECAR) were measured in isolated murine pancreatic acinar cells using a Seahorse XF24 Extracellular Flux Analyser. ROS were generated using the redox-cycler menadione, accumulation of which was inhibited by the antioxidant *N*-acetylcysteine (NAC), or potentiated using an NQO1 inhibitor (2,4-dimethoxy-2-methylnaphthalene: DMN).

Menadione (2.5 - 10 μM) induced a sustained increase of OCR (from basal 334 ± 13.95 pmoles/min to 385.55 ± 29.59 pmoles/min, $p<0.001$ with 10μM) with concurrent increase of ECAR (from basal; 7 ± 0.5 mpH/min, to 12 ± 0.98 mpH/min $p<0.001$ with 10μM) whereas a higher concentration (30 μM) depressed respiration. All effects on OCR and ECAR were inhibited by NAC, indicating a primary role for ROS. DMN, which potentiates menadione-induced ROS elevations, had no effects on basal respiration per se, but augmented the effects of menadione on OCR. Application of a mitochondrial "stress test" showed that menadione (2.5 – 10 μM) increased mitochondrial proton leak and decreased maximal respiratory capacity in a concentration-dependent fashion. At the highest concentration (30 μM), menadione caused bioenergetic collapse, respiratory failure and cell death in a NAC-sensitive manner.

Oxidative stress alters pancreatic acinar cell bioenergetics dependent on the level of ROS production. Excessive stimulation with menadione induced a profound inhibition of mitochondrial bioenergetics, consistent with our previous findings showing ROS-mediated activation of the apoptotic acinar cell death pathway.

Oral Presentation No. 3

Mitochondrial redistribution in live pancreatic tissue: pathophysiological implications

M.C. Cane[1], D. Rajamanoharan[1], A.V. Tepikin[1], R. Sutton[2], D.N. Criddle[1,2]

[1] Department of Cellular and Molecular Physiology.
[2] Liverpool NIHR Pancreas Biomedical Research Unit, Royal Liverpool University Hospital, University of Liverpool, UK.

Mitochondrial dysfunction is implicated in the development of acute pancreatitis, but detailed investigation in intact tissue is lacking. We aimed to assess mitochondrial and cytoskeletal redistribution in live pancreatic tissue.

Real-time cytosolic Ca^{2+} ($[Ca^{2+}]_c$; fluo-4) and mitochondrial membrane potential ($\Delta\Psi_m$; TMRM) were assessed in perfused murine pancreatic tissue

segments using confocal microscopy. Mitochondrial/cytoskeletal distributions were visualised by immunofluorescence. $[Ca^{2+}]_c$, $\Delta\Psi_m$ and cytoskeletal dynamics were modulated pharmacologically.

In resting tissue, mitochondria were distributed diffusely throughout acinar cells, rather than in the distinct peri-granular, peri-nuclear, and sub-plasmalemmal groupings previously documented in unstimulated, isolated cells. The basolateral membrane displayed discrete patches of actin and a rich tubulin decoration. Application of 100 pM cholecystokinin (CCK), which induced $[Ca^{2+}]_c$ oscillations, caused Ca^{2+}/tubulin-dependent redistribution of mitochondria into perigranular, peri-nuclear and sub-plasmalemmal distributions. Toxic stimulation (100 nM CCK or 500 μM TLC-S) induced sustained $[Ca^{2+}]_c$ elevations and dramatic relocation of all mitochondria towards the granular region, the latter being independent of Ca^{2+}, tubulin and $\Delta\Psi_m$, but dependent upon actin/myosin. Actin patches were dispersed from the basolateral membrane and apical mitochondria enclosed by a peri-granular belt of actin and microtubules.

In live, non-dissociated pancreatic exocrine tissue, diffuse mitochondria redistribute differentially in response to physiological and pathophysiological stimulation, effects associated with cytoskeletal alterations. These observations challenge established views derived from studies using isolated cells, and suggest important implications for the role of mitochondrial redistribution in pancreatic health and disease.

Oral Presentation No. 4

Chronic hypoxia induced upregulation of TRPV4 in pulmonary artery contributes to the enhanced myogenic tone and pulmonary hypertension

James S.K. Sham

Division of Pulmonary and Critical Care Medicine, Johns Hopkins School of Medicine, Baltimore, MD21224.

Chronic hypoxia causes pulmonary hypertension with vascular remodeling, increase in vascular tone and altered reactivity to agonists. These changes involve alterations in multiple Ca^{2+} pathways, including TRPC1 and TRPC6 channels, in pulmonary arterial smooth muscle cells (PASMCs). We have previously shown that TRPV and TRPM channels are expressed in pulmonary arteries (PAs). Here we found that TRPV4 was the only member of the TRPV and TRPM subfamilies upregulated in PAs of chronic hypoxic rats. The increase in TRPV4 expression occurred within one day of hypoxia exposure, indicative of an early hypoxic response. TRPV4 are mechanosensitive in PASMCs. Osmo-mechanical stress imposed by hypotonic solution activated Ca^{2+} influx, which was inhibited by TRPV4 specific siRNA, the TRPV blocker ruthenium red, and the cytochrome-P450 epoxygenase inhibitor MS-PPOH. Consistent with TRPV4 upregulation, Ca^{2+} response induced by the TRPV4 agonist 4α-PDD and hypotonicity was potentiated in hypoxic PASMCs. Moreover, significant ruthenium red sensitive myogenic tone was observed in de-endothelized pressurized small PAs of hypoxic but not normoxic rats. The elevated basal $[Ca^{2+}]$i in hypoxic PASMCs was also reduced by ruthenium red. In extension of these results, the development of pulmonary hypertension, right heart hypertrophy, and vascular remodeling were significantly suppressed in hypoxic *trpv4-/-* mice. These results suggest the novel concept that TRPV4 serves as a signal pathway crucial for the development of hypoxia-induced pulmonary hypertension. Its upregulation may provide a pathogenic feed-forward mechanism that promotes pulmonary hypertension via facilitated Ca^{2+} influx, enhanced myogenic tone and vascular remodeling. (Supported by NIH grants R01-HL071835, and R01-HL075134).

Oral Presentation No. 5

Role of TRPM4 channel in urinary bladder function

Amy C. Smith, Kiril L. Hristov, Shankar P. Parajuli, Qiuping Cheng, Wenkuan Xin, John Malysz, and Georgi V. Petkov

Department of Pharmaceutical and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC-29208, USA.

Contraction and relaxation of the detrusor smooth muscle (DSM), which makes up the wall of the urinary bladder, facilitates the storage and voiding of urine. Transient Receptor Potential Melastatin-4 (TRPM4) channel is a Ca^{2+}-activated monovalent selective cation channel implicated in many cellular processes. However, at this time, the expression and function of the TRPM4 channels in DSM have not yet been explored. Using rats and guinea pigs, we employed a multidisciplinary approach to study the role of the TRPM4 channels in DSM, utilizing single-cell RT-PCR, Western blot, immunocytochemistry, Ca^{2+} imaging, patch-clamp, functional studies on

DSM contractility, and the selective TRPM4 channel inhibitor 9-phenanthrol. TRPM4 channel expression at the mRNA and protein level was detected in freshly isolated DSM single cells. In perforated patch-clamp recordings at -70 mV, 9-phenanthrol (30 μM) decreased the spontaneous inward current activity in freshly isolated DSM cells. DSM live-cell Ca^{2+} imaging showed that selective inhibition of TRPM4 channel with 9-phenanthrol (30 μM) significantly reduced intracellular Ca^{2+}. 9-phenanthrol (0.1–30 μM) significantly inhibited the amplitude, muscle force integral, and frequency of the spontaneous phasic and pharmacologically induced contractions of DSM isolated strips. 9-phenanthrol also decreased the amplitude of electrical field stimulation-induced DSM contractions.

In conclusion, this is the first report to examine the expression and provide evidence for the TRPM4 channels as critical regulators of rat and guinea pig DSM excitability and contractility. TRPM4 channels represent novel unexplored therapeutic targets for pharmacological management of overactive bladder, a clinical condition for which effective therapeutics are lacking.

Oral Presentation No. 6

Modeling and Validation Studies of Open, Closed and Open-inactivated

States of hERG1 Channel: A Multi-faceted Approach

Serdar Durdagi[1], Henry J Duff[2], Sergei Y Noskov[1]

[1] Institute for Biocomplexity and Informatics, Department of Biological Sciences, University of Calgary, Alberta, CANADA.
[2] Libin Cardiovascular Institute of Alberta, University of Calgary, Calgary, Alberta, CANADA.

The human ether-a-go-go related gene 1 (hERG1) K ion channel is a key element for the rapid component of the delayed rectified potassium current in cardiac myocytes. Since there is no crystal structures for hERG channels, creation and validation of its reliable atomistic models has been a key target in molecular cardiology for the last decade. In this talk protein engineering studies for hERG1 channel in three different states will be covered. We developed and vigorously validated models for open, closed and openinactivated states of hERG1 using a multi-step protocol. The conserved elements were derived using multiple-template homology modeling utilizing available structures for Kv1.2, Kv1.2/2.1 chimera and KcsA channels. Then missing elements were modeled with a ROSETTA De Novo protein-designing suite and further refined with all-atom Molecular Dynamics simulations. Final ensemble of models was evaluated for consistency to the reported experimental data from biochemical, biophysical and electrophysiological studies. The next step was to study binding of the state-dependent channel blockers to open and open-inactivated forms of the channel. The closed state models were cross-validated against experimental data on the toxin-foot printing with a protein-protein docking using hERG state-selective toxin BeKm-1. Poisson-Boltzmann calculations were performed to determine gating charge and compare it to electrophysiological measurements.

Oral Presentation No. 7

Inflammation mediated oxidative stress in cancer

Sadshib Ghosh, Arkoprovo Paul and Ellora Sen

National Brain Research Centre, Manesar 122 050, Haryana, INDIA.

Inflammation is an indispensable participant in the progression of Glioblastoma multiforme (GBM)- the most malignant of brain tumors. The invasive and metastatic potential- an inherent feature of glioma cells involves cytoskeletal rearrangement. The pro-inflammatory cytokine Tumor Necrosis Factor-alpha (TNF-α) elevates oxidative stress in glioma cells. Interestingly, TNFα induced oxidative stress affects Akt activation to regulate actin cytoskeletal organization through complex interplay of various signaling cascades. Also, TNFα mediated alteration in Ca^{2+} levels affected the transcriptional activation of hypoxia inducible factor (HIF-1α) an important component of oxidative stress. Importantly, fluorescence recovery after photobleaching (FRAP) revealed altered membrane fluidity in TNFα treated glioma cells. The mechanisms by which glioma cells interpret these changes in membrane to modulate signaling cascades critical for its survival under inflammatory conditions are not fully understood. We therefore investigated the association between inflammation mediated oxidative stress, membrane physical properties, changes in cytoskeleton dynamics and cell signaling. As membrane lipid rafts play critical role in signaling events, the effect of TNFα induced alteration in membrane fluidity and cytoskeletal organization on the spatial localization of key signaling mediators associated with glioma cell survival and immune evasion, in the lipid rafts of glioma cells was investigated.

Oral Presentation No. 8

Up-regulation of the type-2 IP_3R isoform dictates the apoptotic response to disrupting IP_3R/Bcl-2 complexes in diffuse-large B-cell lymphoma

Haidar Akl[1], Rita Larovere[1], Giovanni Monaco[1], Alexandre Kauskot[2], Jordi Molgo[4], Clark Distelhorst[3], Ludwig Missaen[1], Jan B. Parys[1], Humbert De Smedt[1], Geert Bultynck[1]

[1] Lab. Molecular and Cellular Signaling, Dep. Cellular and Molecular Medicine, Campus Gasthuisberg O/N-1, KU Leuven, BELGIUM.
[2] Center for Molecular and Vascular Biology, Campus Gasthuisberg O/N-1, KU Leuven, BELGIUM.
[3] Dep. Medicine, Case Western Reserve University, Cleveland, OH.
[4] Lab. de Neurobiologie et Développement; Institut de Neurobiologie Alfred Fessard; CNRS; FRANCE.

IP_3Rs play a critical role in cell survival and death by controlling the mitochondria and are tightly regulated by anti-apoptotic proteins (like Bcl-2). Recently, we found that Bcl-2 through its BH4 domain suppresses IP_3R activity, protecting cells against pro-apoptotic Ca^{2+} signaling. Based on the Bcl-2-binding site on the IP_3R, we developed a novel peptide tool (TAT-IDPS) targeting BH4-Bcl-2, reversing Bcl-2's inhibitory action on the IP3R channel and sensitizing cells to apoptotic stimuli (1-3). Using chronic lymphocytic leukemia (CLL) cells, we found that TAT-IDPS was much more effective in triggering pro-apoptotic Ca^{2+} signaling and cell death in CLL cells than in peripheral mononuclear blood cells (4).

Here, we explored the potential of targeting the BH4-Bcl-2 in diffuse large B-cell lymphoma (DLBCL) cells, a much more heterogeneous B-cell malignancy than CLL, by using "primed-to-death" DLBCL cell lines expressing high levels of Bcl-2, previously BH3 profiled (KARPAS, TOLEDO, PFEIFFER, SU-DHL-4 and OCI-LY-1) (5). Strikingly, we found that some cancer cell lines were very sensitive to TAT-IDPS exposure (like SU-DHL-4) resulting in apoptotic cell death, while others were very resistant (like OCI-LY-1). The differences correlated with TAT-IDPS-induced Ca^{2+} release in SU-DHL-4, which was lacking in OCI-LY-1. This Ca2+ release originated from the ER and was blocked using IP_3R antagonists (Xestospongin B; XeB), which also protected SU-DHL-4 against TAT-IDPS-induced cell death, indicating a central role for IP_3Rs. Thus, we analyzed IP_3R isoforms in SU-DHL-4 and OCI-LY-1, which displayed similar total IP_3R levels. SU-DHL-4 expressed high levels of IP3R2, the IP_3R isoform most sensitive to IP_3, while OCI-LY-1 expressed high levels of IP_3R_3, the IP_3R isoform least sensitive to IP_3. We also examined IP3R/Bcl-2 complexes and found that IP3R/Bcl-2 complexes from SU-DHL-4 were particularly affected by TAT-IDPS, while those from OCI-LY-1 not. Thus, SU-DHL-4 seem to be addicted to high levels of Bcl-2 to suppress chronic Ca^{2+} signaling through IP3R2 like during chronic B-cell receptor stimulation (6). These observations also correlate with published findings, showing that in OCI-LY-1 Bcl-2 is directed to Bax and Bim (5). Plotting the responsiveness to TAT-IDPS to the IP_3R2/IP_3R_3 ratio of the different DL-BCL cell lines revealed a positive correlation. To underpin the critical role of IP_3R_2 in SU-DHL-4, we found that reducing IP_3R_2 levels using siRNA correlated with increased resistance to TAT-IDPS.

In summary, these data point towards a critical role for the IP_3R_2/IP_3R_3 ratio as a rheostat dictating the sensitivity of DL-BCL cells to peptides disrupting IP_3R/Bcl-2 complexes.

References

1. Rong YP et al. Targeting Bcl-2-IP_3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals. Mol Cell 2008; 31:255-265.
2. Rong YP et al. The BH4 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling domain of the IP_3 receptor. Proc Natl Acad Sci U S A 2009;106:14397-14402
3. Monaco G et al. Selective regulation of IP3-receptor-mediated Ca^{2+} signaling and apoptosis by the BH4 domain of Bcl-2 versus Bcl-Xl. Cell Death Differ 2012;19:295-309.
4. Zhong F et al. Induction of Ca^{2}+-driven apoptosis in chronic lymphocytic leukemia cells by peptide-mediated disruption of Bcl-2-IP_3 receptor interaction. Blood 2011;117:2924-2934.
5. Deng J et al. BH3 profiling identifies three distinct classes of apoptotic blocks to predict response to ABT-737 and conventional chemotherapeutic agents. Cancer Cell 2007;12:171-185.
6. Davis RE et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 2010;463:88-92.

Oral Presentation No. 9

Calpastatin reduces methamphetamine-induced induction in c-Jun phosphorylation, Bax and cell death in neuroblastomam SH-SY5Y cells

W Suwanjang

(London, UK)

Oral Presentation No. 10

Molecular expression and calcium signalling roles of native TRP channels in vascular cells

D. Melanaphy[1], L. Borisova[3], M. Kustov[4], C. Watson[1,2], C. Johnson[2], T. Burdyga[3], A. Zholos[1,4,5]

[1] Centre for Vision and Vascular Science.
[2] Centre for Biomedical Sciences Education, Queen's University Belfast, Belfast BT12 6BA, UK.
[3] Department of Cellular and Molecular Physiology, University of Liverpool, Liverpool L69 3BX, UK.
[4] A.A. Bogomoletz Institute of Physiology, Kiev 01024, UKRAINE.
[5] Institute of Biology, Taras Shevchenko Kiev National University, Kiev 01601, UKRAINE.

In the vasculature, various members of the TRP-superfamily of non-selective cation channels mediate diverse non-voltage-gated Ca^{2+}-entry pathways and functions, which involve both vascular myocytes and communicating endothelial cells (1,2). We and others have previously found that TRPM8, the principal neuronal cold receptor, is widely expressed, both at the mRNA and protein level, in rat arteries (3,4). Thus, it is likely involved in complex thermal behaviour of blood vessels, better understanding of which is relevant to hypothermic and cardiovascular surgery conditions. Indeed, our contractile studies have revealed a mixture of contraction and relaxation in response to TRPM8 agonists (4). Thus, in the next stage we focused on the details of subcellular TRPM8 localisation, intracellular Ca^{2+} signals and associated electrical responses induced by TRPM8 agonists.

With the use of immunostaining of isolated rat tail artery myocytes, laser confocal imaging and patch-clamp techniques we found that (i) TRPM8 co-localises with intracellular Ca^{2+}-release channels, ryanodine (RyR) and inositol 1,4,5-trisphosphate receptors; (ii) menthol (100-300 μM) applied to cells loaded with the intracellular Ca^{2+} indicator fluo-4 AM induces a transient increase in $[Ca^{2+}]_i$ that was associated with an increase in the frequency of local Ca^{2+} release events known as "Ca^{2+} sparks" and that these effects depended on both Ca^{2+} influx and Ca^{2+}-release; (iii) in voltage-clamped cells, menthol accelerates STOCs discharge due to Ca^{2+} sparks/BKCa coupling, while under current-clamp conditions it causes sustained membrane depolarisation frequently interrupted by spontaneous large-amplitude hyperpolarisations; (iv) the vasodilatory effects of TRPM8 agonists, menthol and icilin, on pre-constricted vascular rings (4), are largely due to their direct inhibitory action on L-type Ca^{2+} channels. Finally, our current research is revealing expression and Ca^{2+} signalling roles of TRPM8 in microvascular endothelial cells. We conclude that TRPM8-mediated Ca^{2+} signals could contribute to the mechanisms controlling vascular tone, likely via dual channel expression and function in smooth muscle and endothelial cells.

We thank the British Heart Foundation for support (FS/07/040 & PG/09/063).

References

1. Yao X, Garland CJ. 2005. Recent developments in vascular endothelial cell transient receptor potential channels. Circ Res 97:853-863.
2. Albert AP, Large WA. 2006. Signal transduction pathways and gating mechanisms of native TRP-like cation channels in vascular myocytes. J Physiol 570:45-51.
3. Yang XR, Lin MJ, McIntosh LS, Sham JSK. 2006. Functional expression of transient receptor potential melastatin- and vanilloid-related channels in pulmonary arterial and aortic smooth muscle. Am J Physiol 290:L1267-L1276.
4. Johnson CD, Melanaphy D, Purse A, Stokesberry SA, Dickson P, Zholos AV. 2009. Transient receptor potential melastatin 8 channel involvement in the regulation of vascular tone. Am J Physiol 296:H1868-H1877.

Oral Presentation No. 11

Oxidative regulation of the Na^+-K^+ pump in the cardiovascular system

Gemma A Figtree[1,2]

[1] North Shore Heart Research Group, Kolling Institute of Medical Research, University of Sydney, Australia.
[2] Department of Cardiology, Royal North Shore Hospital, Sydney, AUSTRALIA.

The Na^+-K^+ pump is an essential heterodimeric membrane protein which maintains electrochemical gradients for Na^+ and K^+ across cell membranes in all tissues. We have identified glutathionylation of the Na^+-K^+ pump's β1 subunit as a key molecular mediator of Na^+-K^+ pump inhibition with particular significance in the cardiovascular system. Oxidative inhibition of the Na^+-K^+ pump via NADPH oxidase mediates physiological and pathophysiological effects of Angiotensin II and β1-adrenergic receptor agonists on the cardiac myocyte. This has implications for dysregulation of intracellular Na^+ and Ca^{2+} in heart failure, myocardial ischaemia and conditions of elevated oxidative stress. Treatment strategies that are able to reverse this oxidative inhibition of the Na^+-K^+ pump have the potential to improve cardiac function.

Oral Presentation No. 12

Lifelong caloric restriction alleviates increased oxidative stress with age in sympathoadrenal system of rats

Nihal Tümer, Melissa Whidden, Philip Scarpace, Nataliya Kirichenko, Zekai Halici, Benedek Erdos, and Thomas Foster

GRECC, Veterans Affairs Medical Center, Dept. Pharmacology, University of Florida, Gainesville, FL, UNITED STATES.

Aging is associated with oxidative damage and an imbalance in redox signaling in a variety of tissues, yet little is known about the extent of age-induced oxidative stress in the sympathoadrenal system. Lifelong caloric restriction has been shown to lower levels of oxidative stress and slow the aging process. Therefore, we examined the effect of aging on oxidative stress in the adrenal medulla and hypothalamus and whether lifelong 40% caloric restriction (CR) reverses the adverse effects of age-induced oxidative stress in the sympathoadrenal system. Adult (18 months) and senescent (38 months) male Fischer 344 x Brown Norway rats were divided into *ad libitum* or 40% CR groups and parameters of oxidative stress were assessed in the adrenal medulla and the hypothalamus. Age related lipid peroxidation (+20%, $P<0.05$) and tyrosine nitration (+111%, $P<0.001$) were significantly increased in the adrenal medulla while aging resulted in a reduction in the protein expression of key antioxidant enzymes, CuZnSOD (-27%, $P<0.01$) and catalase (-27%, $P<0.05$) in the hypothalamus. Lifelong CR completely prevented the age-related increase in lipid peroxidation in the adrenal medulla and alleviated the age-related decline in antioxidant enzymes in the hypothalamus. In summary, these data indicate that aging results in a significant increase in oxidative stress in the sympathoadrenal system. Importantly, lifelong CR prevented the age-related changes in oxidative stress in the adrenal medulla and hypothalamus. Caloric restriction could be a potential non-pharmacological intervention to prevent increased oxidative stress in the sympathetic adrenomedullary system with age.

Oral Presentation No. 13

S-glutathionylation is an important post-translational protein modification mechanism in oxidative stress.

Chun Jiang and Yang Yang

Department of Biology, Georgia State University, 100 Piedmont Avenue, Atlanta, GA 30302-4010, USA.

S-glutathionylation is a thiol modification process, targeting at cysteine residues of cytosolic protein domains. Although such a protein modification is normally limited by several antioxidant systems inside the cell, it can occur under several conditions such as inflammation, ischemia-reperfusion, trauma, diabetes, neurodegenerative diseases, etc. Within the cell the S-glutathionylation may activate certain cascade of events, leading to excitotoxicity, autophagy and tissue injuries. If the affected proteins also play a role in systemic function such as the blood circulation, the consequences can be deleterious and widespread. Our studies suggest that the ATP-sensitive K^+ (K_{ATP}) channel in vascular smooth muscles (VSM) is one of the proteins. The channel regulates membrane potentials of VSM cells and the VSM contractility. It is a common target of various vasodilators and vasoconstrictors that affect regional blood flows by activating and inhibiting this K^+ channel, respectively. The K_{ATP} is sensitive to metabolites, allowing it to regulate vascular tones according to the metabolic state. Previous studies indicate that the vascular K_{ATP} is inhibited in oxidative stress. However, the molecular mechanism underlying the K_{ATP} inhibition is unclear, which we performed studies to address. An exposure of isolated mesenteric rings to H_2O_2 impaired the K_{ATP} channel-mediated vascular dilation. In whole cell recordings and inside-out patches, H_2O_2 or diamide caused strong inhibitions of the vascular K_{ATP} channel (Kir6.1/SUR2B) in the presence, but not absence, of glutathione (GSH). Similar channel inhibition was seen with oxidized glutathione (GSSG) and thiol oxidation reagents. The oxidant-mediated channel inhibition was reversed by the reducing agent dithiothreitol and the specific deglutathionylation reagent glutaredoxin-1. Consistent with the S-glutathionylation, pull-down assays with biotinylated glutathione ethyl ester (BioGEE) showed incorporation of GSH to the Kir6.1 subunit in the presence of H_2O_2. Systematic mutational analysis revealed that Cys176 was the critical player. Simulation modeling suggest that after incorporation to residue 176, the GSH moiety occupies a space between the slide helix and two transmembrane helices, and prevented the helices from movements necessary for channel gating. Thus a novel modulation mechanism of the vascular K_{ATP} channel is suggested via S-glutathionylation in oxidative stress.

Oral Presentation No. 14

Therapeutic targeting of Krüppel like factor 4 abrogates inflammation and oxidative stress in brain

Deepak Kumar Kaushik, and Anirban Basu

National Brain Research Center Manesar, Haryana-122050, INDIA.

Neuroinflammation occurs as a result of microglial activation which responds to invading organisms

or other inflammatory stimuli within the Central Nervous System (CNS). Microglial activation results in the production of pro-inflammatory cytokines in order to combat the ongoing infection. However, an exaggerated response can lead to neuronal death and subsequent neurological sequelae. Therefore, it is important to target the pro-inflammatory factors and check the exaggerated response by targeting the production of these cyto-chemokines. We have observed that Krüppel like factor 4 (Klf4), a zinc finger transcription factor gets upregulated upon stimulation with lipopolysaccharide (LPS) and IL-1β in BV-2 microglial cells. We have shown for the first time that Klf4 is involved in microglial activation and subsequent release of pro-inflammatory cytokines, TNF-α, MCP-1 and IL-6 as well as pro-inflammatory enzymes, iNOS and Cox-2 in LPS treated microglial cells. Our ongoing studies focus on finding out whether Klf4 is a potential target to therapeutic agents. For these studies, we have used a traditional herbal anti-inflammatory medicine, honokiol which is a biphenolic compound and can easily cross the BBB. We have found that honokiol can substantially downregulate the production of pro-inflammatory cytokines and enzymes in LPS stimulated BV-2 microglial cells. In addition, Honokiol was shown to downregulate LPS induced upregulation of both Klf4 and pNF-κB. Interestingly, overexpression of Klf4 using pcDNA3.1-Klf4 construct (K-10) is shown to reverse the anti-inflammatory action of honokiol suggesting that honokiol's anti-inflammatory properties are targeted via Klf4. We hereby report a novel therapeutic approach for treating neuro-inflammatory conditions of CNS.

Oral Presentation No. 15

Oxidative stress biomarkers in rat erythrocytes and plasma during aging

Syed Ibrahim Rizvi and Dileep Kumar

Department of Biochemistry, University of Allahabad, Allahabad 211002, INDIA.

Despite considerable research effort, aging continues to be the most intriguing biological phenomenon. The 'Oxidative Stress Theory of Aging' is the best possible explanation for the processes which accompany aging however recent data concerning life span determinations and the effect of antioxidant supplementation on longevity have failed to validate this theory. The study on aging related biochemical parameters in humans is dependent on many variables such as genetic factors, temperature, activity, and nutrition. Thus the conclusions drawn from human based studies may not be very relevant when validation of oxidative stress theory of aging is at stake. The present study was undertaken to determine markers of oxidative stress of plasma and erythrocytes in rats, kept in controlled laboratory conditions, at different ages ranging from 4 weeks to 24 months. We report the age dependent changes in plasma total antioxidant capacity, plasma membrane redox system (PMRS), protein carbonyl, advanced oxidation protein products (AOPP), reduced glutathione (GSH), lipid peroxidation product malondialdehyde, and sialic acid. Our results provide evidence of an increase in erythrocyte PMRS, AOPP, protein carbonyl and lipid peroxidation. There was decrease in intracellular reduced glutathione, membrane sialic acid and plasma antioxidant capacity. Our observations validate the Oxidative Stress Theory of Aging. To the best of our knowledge, our observation of the activation of erythrocyte PMRS during rat aging is a novel finding. The activation of erythrocyte membrane PMRS may be a protective mechanism to protect the organism from oxidative stress.

Oral Presentation No. 16

The role of sleep deprivation and the resultant oxidative stress in diminished hippocampal long-term potentiation

Mohammad Torabi Nami[1],*, Mohammad Nasehi[2], Mohammad-Reza Zarrindast[2,3]

[1] MD, PhD candidate, Department of Neuroscience, Institute for Cognitive Science Studies, Tehran, 15948 IRAN.
[2] Faculty, Department of neuroscience, Institute for Cognitive Science Studies, Tehran, IRAN.
[3] Professor of neuroscience, Neuropharmacology, Tehran university of Medical Sciences.

Sleep deprivation (SD) in animal models is proven to cause oxidative stress in the brain. Post SD accumulation of free radicals causes hippocampal oxidative stress leading to lipid peroxidation in the neural membrane which could translate to neuro-degeneration. It has been suggested that reactive oxygen species (ROS) may be the culprit for some of these oxidative stress. Amyloid beta (Aβ) will be then formed as a resultant product. The concept of oxidative stress and lack of neurotrophic factors is perceived as the main determinant of the impact of chronic stress on the plasticity (hippocampal long-term potentiation) of neural networks and consequently Alzheimer's Disease (AD) pathogenesis . It has been clearly shown that there is a positive linear correlation between Amyloid â fibril formation and phosphorylation of

tau protein. There is a direct relationship between the amount of amyloid plaques, and neurofibrillary tangles in hippocampus or entorinal cortex and the memory performance of the brain subjected to an oxidative stress caused by SD. The dentate gyrus (DG) is known for the cluster of progenitor cells, which can potentially differentiate into neurons. There are available evidence supporting the notion that sleep deprivation has caused reduction in cell proliferation of the posterior hippocampus namely the DG. To examine the relation between sleep deprivation, hipocampal oxidative stress and hastened neurodegenerative process towards AD, a comprehensive neurochemical assessment and histopathological study of DG of the brain of the sleep deprived rats should be completed. This is what we currently are pursuing.

Oral Presentation No. 17

Hemolymph cells Apoptosis in imported shrimp *Litopenaeus vannamei* from Hawaii to Iran, exposed to White Spot Virus

S. Kakoolaki[1]*; T. Z. Selamoglu[2]; I. Sharifpour[1]; M. Afsharnasab[1]; M.R. Mehrabi[1]

[1] Department of Aquatic Animal Health and diseases, Iranian Fisheries Research Organization, P.O. Box: 14155-6116, Tehran, IRAN.
[2] Department of Biology, Faculty of Arts and Science, Nigde University, Campus, Nigde, 51200, TURKEY.

This study was aimed to show *hemolymph Apoptosis* in imported shrimp *Litopenaeus vannamei* from Hawaii to Iran. One hundred and eighty shrimps (7.98±0.54 g) which were collected from a research shrimp farm located in Heleh site in north of Bushehr Province distributed equally to each 6 glass aquariums (50×50×60cm) as group *A* in triplicate (imported batch in 2011, without crossing with other generations) with well clean aerated sea water (100 L per each aquarium), salinity of 40 ppt and temperature of 29°C. Group B (produced by crossing the adults of imported batches in 2009 up to 2011) were distributed also among 6 aquariums with the same conditions. Both shrimp groups were injected with concentration of LD_{50}=1×10$^{5.4}$ White Spot Virus (WSV). Our results showed that in group *A*, the mortality began approximately 24 h after exposure and reached 100% after 36 h but no mortality was occurred up to 15 days in shrimps of group *B*. The slide evaluation of hemolymph of group *B* showed an increasing trend of apoptosis occurrence in all three types of hemolymph cells, Hyalinocytes, Semi-Granulocytes and Granulocytes from 24 h to 72 h in contrary to group *A* that no any apoptosis was observed during the course of the study (15 days). It is concluded that crossing among the SPF generations could induce the increasing immunity level through apoptosis to protect them against White Spot Disease.

Oral Presentation No. 18

Antioxidant and small molecules transportation and interactions with models of domains in natural membranes: calorimetric, UV-vis and DLS studies

H.Wilczura-Wachnik

Faculty of Chemistry, University of Warsaw, POLAND.

Reversed micelles (RM) as ones of the intermediate biomembraneous structures have been found by Sigel in 1984 during investigations on rearrangement of lipid bilayer. Synthetic RM similarly to *in vivo* membranous environment builds a dynamic system. They are formed by amphiphilic molecules (surfactants) in apolar organic solvents and are very useful for the study of various aspects of life science viz. molecular transportation processes, interactions, activities and location of antioxidants in a structures mimicking a natural membranes. In RM systems there are many controllable variables: detergent type and concentration, water content, organic solvent etc. by manipulating which in a simple way can be obtained information under different condition about processes taking place in the system being mimicked. The RM is not the only structures employed in such investigations. In a polar solvents normal micelles (spherical) and rod-like micelles are formed as well as monolayer, bilayer and vesicles are possible. There are different surfactants and solvents employed in such studies. The most popular are AOT/solvent/water reversed micelles which have been used to study enzymatic reactions, antioxidants solubilization and preferring binding sites in membranous interfaces, diffusion effects in drug delivery system etc. Except AOT there are widely used SDS, Triton X-100, Brij, TTAB and other. Recently as a containers of hydrophobic drugs have been reviewed a copolymeric surfactants as for example a commercially available (PEO-PPO-PEO) triblock copolymer.

The problem of small molecules transportation and interactions with RM will be present in the context of methods (calorimetry, DLS, FTIR and UV-vis, cloud point and viscosity measurements) employed in investigations mentioned above.

References

1. Siegel D.P. Inverted micellar structures in bilayer membranes. Biophys J 1984; 45:399-420.
2. ChangGG, Huang TM, Hung HC, Reverse micelles as life-mimicking systems. Proc Natl Counc ROC(B) 2000;24(3):89-100.
3. Parmar A, Singh K, Bahadur A, Marangoni G, Bahadur P, Interaction and solubilization of some phenolic antioxidants in Pluranic micelles. Colloids and Surfaces B: Biointerfaces 2011; 86:319-326.
4. Wilczura-Wachnik H, Yavuz AG, Myslinski A, Calorimetric study on the α-tocopherol solubility in reversed AOT micelles. J Colloid and Interface Science 2008; 319:520-525.
5. D'Aprano A, Donato ID, Pinio F, Liveri TV, Calorimetric study oft he solubilization of cholesterol, retinol and retinal by reversed AOT micelles. J Solution Chemistry 1990; 19(11):1055-1061.
6. Leodidis EB, Hatton TA, Amino Acids in AOT reversed micelles. 1. Determination of interfacial partition coefficients using the pase-transfer method. J Phys Chem 1990; 94:6400-6411.
7. Singh K, Marangoni DG, Microcalorimetric determination of effect of antioxidant (Quercetin) on polymer/surfactant interactions. J Therm Anal Calorim 2010; 102:729-737.
8. Avellone G, Bongiorno D, Ceraulo L, Ferrugia M, Liveri VT, Spectrophotometric investigation of the binding of vitamin E to water-containing reversed micelles. Int J Pharmaceutics 2002; 234:249-255.

Poster Presentations

Poster No. 1

Regulation of calcium intracellular signaling by usnic acid in cardiomyocytes

Thássio R. Mesquita[1], Valéria A. Fernandes[1], Aline Lara[2], Luciano Capettini[2], Thales Prímola-Gomes[3], Judson Quintão-Júnior[3], Antônio J. Natali[3], Adriano Antunes[1], Silvia Guatimosim[2], Jader Cruz[2], Sandra Lauton-Santos[1]

[1] Department of Physiology, Federal University of Sergipe - BRAZIL.
[2] Department of Physiology and Biophysics, Federal University of Minas Gerais - BRAZIL.
[3] Department of Physical Education, Federal University of Viçosa - BRAZIL.

Usnic acid (UA) is a secondary lichen metabolite that exhibit antiviral, antiprotozoal, antiproliferative, anti-inflammatory and analgesic activity. UA as a pure substance has been formulated in cosmetics, sunscreen products and health food supplements. Our aim is to evaluate the acid usnic effects on the cardiac cell contraction and transient calcium intracellular measures. Ventricular cardiac cells of wistar rats were enzymatically isolated for cell contractility evaluation. The cells were treated with UA and contractions were elicited via bath electrodes. Cells were visualized and the image was used to measure cell shortening in response to electrical stimulation using a video motion edge detector. We found a decrease on the cell shortening contraction at different UA concentrations used (1nM decreased 22%, 10nM decreased 24%, 100nM decreased 22% and 1μM decreased 37%) and a decrease on the time to peak at all UA concentrations used (1nM decreased 18%, 10nM decreased 19%, 100nM decreased 21% and 1μM decreased 33%), when compared with control cells. For intracellular Ca^{2+} evaluation, myocytes were loaded with fluo-4 AM treated with UA. Cells were stimulated at 1 Hz. A Meta LSM 510 scanning system with an oil immersion objective was used for confocal fluorescence imaging. We found treatment with UA decreased intracellular calcium transient in cardiac myocytes at all concentrations tested (1nM decreased 30%, 10nM decreased 26%, 100nM decreased 28% and 1μM decreased 28%) when compared with control cells. In conclusion, usnic acid, in all concentrations used, decreases both the cellular contraction and intracellular calcium transient in cardiac myocytes.

References

1. Guatimosim S, Dilly K, Santana JF, Jafri MS, Sobie EA, Lederer WJ. Local Ca^{2+} Signaling and EC Coupling in Heart: Ca^{2+} Sparks and the Regulation of the $[Ca^{2+}]$i Transient. J Mol Cell Cardiol 2002; 34(8):941–950.
2. Ingólfsdóttir K. Usnic acid. Phytochemistry 2002; 61: 729–736.

Poster No. 2

Devolepmental changes of TRPM2 expression in mouse cochlear nucleus and inferior colliculus

Ramazan Bal[1], Ebru Onalan Etem[2], Funda Bulut[1], Yasemin Ustundag[3], Esin Oz[2]

Department of [1]Physiology, Medical Biology[2], Faculty of Medicine, Firat University, 23119 Elazig, TURKEY. [3]Department of Anatomy, Faculty of Veterinary Medicine, Firat University, 23119 Elazig, TURKEY.

The aim of the present study was to examine the developmental expression of TRPM2 and TRPM7 in the postnatal mouse cochlear nucleus (CN) and inferior colliculus (IC). The CN is a first relay of the central auditory system as well as a site for integration of multimodal information. The CN receive input from the cochlea, which carry sound information from the cochlea and the outputs from the CN are to higher regions of the auditory brainstem including IC.

Here, by using quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR), we explored the changes in the expression of TRPM2 and TRPM7 in mouse CN and IC from postnatal day 0 to 13 months.

mRNAs for TRPM2 and TRPM7 channels were detected in both CN and IC. TRPM2 channel displayed transient high levels during early postnatal day 0, but the expression decreased from PN day 0 to PN day 16, at which maturation of the auditory pathway nearly finalized. TRPM2 expression increases strikingly after PN day 24 till 13 months when compared to postnatal day 0. Whereas, the expression of TRPM7 gene decreases after PN day 0, peaking on PN day 16.

Our results, showing the expression and dynamic regulation of TRPM2 and TRPM7 during development, indicate that TRPM2 and TRPM7 could participate during development of auditory pathway by determining distinct physiological properties of auditory pathway.

Poster No. 3

Selenium attenuates calcium ion influx and oxidative stress through voltage gated and TRPM2 cation channels in transfected cells

Mustafa Nazıroğlu[1,2], Cemil Özgül[1], Mustafa Küçükayaz[1], Bilal Çiğ[1], Simon Hebeisen[3], Ramazan Bal[4]

[1] Department of Biophysics, Medical Faculty, Süleyman Demirel University, Isparta, TURKEY.
[2] Neuroscience Research Center, Süleyman Demirel University, Isparta, TURKEY.
[3] B'SYS GmbH, Benkenstrasse 254, CH-4108 Witterswil, Switzerland.
[4] Department of Physiology, Medical Faculty, Fırat University, Elazığ, TURKEY.

It has been recently reported protective effects of essential antioxidant element selenium on cytosolic Ca^{2+} release in a cell lines although the effects of selenium on melastatin-like transient receptor potential 2 (TRPM2) channel actions in response to oxidative stress (H_2O_2) have not been understood. We investigated the effects of selenium on H_2O_2-induced TRPM2 channel currents in Chinese hamster ovary (CHO) cell line using patch-clamp and fura-2 fluorescence imaging techniques.

The CHO cell line was incubated by selenium for 36-48 hours before patch-clamp and Ca^{2+} signaling analysis. Selenium was also given into the cells by the patch pipette. H_2O_2-induced TRPM2 currents in selenium incubated cells were completely inhibited. Intracellular selenium, 2-aminoethoxydiphenyl borate (2-APB) and N-(p-amylcinnamoyl)anthranilic acid (ACA) also inhibited the H_2O_2-induced TRPM2 currents. The cytosolic Ca^{2+} release and lipid peroxidation levels were decreased by selenium incubations but not 2-APB although glutathione peroxidase activity and reduced glutathione levels increased.

In conclusion, selenium supplementation in the transfected CHO cells seems to have protective effects on the H_2O_2-induced increase of Ca^{2+} influx and oxidative stress through regulation of TRPM2 channels. The findings may contribute the use of selenium in treatment of TRPM2 channels activation induced-cellular oxidative toxicity.

Poster No. 4

N-Acetyl Cysteine Attenuates Chronic Diazinon Exposure-Induced Oxidative Stress in Rat Testis

Taylan Oksay[1], Mustafa Nazıroğlu[2], Osman Ergün[1], Salih Doğan[2], Orhan Özatik[3], Abdullah Armağan[4], Alper Özorak[1], Ömer Çelik[2]

[1] Department of Urology, Faculty of Medicine, Suleyman Demirel University, Isparta, TURKEY.
[2] Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, TURKEY.
[3] Department of Obstetrics and Gynecology, Faculty of Medicine, Suleyman Demirel University, Isparta, TURKEY.
[4] Department of Urology, School of Medicine, Bezmialem Vakif University, Istanbul, TURKEY.

Diazinon is not only widely used in industrial agriculture worldwide but also used in ectoparasiticide formulations for sheep and cattle resulting in distribution of diazinon in the environment and deleterious effects on biological systems. Although several reports about the toxicity of diazinon have been published, little study has been performed about its testicular effects and acting mechanism in testes; particularly in reference to its possible oxidative stress generating potential. The aim of this study is to investigate the protective effect of N-Acetyl Cysteine (NAC) on testicular oxidation damage induced by chronic exposure to diazinon for the period of four weeks.

Thirty six male rats divided into four groups. The control group was received the vehicle orally for four weeks. Three treatment groups received NAC, diazinon, combination of NAC and diazinon, respectively for 4 weeks.

Testis lipid peroxidation levels were higher in diazinon group than in control although lipid peroxidation levels were lower in diazinon+NAC group than in diazinon group. The reduced glutathione levels were lower in diazinon group than in control and NAC group although its levels were higher in diazinon+NAC group than in diazinon group. Vitamin C concentrations were lower in diazinon group than in control. Vitamin E and β-carotene concentrations were also lower in diazinon group than in control and NAC groups although their concentrations are higher in diazinon+NAC group than diazinon group. Glutathione peroxidase activity and vitamin A concentrations in the testis did not change in the four groups, significantly.

In conclusion, we observed that NAC treatment modulated diazinon-induced oxidative injury in the rat testis. These findings suggest that NAC supplementation can be useful in testis oxidative injury caused by the organophosphate.

Poster No. 5

Protective Effects of Caffeic Acid Phenethyl Ester (CAPE) on Diazinon-Induced Oxidative Stress and Antioxidant Enzymes in Rat Tissues: A Comparative Study

Gokhan Cesur[1], Bora Buyukvanli[2], Berit Gokce Ceylan[2], Mustafa Kabu[3], Ismail Kucukkurt[4], Sinan Ince[5], Kurtulus Ongel[6]

[1] Department of Physiology, Adnan Menderes University, Faculty of Medicine, Aydin, TURKEY.
[2] Department of Anesthesiology, Suleyman Demirel University, Faculty of Medicine, Isparta, TURKEY.
[3] Department of Internal Medicine, Afyon Kocatepe University, Faculty of Veterinary, Afyonkarahisar, TURKEY.
[4] Department of Biochemistry, Afyon Kocatepe University, Faculty of Veterinary, Afyonkarahisar, TURKEY.
[5] Department of Pharmacology and Toxicology, Afyon Kocatepe University, Faculty of Veterinary, Afyonkarahisar, TURKEY.
[6] Department of Family Medicine, Izmir Tepecik Research and Implementation Hospital, Izmir, TURKEY.

An organophosphate insecticide (OPI), diazinon, has been used in agriculture and domestic for many years. Aim of the study is to examine diazinon-induced oxidative stress that promotes production of reactive oxygen species (ROS). Moreover; role of caffeic acid phenetyl ester (CAPE) on tissues (brain, heart and lung) against possible oxidative damage in rats was examined.

Thirty rats were used in the study. Animals were randomly grouped as: sham-operated control group (n=10), diazinon inducement experimental group (group II, n:10) and diazinon inducement + CAPE treated group (group III, n:10). Brain, heart and lung tissues were investigated for antioxidant activity and the levels of lipid peroxidation.

In the diazinon inducement group; malondialdehyde (MDA) and nitric oxide (NO) levels were higher, glutathione (GSH), superoxide dismutase (SOD) were lower than control group in heart tissue. MDA levels in brain and lung tissues were increased for diazinon inducement group compared to control group. GSH levels in brain and lung tissues were lower in diazinon inducement group than control group. In diazinon inducement + CAPE treated group; MDA levels were lower and GSH levels were higher than diazinon inducement group for all of the tissues. SOD levels were increased in diazinon inducement + CAPE treated group for heart tissue.

The potent free radical scavenger and antioxidant agent, CAPE, seems to be a highly promising agent for protecting brain, heart and lung tissues from oxidative damage and preventing organ dysfunction as a result of diazinon inducement.

Poster No. 6

The effect of low-frequency electromagnetic field (ELF-EMF) on serum total antioxidant capacity (TAOC) and on total oxidant stress (TOS)

Veysi Akpolat[1], M.Salih Çelik[1], Birgül Işik[2], M. Cihan Yavaş

[1] Biophysics Department, Medical Faculty of Dicle University, 21280 Diyarbakir, TURKEY.
[2] Biochemical Department, Medical Faculty of Dicle University, 21280 Diyarbakir, TURKEY.

This study was carried out on individuals who constantly use Blow/Hair-dryers in Hair-dressing saloons. In the study, 32 subjects selected randomly (mean age:24±6) were divided into two groups:Group 1(n=16, 8 males and 8 females using Blow-dryers) as experimental group; and Group 2 (n=16, 8 males and 8 females never using Blow-dryers) as control group.

Total Antioxidant Capacity (TAOC) mmol Trolox Eqv/L and Total Oxidant Stres (TOS)μmol H_2 O_2 Eqv/L levels of the sera taken from the subjects were measured by an autoanalyzer. Statistical analyses of the data obtained were performed by using SPSS 15.0 Windows Package Program.

In the study, TAOC and TOS values measured for males and females in both groups were compared separately by taking the same-genders into consideration.

TAOC levels for males in control group were measured as mean±std= 0.72±0.05, for males in experimental group as mean±std=0.49±0.07, p<0.0001. TOS levels for males in control group were found as mean±std=9.92±1.5, for males in experimental group as 6.77±1.5, p<0.001.

TOS levels for females in control group were found as mean±std=0.58±0.08, for females in experimental group as mean±std=0.40±0.05, p<0.0001.

In the study, it was seen that TAOC and TOS values of individuals who use constantly blow/hair dryers in hairdressing saloons and who are exposed to electromagnetic field varied significantly.

It is concluded that radiation plays a significant role in revealing these molecules that play a part in the etiopathogenesis of many diseases, and thus preventive measures should be strictly taken, and exposure time should be taken under control through a limitation in workers

References

1. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more s ABTS radical cation. Clin Biochem. 2004;37:277-85.
2. Erel. O A new automated colorimetric method for measuring total oxidant status, Clinical Biochemistry 38 (2005) 1103–1111

This study was conducted with permission of Medical Facultiy's Ethics Committee. (13.01.2012 / 4-352).

Poster No. 7

The Redox State of Transglutaminase Controls Arterial Remodeling

Jeroen van den Akker[1], Ed VanBavel[1], Remon van Geel[1], Hanke L Matlung[1], Bilge Guvenc Tuna[1], George MC Janssen[3,4], Peter A van Veelen[3], Wilbert C Boelens[2], Jo GR De Mey[5] and Erik NTP Bakker[1]

[1] Dept of Biomedical Engineering and Physics, Academic Medical Center, Amsterdam.
[2] Dept of Biomolecular Chemistry, Radboud University, Nijmegen.
[3] Dept of Immunohematology and Blood Transfusion, Leiden University Medical Centre.
[4] Netherlands Proteomics Centre.
[5] Dept of Pharmacology & Toxicology, Maastricht University, THE NETHERLANDS.

Inward remodeling of small arteries in response to low blood flow and hypertension depends on type 2 transglutaminase (TG2). We studied the regulation of TG2 activity, its localization and substrates during remodeling. Isolated mouse mesenteric arteries exposed to exogenous TG2 required a reducing agent to induce inward remodeling. The effect of TG2 depended on its cross-linking activity, as indicated by the lack of effect of mutant TG2. The cell-impermeable reducing agent TCEP did not induce remodeling. However, the cell-permeable reducing agent DTT induced translocation of endogenous TG2 and transglutaminase activity at the smooth muscle membrane. This resulted in inward remodeling, which could be inhibited by a TG2 inhibitor or the nitric oxide donor SNAP. Using mass spectrometry, 21 proteins were identified as cross-linking substrates, including collagen, fibronectin and nidogen. Finally, inward remodeling induced by low blood flow was associated with the upregulation of several reducing enzymes in vivo. In conclusion, these results suggest that a reduced state induces TG2 activity at the smooth muscle cell surface. Here the cross-linking of matrix proteins may induce inward remodeling. Supported by the Netherlands Heart Foundation, NHS.2005B080.

Poster No. 8

Connexin hemichannels-mediated nitric oxide synthesis in the injured endothelium of excised rat aorta

Roberto Berra-Romani[a], José Everardo Avelino-Cruz[b], Abdul Raqeebb, Francesco Moccia[b], Franco Tanzi[b]

[a] School of Medicine, Department of Biomedicine, Benemérita Universidad Autónoma de Puebla, Puebla, MEXICO; [b] Department of Physiology, University of Pavia, Pavia, ITALY.

Endothelial injury is regarded as the early event that leads to the onset and progression of severe vascular disorders. The signal transduction pathway which drives the subsequent healing process provides an ideal target to design novel pharmacological strategies to promote re-endothelization. We have recently exploited the excised rat aorta to investigate injury-induced Ca^{2+} elevation within ex vivo ECs and hinted for the first time at role for Ca^{2+} entry through unpaired connexons. In this study, we sought to determine whether such Ca^{2+} entry results in nitric oxide (NO) synthesis at the wound edge by loading ECs with the NO-sensitive fluorochrome, DAF-FM diacetate. Mechanical scraping augments intracellular NO levels within surviving ECs. Injury-elicited NO synthesis was prevented in presence of the endothelial NO synthase inhibitor, L-NAME, and in absence of extracellular Ca^{2+}. Unlike ATP-dependent NO production, the NO response to injury was insensitive to BTP-2, a selective blocker of store-operated Ca^{2+} inflow. However, injury-induced NO synthesis was significantly reduced by the gap junction blockers, octanol, palmitoleic acid and oleamide. The data presented provide the first evidence that endothelial scraping stimulates NO synthesis at the wound edge, which might both exert an immediate anti-thrombotic and anti-inflammatory action and promote the subsequent re-endothelization.

Poster No. 9

The effect of vitamin E on Ca^{2+} signaling and oxidative stress in neuthrophils of patients under ischemia-reperfusion injury and sevoflourane anesthesia

Ömer Osmanlıoğlu[1], Mustafa Nazıroğlu[2], Lütfi Yavuz[1], Bilal Ciğ[2]

[1] Department of Anaesthesiology and Reanimation, Süleyman Demirel University Faculty of Medicine, Isparta, TURKEY.

[2] Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, TURKEY.

Polimorphonuclear leucocytes are the main role performing cells in the inflamatory process. The cytosolic Ca^{2+} concentration enhance that adequates the formation of Ca^{2+} signal constitutes an important step in neutrophil activation. The Ca^{2+} signalisation and the increase in neutrophil activation induce the reactive oxygen radicals formation that causes tissue damage.

In this study, we aimed to investigate the effect of preoperatively administered vitamin E on oxidative stress parameters such as lipid peroxidation, reduced glutathione, glutathione peroxidase and cytosolic free Ca^{2+} release in neutrophil which were freshly isolated from surgical arthroscopy performed patients.

Twenty healthy arthroscopy patients were included to the study. They were divided into four groups. Blood samples in the first (preoperative) and second (postoperative) groups were taken before and after arthroscopy (ischemia-reperfusion injury) without treatments, respectively. Blood samples in the third groups were taken and then they received intramuscular 300 IU vitamin E (D-Alpha-tocopherol acetate) before arthroscopy. About 2 hours later (postoperative+treatment group) of arthroscopy, blood samples were taken the patients again.

Cytosolic free Ca^{2+} levels and oxidative stress markers in the postoperative period were found significantly ($p<0.001$) higher according to preoperative levels. Lipid peroxidation in neuthrophils were not different between postoperative and treatment+postoperative groups while reduced glutathione and glutathione peroxidase values increased significantly ($p<0.001$) in treatment+postoperative group as compared to postoperative groups.

In conclusion, we observed that preoperative vitamin E supplementation may be beneficial in tourniquet (ischemia-reperfusion injury) performed patients by regulating Ca^{2+} influx, reduced glutathione and glutathione peroxidase values.

Poster No. 10

Inhibitory effect of prenatal stress on field excitatory postsynaptic potentials in infant rats

Saboory E., Roshan-Milani S.

Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, IRAN.

Exposure to stress is known to change synaptic plasticity and results in long-term depression; further, this stress precipitates seizures. In the study described here, the prenatal restraint and predator stress models were used to test the hypothesis that indirect prenatal stresses influence hippocampal synaptic potentiation and may affect seizures susceptibility in infant rats. Pregnant female Wistar rats were divided into 3 groups: control, restraint-stressed, and predator-stressed groups. Both stressed groups were exposed to the stressor on gestation days 15, 16, and 17. The restraint stress involved 1-h sessions twice daily in a Plexiglas tube and the predator stress involved 2-h sessions once daily in a cage placed within the visual range of a caged cat. Blood corticosterone (COS) levels were measured in different time points. Hippocampal slices were prepared and field excitatory postsynaptic potentials (fEPSP) were studied on postnatal day 15. Pilocarpine was administered on postnatal day 25 and mortality rates were measured after 2 and 24 h. Restraint and predator stresses resulted in significantly elevated COS blood levels in dams and pups. Both the amplitude and slope of fEPSP in the CA1 area decreased significantly in the stressed groups as compared to the control. Prenatal restraint and predator stresses significantly increased the fatal effect of pilocarpine at 24 h after injection. Exposure to prenatal stresses and COS blood levels elevation reduce hippocampal synaptic potentiation and increase mortality rate of seizure in infant rats and may affect on later seizure susceptibility and prognosis.

Hippocampal slices, Prenatal stress, Corticosterone, Postsynaptic evoked potentials, Rat.

Poster No. 11

In vivo prevention of adriamycin renal toxicity by selenium

Eylem Taşkın, Nurcan Dursun, Elvan Kunduz

Department of Physiology, Faculty of Medicine, University of Erciyes, Kayseri, TURKEY.

Adriamycin clinical use in cancer chemotherapy is limited due to its high renal toxicity. This study investigated the mechanism of ADR nephropathy and the protective effect of selenium on ADR-induced kidney damage. Rats were divided into four groups. The first group was injected with saline i.p. for 21 days, the second group received the 4 mg/kg i.p. ADR every alternate day for 8 days, the third group received the 50 μg/kg i.p. Se for 21 days, and the fourth group received the Se. ADR co-administration i.p. blood pressures were assessed, the mitochondrial membrane potential (MMP) was

assessed, and the adenosine triphosphate (ATP) levels were determined. The total antioxidant (TAS) and oxidant status (TOS) in cytosol, the mitochondria of kidney cells, and plasma were measured. Mitochondrial TAS decreased and TOS increased in the ADR group compared to the Se group. ADR-treated rats showed significantly lower MMP than did the control and Se groups. MMP was significantly restored in the Se+ADR group through selenium treatment compared to the ADR group ($p < 0.01$). In the ADR group, a reduction in ATP content was seen compared to the control and Se groups ($p < 0.01$). ATP level was significantly restored through treatment with selenium in the Se+ADR group compared to the ADR group ($p < 0.01$). We concluded that selenium is effective in vivo against ADR-induced kidney damage via the restoration of TAS and TOS, which prevented mitochondrial damage.

Adriamycin, selenium, mitochondrial membrane potential, mitochondrial ATP levels, total antioxidant status, total oxidant status, rat, kidney.

Poster No. 12

The effect of morphine on action potential properties of dorsal root ganglion neurons in mice

Ehsan Saboory[1], Oxana Garilova-Ruch[2], Stefan Heinnemann[2]

[1] Department of physiology, faculty of medicine, Urmia University of medical sciences, IRAN.
[2] Center for molecular biomedicine, Department of Biophysics, Friedrich-Schiller University Jena, GERMANY.

Midbrain periaqueductal gray and spinal cord dorsal horn are major action sites of opioid analgesics in the pain pathway. Recent investigations demonstrate an important role of peripheral mechanisms of opioid analgesia. Peripheral sensory neurons in the dorsal root ganglia (DRG) are among the critical targets of opioids acting on opioid receptors. We investigated the effect of morphine on properties of action potential (AP) in medium size DRG neurons of mice. DRGs were obtained from adult mice by the spinal cord dissection and plated on poly-L-lysine coated glass coverslips in HAM`s F12 supplemented medium. Electrophysiological measurements were performed within 48 h of culture using current clamp mode of EPC-10 amplifier. Holding potential for all experiments kept at -70 mv. The resting membrane potential (RMP) was 62.46 ± 7.5 mv (n=29). Twenty one percent (6 from 29) of DRGs had bursting activity following an appropriate stimulus (-20 to 500 pA, 100 ms). Morphine (1-20 μM) neither affect the RMP nor bursting activity in DRGs. Morphine significantly increased the duration of AP and threshold for evoke an AP. In voltage clamp mode, morphine significantly decreased sodium and potassium currents. Bath application of 5-20 μM morphine significantly decreased sodium currents from 19.25 ± 2.5 to 10.36 ± 2.8 nA ($p < 0.01$). We conclude that morphine reduces sodium and potassium currents and at least in part, slows the repolarization process. Meanwhile, morphine increases the firing threshold in DRG neurons and probably reduces or prevents conduction of pain signals to spinal cord.

Action potential, current clamp, DRG, morphine, mouse, Somatic sensory cell.

Poster No. 13

The effect of low-frequency electromagnetic field (ELF-EMF) on serum paraoxanase (PON1) and malondialdehyde (MDA) levels

Mustafa Salih Çelik[1], Veysi Akpolat[1], Birgül Işik[2], M.Yusuf Çelik[3], M.Cihan Yavaş[1], Osman Evliyaoğlu[1]

[1] Biophysics Department, Medical Faculty of Dicle University, 21280 Diyarbakir, TURKEY.
[2] Biochemical Department, Medical Faculty of Dicle University, 21280 Diyarbakir, TURKEY.
[3] Biostatistics Department, Medical Faculty of Dicle University, 21280 Diyarbakir, TURKEY.

This study was carried out on individuals using constantly blow-dryers and hair-dryers in hair-dressing saloons. We aimed to investigate the effects of ELF-EMF caused by blow/hair dryers on PON1 and MDA serum levels of users.

The study was conducted on 32 individuals (mean age; 24± 6) selected randomly from among those constantly using blow/hair dryers in haircutting and hairdressing saloons.

The subjects included into the study were divided into two groups: group 1 (n=16, 8 males, 8 females using hairdresser) as experimental group; Group 2 (n=16, 8 males, 8 females non-using hairdresser) as control group.

In the sera of the subjects, PON 1 serum levels (U/L) were measured spectrophotometrically.

MDA serum levels (μMol/L) , however, were measured by inducing MDA-TB (Thiobarbituric acid) complex of peroxidation products.

The statistical analyses of the data obtained in the study were performed by using SPSS 15.0 Windows Package Program. P ($p<0.05$) was considered significant.

In the study, PON 1 and MDA values of the

subjects (Males and Females) in control and experimental groups were compared separately by taking the same-genders into account.

PON 1 levels for males in control group were found as mean±std=52,71±14,43; and for males in experimental group as mean±std=40.32±6.18, p<0.043. MDA levels for males in control group were found as mean±std =14.37±4.12, for males in experimental group as mean±std=18.17±3.49, p<0.05.

PON 1 levels for females in control group were measured as mean±std=67.87±8.10, for females in experimental group as mean±std=32.67±8.01, p<0.0001. MDA levels for females in control group were determined as mean±std=21.12±6.58, for females in experimental group as mean±std=18.42±4.08, p<0.34.

In the study, it was observed that PON 1 and MDA values measured for those individuals who constantly use blow/hair dryers and thus are exposed to ELF-EMF varied considerably.

It was concluded that individuals who work constantly with electric appliances producing electromagnetic field should be informed of the hazards of these machines, and preventive measures should be taken against these hazards

References

1. Eckerson HW, Romson J, Wyte C, La Du BN. 1993, The human serumparaoxonase polymorphism: identification of phenotypes by their response to salts. Am J Hum Genet;35:214-27.

2. Jentzsch,A.M., et al. 1996, Improved Analysis of Malondialdehyde in Human Body Fluids, Free Rad. Biol. Med. 20:251-256.

- This study was conducted with permission of Medical Facultiy's Ethics Committee. (13.01.2012 / 4-352).

Poster No. 14

Investigation of the protective effect of vitamin c on methotrexate induced liver injury in rats

Mehtap Buldac[1], Ekrem Cicek[2] , Duygu Kumbul Doguc[3], Halil Asci[2], Sukriye Yesilot[2], I. Aydın Candan[4], Birsen Dagdeviren[3], Nihan Cankara[2], Meral Oncu[4], M.Kaya Ozer[5]

[1] Ministry of Health of TURKEY, General Directorate of Pharmaceuticals and Pharmacy, Department of Clinical Drug Trials, Ankara, TURKEY.
[2] Süleyman Demirel University Faculty of Medicine Department of Pharmacology, Isparta, TURKEY.
[3] Süleyman Demirel University Faculty of Medicine Department of Biochemistry, Isparta, TURKEY.
[4] Süleyman Demirel University Faculty of Medicine Department of Histology and Embryology, Isparta, TURKEY.
[5] Firat University Faculty of Medicine Department of Pharmacalogy, Elazığ, TURKEY.

Methotrexate (MTX), a folat antagonist used for the treatment of cancer and inflamation, causes potential side effects like hepatotoxicity and in previous studies, it has been shown that oxidative damage is the causative reason for the MTX toxicity. Vitamin C (Vit C) is a well-known antioxidant and it has been used in various studies for this property. Thereby Vit C could be an alternative prophylactic agent against MTX-induced hepatotoxicity.

A total of thirty-six male Wistar rats weighting 200–250 gr were equally divided into the six groups as follows: Controls (saline i.p. for 5 days), MTX (20 mg/kg, i.p, single dose), Vit C (250 mg/kg Vit C, orally, 1 day), Vit C (250 mg/kg Vit C, orally, 3 days), MTX +Vit C group (250 mg/kg Vit C orally 1 hour prior to 20 mg/kg single dose MTX i.p), MTX +Vit C group (250 mg/kg Vit C orally 1 hour prior to 20 mg/kg single dose MTX i.p. and continued for 2 days). At the end of the study, liver tissues were evaluated. Histopathological and the biochemical analysis for malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were performed.

In hepatic tissues, MDA levels were increased and SOD, CAT and GPx levels were decreased by MTX. All parameters but except CAT levels, were significantly restored after the administration of Vit C for three days (Table 1). Similar to the biochemical findings, by the use of MTX, the evidences of oxidative damage were seen in liver tissues by histopathologic examination (Fig 2). Compare to the control group (Fig 1), the findings of oxidative damage were not seen in any MTX plus Vit C treatment groups (Fig 3 & Fig 4)

The use of MTX is often limited because of the drug-induced severe hepatotoxicity. Despite the exact mechanism of MTX-induced hepatotoxicy is not well-understood, recent studies have indicated the oxidative damage as the causative factor. This study was aim to find out whether Vit C, a potent antioxidant could restorate the MTX-induced oxidative damage in the rat liver. As a result of this study, Vit C at various doses can ameliorate the toxic effect of MTX on the rat liver tissues

References

1. Jahovic N, Cevik H, sehirli AO, Yegen BC, Sener G. Melatonin prevents Methotrexate-induced hepatorenal oxidative injury in rats. J Pineal res. 2003 May; 34(4),: 282-7
2. Uraz S,Tahan V,Aygun C,Eren F,Unluguzel G,Yuksel M,Senturk O,Avsar E,Haklar G,Celikel C,Hulagu S,Tozun N,3Scheiman JM. Role of ursodeoxycholic acid in prevention of methotrexate-induced liver toxicity. Dig Dis Sci. 2008 Apr;53(4):1071-7. Epub 2007 Oct 13.
3. Ozturk IC, Ozturk F, Gul M, Ates B, Cetin A. Protective effects of ascorbic acid on hepatotoxicity and oxidative stres caused by carbon tetracholoride in the liver of Wistar rats. Cell Biochem Funct 2009; 27: 309-315

4. Kaida S, Ohta Y, Imai Y, Kawanishi M. Protective effect of L-ascorbic acid against oxidative damage in the liver of rats with water-immersion restraint stress. Redox Rep. 2010;15(1):11-9.

Poster No. 15

Cyclosporin A-mediated mitochondrial permeability transition pore (mPTP) protection against doxorubicin-induced cardiac mitochondrial toxicity

Dilek Sivri, Nurcan Dursun, Kalender Özdoğan, Ali Baykan, Songül Biltekin

Department of Physiology, Medical Faculty, Erciyes University, Kayseri, TURKEY.

The use of doxorubicin (DOX), an antibiotic used in oncological treatments, is limited by a dose-related cardiotoxicity. We tested whether inhibition of mitochondrial permeability transition pore by cyclosporin A (CsA) would prevent doxorubicin-induced myocardial and mitochondrial dysfunction.

Twenty rats were divided into 4 groups. Acute model of DOX exposition were performed in rats with a single intraperitoneal bolus (10mg/kg body weight). Follow-up was 8 days. Rats received either CsA (1 mg/kg of body weight) or tacrolimus (0,1 mg/kg of body weight FK506, a ciclosporin derivative with no inhibitory effect on the mitochondrial transition pore) or saline until follow-up. The general observations, mortality, electrocardiographic changes, blood pressure, biomarker enzymes' activities like lactate dehydrogenase (LDH) and creatine phosphokinase (CPK), biochemical parameters such as aspartate aminotransferase (AST) alanine aminotransferase (ALT) were monitored after last dose.

The acut model, doxorubicine decreased left ventricular function and incresased biomarker enzymes' activities especially LDH and CPK. Heart function and enzyme activities were improved by tacrolimus but not by CsA.

These findings suggest that co-administration of CsA with doxorubicin did not improved heart tolerance to doxorubicin manifested in vivo, co-administering of CsA or the less toxic FK506 with doxorubicin may not be useful in protecting heart from doxorubicin-induced-cardiotoxicity in humans.

Poster No. 16

The role of renin-angiotensin system on ischemia reperfusion

Kalender Özdoğan, Eylem Taşkın, Elvan Kunduz, Nurcan Dursun

Department of Physiology, Medical Faculty, Erciyes University, Kayseri, TURKEY.

The aim of this study was to prevent the effect of Angiotensin II (Ang II) on the heart with different ways before IR and to compare the frequency of arrhytmia seen in the groups.

Twenty rats were divided into 4 groups. Serum physiologic (SF; 0.1 ml) was administered to the ischemia group (IS), 5 mg/0.1 ml SF to the Angiotensin Converting Enzyme inhibitor; the Kaptopril group (KAP), 25 mg/0.1 ml SF to the Rennin inhibitor; Aliskrein group (AL) and finally KAP+AL group which were given both AL and KAP substances in the same dosage. During the IR, blood pressure was measured, electrocardiography (ECG) records and in the end of the study, blood samples were taken. The hearts of the rats were removed and kept so as to evaluate the oxidant and antioxidant status.

The IR arrhythmia (ventricular ectopic shot, ventricular tachycardia values) occurred with the prevention of Ang II effect was not found to be significantly different compared with the IR procedures performed alone. Total antioxidant formation occurred in IR of the prevented Ang II effect (KAP, AL, KAP+AL groups) was lower than the rats which were performed IR alone ($p<0.001$), besides no significant change in antioxidant defense. In IR with prevented Ang II effect, oxidant stress index (OSI) was lower than the group which were performed IR alone ($p<0.001$).

Consequently, Ang II effect was not significantly high during acute IR occurred in heart arrhythmia however it was found to diminish the healing of heart during IR as it increased the damage.

Poster No. 17

Oxidative stress is a hallmark of cadmium-induced toxicity in different tissues

Hala Hamouda, Amal Baalash, Khalid Saad[1], Ashraf Emara[1]

Departments of Biochemistry and [1]Toxicology, Faculty of Medicine, Tanta University, EGYPT.

Cadmium (Cd) is a potent cell poison known to cause oxidative stress that cause plasma membrane breakdown (1). It was thus found to be of interest to investigate the possible role antioxidants could play in Cd toxicity. The study was conducted on forty male albino rats, which were divided into four equal groups and treated as follows for sixteen days:

Group I was given normal saline 1 ml subcutaneously, Group II was given 1 mg/kg cadmium chloride subcutaneously, Group III was given 1 mg/kg cadmium chloride subcutaneously and additionally given a daily oral dose of 0.85 mg Antox, and Group IV was given a daily oral dose of 0.85 mg Antox. We measured serum and tissue (liver, kidney, heart, brain, and testis) cadmium, metallothionein, malondialdehyde (MDA), and glutathione levels, and antioxidant enzymes activities.

The results showed that cadmium exposure caused significant increase in metallothionein levels in serum and all tissues except testicular tissue. Serum and tissue malondialdehyde levels showed significant increase in Cd exposed animals, with highest levels in brain and testicular tissues. The activities of the studied antioxidant enzymes showed significant changes in in Cd exposed animals. Antox treatment succeeded to minimize such changes.

We can conclude that oxidative stress plays an important role in the development of Cd toxicity, and the use of antioxidants can to some degree support the body defense system to protect itself against the damaging effect of Cd toxicity.

References

1. Miler E, NudlerS, Quinteros F, Cabilla J, Ronchetti S, Duvilanski B. Cadmium induced-oxidative stress in pituitary gland is reversed by removing the contamination source. Human and Experimental Toxicology 2010; 29: 873 - 880.
2. Chang X, Jin T, Chen L, Nordberg M, Lei L. Metallothionein I isoform mRNA expression in peripheral lymphocytes as a biomarker for occupational cadmium exposure. Exp Biol Med 2009; 234: 666 - 672.

Poster No. 18

TRPV1 and ASICs are master players in peripheral diabetic neuropathy

Beatrice Mihaela Radu[1,2], Diana Ionela Dumitrescu[2], Mihaela Temelie[3], Adela Marin[2], Daniel Dumitru Banciu[2], Adina Daniela Iancu[4], Dorel Lucian Radu[4], Mihai Radu[1,3 #]

[1] University of Verona, Department of Neurological, Neuropsychological, Morphological and Movement Sciences, Section of Anatomy and Histology, Strada Le Grazie 8, 37134, Verona, ITALY. #mihai.radu@univr.it

[2] University of Bucharest, Faculty of Biology, Departament of Anatomy, Animal Physiology and Biophysics, Splaiul Independentei 91-95, 050095, Bucharest, ROMANIA.

[3] Horia Hulubei National Institute for Physics and Nuclear Engineering, Department of Life and Environmental Physics, Reactorului 30, P.O. Box MG-6, 077125, Bucharest-Magurele, ROMANIA.

[4] Cantacuzino National Institute for Microbiology and Immunology, Laboratory of Cellular Immunity, Splaiul Independentei, 103, Bucharest, 050096, ROMANIA.

Acid-sensing ion channels (ASIC) and the transient receptor potential cation channel subfamily V member 1 (TRPV1) are important molecular detectors of chemical / nociceptive stimuli in sensory neurons. Many studies on streptozotocin-induced diabetes have proved the role played by TRPV1 (1), but no study has taken into account the importance of ASICs in peripheral diabetic neuropathy. We have focused on the role played in type 1 diabetes by these channels expressed in sensory neurons from dorsal root ganglia, by means of patch-clamp, immunofluorescence microscopy and qRT-PCR. An autoimmune TCR-HA$^{+/-}$/Ins-HA$^{+/-}$ mice was used as a model for type 1 diabetes (2). Whole-cell patch-clamp studies have indicated: TRPV1 current amplitude is significantly larger in diabetic than in Balb/c mice, and the desensitization is more reduced in diabetic condition; there are significant changes in the shape and time recovery of ASIC isoforms between normal and diabetic mice. The immunofluorescence microscopy indicated that TRPV1 and ASIC2 expression is decreased in diabetic animals, while ASIC1 and ASIC3 expression is increased. The mRNA levels for ASICs are varying in the following sequence ASIC2>ASIC3>ASIC1 for Balb/c and diabetic mice. In addition, the mRNA levels for TRPV1 are significantly decreased in diabetes. In diabetic condition, the non-enzymatic glycosylation of the neuronal membrane-receptors (3) impairs the antibody linkage to the active site, therefore distinct results can be obtained by immunofluorescence and qRT-PCR. Our study points out that beside the TRP channels various ASICs heteromers are active players in the early stages of diabetes.

References

1. Pabbidi RM, Yu SQ, Peng S, Khardori R, Pauza ME, Premkumar LS. Influence of TRPV1 on diabetes-induced alterations in thermal pain sensitivity. Molecular Pain 2008; 4:9.
2. Radu DL, Noben-Trauth N, Hu-Li J., Paul WE, Bona CA. A targeted mutation in the IL-4Ra gene protects mice against autoimmune diabetes. Proc. Natl. Acad. Sci. USA 2000; 97(23):12700-12704.
3. Duran-Jimenez B, Dobler D, Moffatt S, Rabbani N, Streuli CH, Thornalley PJ, Tomlinson DR, Gardiner NJ. Advanced glycation end products in extracellular matrix proteins contribute to the failure of sensory nerve regeneration in diabetes. Diabetes. 2009; 58(12):2893-903.

Poster No. 19

Selenium-Vitamin E combination modulates endometrial lipid peroxidation and antioxidant enzymes in streptozotocin-induced diabetic rat

Mehmet Güney

Department of Obstetrics and Gynecology, Faculty of Medicine, Süleyman Demirel University, Isparta, TURKEY.

Diabetes mellitus is associated with diabetic impairment of uterine function, ultimately leading to reduced fertility. Its etiology may involve oxidative damage by reactive oxygen substances, and protection against this damage can be offered by antioxidant supplementation. In the present study, the effects of a vitamin E-plus-selenium (VESe) combination on lipid peroxidation (MDA) and the scavenging enzyme activity in the uterine endometrium of streptozotocin (STZ)-induced diabetic rats were investigated.

Twenty-four female rats were equally divided into three groups as follows: group I (control); group II (diabetic); group III (diabetic+VESe), STZ+vitamin E (60 mg/kg over 1d) + selenium-treated (Na_2SeO_3, 1 mg/kg over 1 d). After 4-weeks of receiving the VESe-treatment, endometrium samples were taken from the uterus. Although the VESe-treatment decreased the MDA and blood glucose levels in the STZ-group, the observed values remained significantly higher than in the controls. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and body weight gain were significantly ($p<0.01$) lower in STZ groups as compared to control group, whereas their activities were ($P<0.01$) increased by VESe treatment. However, there was no significant difference on body weight gain and uterine weights between control and STZ+VESe groups.

In conclusion, the endometrial complications caused by oxidative stress, and the abnormal blood glucose levels in diabetic of rats, can be alleviated by strengthening the physiological antioxidative defense through the administraton of vitamin E and Se.

Poster No. 20

The Effect of Plasma Membrane Ca^{2+} ATPase on ACh Induced Calcium Oscillations in HEK 293 Cells

Figen Amber Çiçek, Mehmet Uğur

Ankara University Faculty of Medicine, Dpt. Biophysics Sihhiye, Ankara, TURKEY.

In response to agonist stimulation the generation of Inositol (1,4,5)-Trisphosphate (IP_3) activates its own receptor channel (IP3R) on Endoplasmic Reticulum (ER) membrane. Due to this activation stored Ca^{2+} release from ER into the cyoplasm and $[Ca^{2+}]i$ increase (1). This increase exhibit in many different spatial and temporal dynamics: They will be global (wave) or restricted to local part of the cell (sparks) or arise of $[Ca^{2+}]i$ may be periodicly called as oscillations. Cells may use these different Ca^{2+} signalling dynamics to encode informations arriving at the cell surface to cytoplasmic targets (2). In this study we tried to understand the contributions of Plasma Membrane Ca^{2+} ATPase (PMCA) on ACh induced Ca^{2+} oscillations in HEK 293 cells. Cells which were incubated with Fluo-3 AM stimulated by acetylcholine, and changes in fluorescent intensity was detected by a confocal microscope. All the experiments were done in the nominal Ca^{2+} free experiment solution. In order to block PMCA, 1 mM La^{3+} was used which is a high enough concentration for insulating PMCA. Even in a single cell type, different oscillation patterns to the same agonist concentration can be obtained. Hence, we classified Ca^{2+} release patterns after the submaximal (1 μM) and maximal (100 μM) doses ACh treatments. We grouped Ca^{2+} responses as their types (qualitatively), oscillation frequencies (number of oscillations) and release times. After this identification, we analysed the effect of the La^{3+} through these classifications. The frequency and time courses of the Ca^{2+} oscillations remained nearly same for the submaximal dose of ACh in the presence of 1 mM La^{3+}. While, for the maximal dose of ACh with 1 mM La^{3+}, Ca^{2+} oscillations showed an increase in which superimposed on sustained release, and also time courses increased. The effect of PMCA on temporal dynamics of $[Ca^{2+}]i$ have become more apparent with the maximal dose of ACh.

References

1. Berridge MJ. Inositol trisphosphate and calcium signalling. Nature, 1993, 28;361(6410):315-25.
2. Falcke M. Reading the patterns in living cells - the physics of Ca^{2+} signaling, 2004. Advances in Physics, 53:3, 255 – 440.

Poster No. 21

Neuroprotection induced by glutathione (GSH) and N-acetylcysteine against cytosolic GSH depletion dependent TRPV1 currents and Ca^{2+} influx in dorsal root ganglion neuron

Mustafa Nazıroğlu, Cemil Özgül, Bilal Çiğ

[1] Department of Biophysics, Faculty of Medicine, University of Suleyman Demirel, Isparta, TURKEY.

Thiol depletion-induced neuropathy is associated with peripheral demyelination and degeneration of nerve fibers. The mechanism underlying dorsal root ganglion (DRG) injury through glutathione (GSH) depletion remains unclear (1,2). Immunohistochemical studies confirmed highly the presence of transient receptor potential vanilloid 1 (TRPV1) in dorsal root ganlion (DRG) neurons. GSH and N acetyl cysteine (NAC) may have a regulator role on TRPV1 channels in the neurons. Therefore, we tested the effects of GSH and NAC on TRPV1 channel currents in intracellular GSH depleted DRG in rats.

DRG neurons were freshly isolated from rats and the neurons were incubated for 24 hrs with buthionine sulfoximine (BSO). Cytosolic treatment of cultured DRG neurons with GSH and NAC, results in a protection against capsaicin activation. This neuroprotection is associated with the attenuation of a Ca^{2+} influx triggered by capsaicin and GSH depletion via BSO. Here, we evidence the contribution of thiol groups on activation of TRPV1 channels in this mechanism. TRPV1 channels are activated by various agents including capsaicin, the pungent component of hot chili peppers and blocked by capsazepine. The capsazepine, anthralic acid and 2-aminoethyl diphenylborinate, TRPV1 inhibitors, strongly reduced capsaicin-induced currents and Ca^{2+} influx, in the same way as to GSH and NAC treatments.

In conclusion, in our experimental model, TRPV1 channels are involved in the capsaicin-induced neuronal death and a negative modulation of this channel activity by GSH and NAC treatment may account, at least in part, for the neuroprotection against GSH depletion induced oxidative toxicity.

References

1. Özgül C, Nazıroğlu M. TRPM2 channel protective properties of N-acetylcysteine on cytosolic glutathione depletion dependent oxidative stress and Ca^{2+} influx in rat dorsal root ganglion. Physiol Behav. 2012;106:122-128.
2. Nazıroğlu M, Özgül C, Çiğ B, Doğan S, Uğuz AC. Glutathione modulates $Ca^{(2+)}$ influx and oxidative toxicity through TRPM2 channel in rat dorsal root ganglion neurons. J Membr Biol. 2011; 242:109-118.

Poster No. 22

Redox signal-mediated sensitization of Transient Receptor Potential Melastatin 2 (TRPM2) to temperature affects macrophage functions

Makiko Kashio[1], Takaaki Sokabe[1], Yasuo Mori[2], Makoto Tominaga[1]

[1]Division of Cell Signaling, Okazaki Institute for Integrative Bioscience (National Institute for Physiological Sciences), National Institutes of Natural Sciences, Okazaki, Japan, [2]Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, JAPAN.

The ability to sense temperature is essential for organism survival and efficient metabolism. Body temperatures profoundly affect many physiological functions, including immunity. Transient receptor potential melastatin 2 (TRPM2) is a thermosensitive, Ca^{2+}-permeable cation channel expressed in a wide range of immunocytes. TRPM2 is activated by adenosine diphosphate ribose (ADPR) and hydrogen peroxide (H_2O_2), although the activation mechanism by H_2O_2 is not well understood. In patch-clamp and Ca^{2+}-imaging studies using TRPM2-expressing HEK293T cells, we have clarified a novel activation mechanism in which H_2O_2 lowers the temperature threshold for TRPM2 activation, termed "sensitization", through Met oxidation and ADPR production. This sensitization was completely abolished by a single mutation at Met 214, indicating that the temperature threshold of TRPM2 activation is regulated by redox signals that enable channel activity at physiological body temperatures. In addition, functional analyses using mouse peritoneal macrophage have shown that loss of TRPM2 attenuates zymosan-evoked macrophage functions, including cytokine release and fever-enhanced phagocytic activity. These results suggest that redox signals sensitize TRPM2 downstream of NADPH oxidase (Nox) activity and make TRPM2 active at physiological body temperature, leading to increases in cytosolic Ca^{2+} concentrations. Our results suggest that TRPM2 sensitization plays important roles in macrophage functions.

Reference

1. Makiko Kashio, Takaaki Sokabe, Kenji Shintaku, Takayuki Uematsu, Naomi Fukuta, Noritada Kobayashi, Yasuo Mori, Makoto Tominaga (2012).
2. Redox signal-mediated sensitization of Transient Receptor Potential Melastatin 2 (TRPM2) to temperature affects macrophage functions. *Proc Natl Acad Sci U S A*. Apr 24;109(17):6745-50.

Poster No. 23

Interaction between endogenous STIM2 and ORAI1 proteins in primary cortical neurons

Joanna Gruszczynska-Biegala[1], Jacek Kuznicki[1,2]

[1] International Institute of Molecular and Cell Biology, 4 Ks. Trojdena Street, 02-109 Warsaw, POLAND.
[2] Nencki Institute of Experimental Biology, 3 Pasteur Street, 02-093 Warsaw, POLAND.

Interaction of ER calcium sensors STIM1 and STIM2 with calcium channel-ORAI1 is crucial for store-operated calcium entry (SOCE) in non-excitable cells, but in neurons their localization and

dynamics are not clear. We showed earlier that in neurons STIM1 is involved in thapsigargin induced SOCE, while STIM2 is active after EGTA-driven depletion of extracellular Ca^{2+} (1, 2). To confirm that this is not due to the overexpression of exogenous proteins we used Proximity Ligation Assay to analyze activities of endogenous proteins. Cortical neurons were cultured in 2 mM $CaCl_2$, 2 mM EGTA or 2 μM thapsigargin, fixed and incubated with primary antibodies anti-STIM2 and anti-ORAI1. The pairs of appropriate secondary antibodies with conjugated oligonucleotides were then added and Duolink II was performed to create the fluorescent products. We detected *in situ* the endogenous STIM2/ORAI1 complexes in somata and quantified in single neurons the number of hetero- and homo-complexes. The amount of hetero-complexes increased up to 10-fold in response to calcium depletion by EGTA. The number of STIM2/ORAI1 endogenous complexes correlated well with the number of overexpressed YFP-STIM2/ORAI1 complexes formed under the same conditions (1). To eliminate possible PLA artifacts 4 pairs of different antibodies were used and all gave similar number and pattern of PLA signals. By co-immunoprecipitation we confirmed the *in situ* interaction between endogenous STIM2 and ORAI1 and that the interaction is increased after Ca^{2+} depletion in the medium. In conclusion, endogenous STIM2 interacts in neurons with ORAI1 in store-independent manner, but in response to reduced extracellular Ca^{2+} level.

References

1. Klejman* M, Gruszczynska-Biegala* J, Skibinska-Kijek A, Wisniewska MB, Misztal K, Blazejczyk M, Bojarski L, Kuznicki J. Expression of STIM1 in brain and puncta-like colocalization of STIM1 and ORAI1 upon depletion of Ca2+ store in neurons. Neurochem Int. 2009; 54:49-55.
   * first co-authors
2. Gruszczynska-Biegala J, Pomorski P, Wisniewska MB, Kuznicki J. Differential Roles for STIM1 and STIM2 in Store-Operated Calcium Entry in Rat Neurons. PLoS ONE 2011; 6:e19285.

Poster No. 24

Effects of Microwave Radiation on Mitochondrial Membrane Potential ($\Delta\Psi$m) Disruption

Meriç Arda Eşmekaya[a], Canseven Kurşun[a],Nesrin Seyhan[a]

[a] Department of Biophysics, Gazi University, Faculty of Medicine & Gazi Non-ionizing Radiation Protection (GNRP) Center, Ankara, TURKEY.

In this study, we researched whether or not 2.1 GHz Microwave Radiation has effect on Mitochondrial Membrane Potential ($\Delta\Psi$m). Mitochondria are involved in many processes; cell signaling, the control of the cell cycle, cellular growth, differentiation and cell death. They are also considered as the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (ATP). Depolarization of $\Delta\Psi$m may play a key role in the inititation or execution of most kinds of apoptosis. The breast epithelial cells were exposed to Microwave Radiation at 2.1 GHz for 4 in CO_2 incubator. Sham-control cells were placed in the in same incubator but, no Microwave Signal was applied. The $\Delta\Psi$m was analysed by flow cytometry and fluorescence microscopy using the 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC–1) probe which is a fluorescent cationic dye. Cells with high $\Delta\Psi$m was observed in FL–2 channel. In contrast, apoptotic cells with low $\Delta\Psi$m contained green JC–1 monomers and were detectable in FL–1 channel. Data were processed using CellQuest software (BD Biosciences). We observed significant reduction in $\Delta\Psi$m in Microwave Radiation exposed epithelial cells when compared to sham exposed cells. Our results showed that 2.1 GHz Microwave Radiation may decrease $\Delta\Psi$m. Disruption of the mitochondrial transmembrane potential is one of the earliest intracellular events that occur in the induction of apoptosis.

References

1. John J. Lemasters and Venkat K. Ramshesh 2007 Imaging of Mitochondrial Polarization and Depolarization with Cationic Fluorophores Methods in Cell Biology 80; 283–295.
2. Labajova A, Vojtiskova A, Krivakova P, Kofranek J, Drahota Z, Houstek J. 2006. Evaluation of mitochondrial membrane potential using a computerized device with a tetraphenylphosphonium-selective electrode. Anal Biochem. 353(1):37–42.
3. Kroemer G, Zamzani N, Susin SA. 1997. Mitochondrial control of apoptosis. Immunol Today 18(1):44–51.
4. Mignotte B., Vayssiere J.-L. Mitochondria and apoptosis. Eur J Biochem. 252,1–15 (1998).

Poster No. 25

Effects of Ginkgo Biloba (EGb 761) treatment on 2.1 GHz Microwave Radiation induced mutagenicity in human lymphocytes

Meric Arda Esmekaya[a], Ebru Aytekin[b], Elcin Ozgur[a], Göknur Güler Öztürk[a], Mehmet Ali Ergun[b], Nesrin Seyhan[a]

[a] Department of Biophysics, Gazi University, Faculty of Medicine & Gazi Non-ionizing Radiation Protection (GNRP) Center, Ankara, TURKEY.
[b] Department of Medical Genetics, Gazi University Faculty of Medicine, Ankara, TURKEY.

Several studies have showed that Microwave Radiation (MW) may induce mutagenic effects on

biological cells depending on frequency, magnitude of the radiation and cell type in literature. The objective of the present study was to investigate the effects of MW with and without antioxidant (Ginkgo Biloba, EGB–761) treatment on Sister Chromatid Exchange (SCE) in human peripheral blood lymphocytes (hPBLs). SCE test is a sensitive technique that is used for the investigation of the mutagenic potential of environmental factors such as radiation. The change in frequency of SCE is also one of the most sensitive markers of DNA damage in blood lymphocytes. The cell cultures with and without EGB–761 were exposed to 3G modulated MW at 2.1 GHz for 24 and 48 hours. No MW radiation was applied to control samples. For each subject, 50 cells at metaphase were scored to determine the individual mean SCE frequency. Statistical analyses were carried out using analysis of variance (ANOVA) and student's 't' tests. $p < 0.05$ was considered to be statistically significant. EGb 761 treatment decreased the SCE frequency in MW radiation + EGb 761 group with respect to MW radiation group alone. The results of our study showed that EGb 761 treatment may reduce MW induced mutagenic damage by decreasing SCE frequency in hPBLs. Further studies with different types of antioxidants should be performed to investigate the benetifical effects of antioxidants in MW induced mutagenicity.

References

1. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutat Res 463;2000:111–172.
2. Garaj-Vrhovac V, Horvat D, Koren Z. The relationship between colony-forming ability, chromosome aberrations and incidence of micronuclei in V79 Chinese hamster cells exposed to microwave radiation. Mutat Res 263;1991:143–9.
3. Markovà E, Hillert L, Malmgren L, Persson BR, Belyaev IY. Microwaves from GSM mobile telephones affect 53BP1 and gamma-H2AX foci in human lymphocytes from hypersensitive and healthy persons. Environ Health Perspect 113;2005:1172–7.
4. Tucker JD, Auletta A, Cimmino MC. Sister chromatid exchange: second report of the Gen-Tox program. Mutat Res 297;1993:101–80.

Poster No. 26

Glutathione efflux from human erythrocytes in response to arsenite and vanadate treatment: the role of MRP1 and methylation processes

Yeliz Çakır, Deniz Yıldız

Department of Biology, Arts and Science Faculty, Mustafa Kemal University, Hatay, TURKEY.

The main objective of the present study was to investigate if Arsenite and Vanadate exposure result in glutathione efflux from human erythrocytes. The effects of inorganic arsenic and vanadium compounds on glutathione level in erythrocytes have been investigated. However, glutathione efflux, in the reduced form from erythrocytes following exposure to inorganic arsenic and vanadium compounds have not been investigated. In the present study, we measured the changes in intracellular and extracellular glutathione levels in inorganic arsenic and vanadium compound exposed erythrocytes. Extracellular glutathione levels reached to 0.122 ± 0.0013, 0.226 ± 0.003, 0.274 ± 0.004 μmol/ml erythrocyte with 1, 5, and 10 mM of arsenite respectively. Similarly, extracellular glutathione levels reached to 0.0150 ± 0.0.001, 0.0330 ± 0.001, 0.0576 ± 0.002 μaamol/ml erythrocyte with 1, 5, and 10 mM of vanadate respectively. Dimercaptosuccinic acid treatment of supernatants from erythrocyte suspensions exposed to arsenite and vanadate significantly increased the glutathione levels measured in the extracellular media. Utilization of MK571 an MRP1 inhibitor decreased the rate of glutathione efflux from erythrocytes. Methylation inhibitor periodate-oxidized adenosine decreased the rate of glutathione efflux from erythrocytes. The decrease in extracellular GSH levels suggest that GSH release partly requires a proper cellular methylation process and that part of GSH detected in the extracellular media may arise from GSH-vandium and GSH-arsenic complexes. The results of the present study indicate that human erythrocytes efflux glutathione in reduced free form and in conjugated form/s that can be recovered with dimercaptosuccinic acid when exposed to Arsenite and Vanadate.

Poster No. 27

The Effect of Different Storage Temperature on Sperm Parameters and DNA Damage in Liquid Stored New Zealand Rabbit Spermatozoa

Serpil Sariözkan, Fazile Cantürk, Arzu Yay, Aytaç Akçay, Yusuf Caner

Department of Biophysics, Medical Faculty, Erciyes University, Kayseri, TURKEY.

The effect of two different temperatures (4°C and 15°C) on motility, plasma membrane integrity, acrosome abnormality and DNA damage of rabbit spermatozoa was evaluated at 0 and 24 h of liquid storage. Ejaculates collected from six New Zealand male rabbits by artificial vagina and pooled at 37°C following evaluation. Pooled ejaculate was divided into two equal aliquots and diluted with the Tris based semen extender at a final concentration of approximately $40x10^6$ sperms/ml in a Eppendorf

plastic tube. There were no significant differences in the percentage of above mentioned parameters between 4°C or 15°C at the beginning of liquid storage (0 h). The percentages of motility (75.0±1.83%) and plasma membrane functional integrity (71.2±1.14%) at 15°C was significantly better than that of liquid stored semen at 4°C (67.9±1.01% and 65.3±1.38%, P<0.05, respectively) at 24 h of storage. The percentage of acrosome abnormality at 24 h wasn't affected by the different storage temperature. The influence of storage temperature and the length of time on spermatozoa DNA damage was found statistically significant (P<0.001). The storage period for up to 24 h lead to an increase in the percentage of spermatozoa DNA damage (P<0.001). The percentages of DNA damage at 4°C was statistically higher than 15°C (P<0.001). In conclusion, 15°C may be prefered when liquid stored rabbit semen are used for 24 h.

References

1. Rosato MP, Laffaldano N.Effect of chilling temperature on the long-term survival of rabbit spermatozoa held either in a tris-based or a jellified extender. Reprod Dom Anim 2011; 46: 301-308.
2. Lopez-Gatius F, Sances G, Yaniz J, Santolaria P, Gutierrez R, Nunez M, Nunez J, Soler C. Effect of solid storage at 15 degrees C on the subsequent motility and fertility of rabbit semen. Theriogenology 2005:64;252-260.

Poster No. 28

The effect of sodium nitroprusside on low dose Penicillin induced epileptiform activity in female rats

Aliye Erguvan Arık[1], Süleyman Emre Kocacan[2], Cafer Marangoz[2]

[1] Samsun Teaching and Research Hospital, Samsun, TURKEY.
[2] Department of Physiology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, TURKEY.

In our study we aimed to investigate the effect of sodium nitroprusside (SNP), a Nitric oxide (NO) donor, in penicillin-induced experimental epilepsy. NO is a small gaseous, membrane-diffusible molecule that is widely distributed in the mammalian body, playing a role in a variety of tissues (1). NO is considered to play a role in the pathophysiology of epilepsy (2), although the results of experiments carried out by several authors are often discrepant (3). Undoubtedly further investigations are needed to determine precisely the role of NO in seizure activity. In this study adult female Wistar rats weighing 220±30 g were used. The left cerebral cortex was exposed by craniotomy under urethane-anesthesia. The epileptiform activity was induced by microinjection of 50 IU penicillin G potassium salt (20 IU/1 μl) into the left cerebral lateral ventricle. SNP was given at a dose of 2.5mg/kg intraperitoneally 10 minutes before penicillin G potassium salt injection. Epileptiform activity started within 1-3 min following the intracerebroventricular injection of 50 IU penicillin G (20 UI/1 μl). SNP (2.5mg/kg) significantly reduced the spike frequency between 10th and 30th minutes. Based on the current findings, we can conclude that NO system could have a role in the effects of SNP on penicillin-induced epileptiform activity.

References

1. Bredt D.S., Snyder S.H.: Nitric oxide as a neuronal messenger. Trends Pharmacol. Sci., 1991, 12, 125–128.
2. Yildirim M., Marangoz A.H., Ayyildiz M., Ankarali S., and Marangoz C. The interactions of nitric oxide and adenosine on penicillin-induced epileptiform activity in rats. Acta Neurobiol Exp 2011, 71: 208–219 .
3. Garthwaite J.: Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurosci., 1991, 14, 60–67.

Poster No. 29

Postnatal hippocampus culture protocol

Neşe Ayşit[1] and Gürkan Öztürk[1]

Department of Physiology, Faculty of Medicine, Medipol University, İstanbul, TURKEY.

The hippocampus is closely associated with the cerebral cortex, and in primates is located in the medial temporal lobe, underneath the cortical surface. It belongs to the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation. Since different neuronal cell types are neatly organized into layers in the hippocampus, it has frequently been used as a model system for studying neurophysiology. The form of neural plasticity known as long-term potentiation (LTP) was first discovered to occur in the hippocampus and has often been studied in this structure. The aim of this study standard hippocampus culture.

Hippocampus cultures were set up by dissociating postnatal mouse after incubation every petris were added B27, FCS, B27+FCS, B27+FGF2. The cells fixed after 24 hours were additionally stained with propidium iodide to determine dead /

live cells. At the 7th days of incubation, the axons grown by the neurons cells were fixed at the and immunohistochemically labelled with map-2 and GFAP and antibodies.

After incubation petri which was added B27 suplement the numbers of neurons rise and similar results was observed number of neuron and glia at tahe B27 +FCS petri.

With this study, the role of B27 in the lives of not only neurons, FCS, and FGF used in combination with the sponsor to the criticality of the effect observed in neurons and glia.

References

1. Martin, S. J., Grimwood, P. D. & Morris, R. G. Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci 23, 649-711 (2000).
2. Bliss, T. V., Collingridge, G. L. & Morris, R. G. Introduction. Long-term potentiation and structure of the issue. Philos Trans R Soc Lond B Biol Sci 358, 607-611 (2003).
3. Shepherd, G. M. The synaptic organization of the brain. 5th edn, (Oxford University Press, 2004).

Poster No. 30

The Effect of Tannic Acid on Liver Damage Mediated by Chronic Nitric Oxide Inhibiton

Cansu Özbayer[1], Didem Turgut Coşan[1], Faruk Saydam[1], Ahu Soyocak[1], Fulya Doğaner[1], İrfan Değirmenci[1], Hasan V. Güneş[1], Hülyam Kurt[1], M. Cengiz Üstüner[1], Cengiz Bal[2]

[1]Department of Medical Biology, [2]Department of Biostatistics and Medical Informatics, Medical Faculty, Eskişehir Osmangazi University, Eskişehir, TURKEY.

Acute and chronic inhibition of nitric oxide causes endothelial dysfunction and vasoconstriction and thus tissue and organ damage could occur (1, 2, 3). Impaired function of endothelial cells induced the releasing of free oxygen radicals and lead to oxidative stress (4). Tannic acid which is a naturally occurring plant polyphenol has the antioxidant effect (5). The aim of our study is to investigate the antioxidant effects of tannic acid on nitric oxide inhibition-mediated liver injury. In this study, we used female Sprague-Dawley rats weighing 250-300gr. As a nitric oxide inhibitor, L-NNA, was added to the drinking water (0.5 g / l) for 15 days. 50 mg / kg of tannic acid was administered intraperitoneally for 15 days (a three-day dose). At the end of the experiment samples were collected under ether anesthesia. The activities of superoxide dismutase (SOD), catalase (CAT) and the concentration of malondialdehyde (MDA) was determined in homogenized tissues of the liver. The SOD activity of L-NNA + tannic acid group decreased when compared to L-NNA control group. When compared the MDA levels, there were no differences between control group and L-NNA + tannic acid group. There were no statistical differences in catalase levels between control and other groups. In our study, there was no important effect of nitric oxide inhibition on lipid peroxidation and antioxidant enzymes of liver. It's known that tannic acid has antioxidant effect but it has been considered that this effect depends on the dose.

References

1. Riado SR, Zanesco A, Barker LA, De Luca IM, Antunes E, De Nucci G. 1999. Long-term nitric oxide inhibition and chronotropic responses in rat isolated right artia. Hypertension 34: 802-807.
2. Cleland JG. 1999. Progression from hypertension to heart failure, mechanisms and management. Cardiology 92: 10-9.
3. Fadl YY, Zareba W, Moss AJ, Marder VJ, Sparks CS, Miller Watelet LF, Carroll ER. 2003. History of hypertension and enhanced thrombogenic activity in postinfarction patients. Hypertension 41: 943-98.
4. Yılmaz E, Sarı A, Oktar S, Aksul HE. 2008. Effect of acute and chronic N (omega)-nitro-L-arginine administration on plasma nitrite/nitrate and malondialdehyde levels in rats. Fırat Tıp Dergisi 13(2):88-91
5. Gülçin İ, Huyut Z, Elmastaş M, Aboul-Enein HY. 2010 Radical scavenging and antioxidant activity of tannic acid. Arabian Journal of Chemistry 3(1):43–53

Poster No. 31

Oxidative stress biomarkers during type II diabetes complicated with cardiovascular diseases in Algerian patients

Mokhtari-Soulimane Nassima*, Merzouk Hafida*, Soulimane Fayçal**, Merzouk Sid Ahmed*, Narce Michel***

* PPABIONUT laboratory, Faculty of sciences, University of Tlemcen, ALGERIA.
**Cardiology department, University Hospital Center of Tlemcen, ALGERIA.
**UMR 866, Lipids Nutrition Cancer, Faculty of sciences Gabriel, university of Burgundy, Dijon, FRANCE.

Diabetes mellitus is associated with increased risk for atherosclerotic cardiovascular disease (CVD). Despite several treatments to reduce low-density lipoprotein cholesterol (LDL-CT) and triglycerides (TG) inducing atherogenic dyslipidemia, a substantial number of type 2 diabetes mellitus (DM) patients still experience progression of cardiovascular risk.

Oxidative stress plays a key role in the pathogenesis of atherosclerosis and CVD, which is promoted by the production of reactive oxygen species (ROS) and impaired antioxidant enzymes.

This study proposes to evaluate biomarkers of oxidative stress in normal control and type 2 DM patients complicated with CVD in Algeria.

This study involved 96 patients (mean age 52.2 ± 8.1 years). The control group consisted of 33 healthy subjects (mean age 50.23 ± 7.3 years). Redox markers were determined as plasma levels of malondialdehyde (MDA), protein carbonyl (PC), oxidized low-density lipoprotein (Ox-LDL), Oxygen Radical Absorbance Capacity (ORAC) and enzymes activities; superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase.

Our data showed that plasma MDA, PC and OX-LDL levels were significantly higher in diabetic patients (P<0,001) compared to controls, while ORAC ratio was decreased. Regarding antioxidant enzymes, GPX and SOD activities were significantly decreased (P<0,001 and P<0,01 respectively). Catalase activity remained unchanged.

In conclusion, theses results demonstrated that Type 2 DM associated to cardiovascular diseases leads to an important oxidant/antioxidant imbalance. These abnormalities may be contributing factors in the pathogenesis of CVD and particularly in the generation of the atheroma plaque from the oxidized LDL. Oxidative stress appears to play a central role in the development and progression of CVD and its complications.

Poster No. 32

Association between TRPM1 gene polymorphisms and colorectal cancer

Celaletdin Camcı[1], Esma Özkara[2], Serdar Öztuzcu[2], Abdullah T. Demiryürek[3]

Departments of [1]Medical Oncology, [2]Medical Biology [3]Medical Pharmacology, Faculty of Medicine, University of Gaziantep, Gaziantep, TURKEY.

TRPM1 was originally characterized as the product of a gene whose expression is decreased in melanomas, and is useful as a prognostic marker for metastasis. The aim of this study was to investigate a possible association between TRPM1 gene polymorphisms and CRC development. A total of 84 patients operated due to CRC and 176 healthy controls with similar age and sex were included to this study. Polymorphisms were analyzed in genomic DNA using a BioMark 96.96 dynamic array system. There were significant associations between TRPM1 gene rs11070811 and rs2241493 (Ser32Asn) polymorphisms with CRC development. CT genotype and T allele frequencies were markedly high in patients with rs11070811 polymorphism. In rs2241493 (Ser32Asn) polymorphism, CC genotype and C allele frequencies were significantly high in CRC patients. However, no marked association was found between TRPM1 gene rs28441327 polymorphism and the risk of developing CRC. These results are the first to demonstrate the contribution of TRPM1 channel in CRC development. Our data showed that the TRPM1 channel gene might be a risk factor for CRC, and suggested that genetic polymorphisms in the these genes modify individual susceptibility to CRC in the Turkish population.

Poster No. 33

Investigation of glycoconjugates located in the cell surface and extracellulary matrix of gingiva cells in the rats experimentally diabetes caused

Sabri Fatih Kurşunlu, Ayşegül Bildik[1], Kamil Seyrek[2]

[1] Department of Biochemistry, Veterinary Medicine Faculty, Adnan Menderes University, Aydın, TURKEY.
[2] Department of Biochemistry, Medicine Faculty, Balıkesir University, Balıkesir, TURKEY.

Diabetes mellitus is a chronic disease characterized by chronic hyperglycemia. In this study, we aimed to assess the alternations of galactose, lactose, fructose, mannose, N- acetyl galactoseamine, N- acetyl glucoseamine and sialicacid which are located in the cell surface and extracellulary matrix of gingiva cells belong to the rats experimentally diabetes caused.

In this study, constituted a control group of 10 rats, 10 rats the experimental group. To create diabetes in rats, 0.01 M sodium citrate buffer in a single dose of streptozotocin 50mg/kg was injected intraperitoneally. Similar to the control group animals, the experimental group was administered intraperitoneally at the same rate of 0.01 M sodium citrate buffer. Kriostat-thin sections were obtained 6 microM. Tissues, glucose (ECL, ECA, WGA), galactose (EEL, GSL I, BSL I), lactose (RCA I, RCA120), Fukoz (PSA), mannose (GNL), sialic acid-specific (MAA, SNA, EBL) with the lectins were incubated with biotinylated, lectins, joints DAB (3′-3′-diaminobenzidine) was visualized by color substrate. Evaluation of reactions carried out under the light microscope.

Research at the end of the muscle tissue of the gums in the glucose, lactose, and mannose units with fukoz lectins of specific binding in the control group, more severe than in the experiment; sialic acid units to connect the trial group were more intense. The mechanism by which these changes in gum tissue of rats with diabetes to occur, and how the impact on the pathogenesis of gum disease that is thought to be necessary to establish the new work is done.

Poster No. 34

Resveratrol mitigates the effect of oxidative stress on membrane fluidity and maintains Ca^{2+} homeostasis in human red blood cells

Kanti Bhooshan Pandey, Syed Ibrahim Rizvi

Department of Biochemistry, University of Allahabad, Allahabad-211002, U.P- INDIA.

The precise regulation of intracellular calcium homeostasis is crucial in all accepts of cell function. Plasmalemmal Ca^{2+}-ATPase is the fine and dominant tuner of cytosolic Ca^{2+} concentration[1]. Oxidative stress changes the ion balance that triggers many deleterious events including altered Ca^{2+} metabolism, proteolysis and changed membrane fluidity[2]. The study was conducted to evaluate the effect of resveratrol (3, 5, 4′-trihydroxystilbene) on oxidative stress-induced alterations on membrane deformability and Ca^{2+} homeostasis in red blood cells (RBCs).

Ability of resveratrol to penetrate the RBCs followed by its dose and time dependent effects on protein carbonyls (PCO), lipid peroxodation (LPO), sulphydryl groups (-SH) and calmodulin dependent Ca^{2+}-ATPase were measured in t-BHP induced oxidative stressed RBCs membrane. Furthermore, osmotic fragility and Ca^{2+} homeostasis was also estimated in RBCs.

Significant uptake of resveratrol (81%) within 10 min of treatment by RBCs and protection of ghost ingredients; proteins, lipids and –SH as evidenced by decreased contents of PCO and MDA, reflects ability of resveratrol to mitigate the adverse effects of oxidative stress. Over expression of plasmalemmal Ca^{2+}-ATPase and restoration of Ca^{2+} homeostasis after loading of resveratrol at 0.1 to 100 μM, provide evidence towards its strong antioxidant potential.

References

1. Moreau B, Straube S, Fisher RJ, Putney JW Jr, Parekh AB. Ca^{2+}-calmodulin-dependent facilitation and Ca^{2+} inactivation of Ca^{2+} release-activated Ca^{2+} channels. J Biol Chem. 2005; 280:8776-83.
2. Pandey KB, Rizvi SI. Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxid Med Cell Longev. 2010;3:2-12.

Poster No. 35

Hypo-osmolarity regulates Ca^{2+}-mediated RANKL expression via TRPM3 and TRPV4 in primary cultured mouse osteoblastic cells

Aran Son, Yu-Mi Yang, Syng-Ill Lee, Dong Min Shin

Department of Oral Biology, Yonsei University College of Dentistry, Seoul, 120-752, KOREA.

Mechanical stimulation, such as shear stress, membrane stretch, or hypotonic swelling, changes the shape of osteoblasts, and subsequently activates related intracellular signaling that regulates bone remodeling. However, the mechanism underlying the hypo-osmotic stress-induced cellular response in osteoblasts remains poorly understood. In this study, we investigated hypotonic stress-induced receptor activator of nuclear factor-kappa B ligand (RANKL) expression and determined which transient receptor potential (TRP) channels are affected by hypotonic stress in mouse primary cultured osteoblasts using reverse transcriptase-polymerase chain reaction, fluorometric Ca^{2+} imaging, and electrophysiology. Hypotonic stress increased RANKL mRNA expression and the intracellular calcium concentration ($[Ca^{2+}]_i$). Furthermore, thapsigarsin, an inhibitor of sarco/endoplasmic reticulum Ca^{2+} -ATPase , induced increases in RANKL expression. Treatment with gadolinium and lanthanum, non-specific plasma membrane Ca^{2+} channel blockers, completely inhibited these hypotonic stress-induced effects. In contrast, ruthenium red and 2-aminoethoxydiphenyl borate, inhibitors of TRPV4 and TRPM3, partially inhibited the hypotonic stress-induced $[Ca^{2+}]_i$ increase. TRPM3 and TRPV4 activity was confirmed using the whole-cell patch-clamp recordings. Moreover, knockdown of TRPM3 or TRPV4 with siRNA abrogated the hypotonic stress-induced $[Ca^{2+}]_i$ increase. These results suggest that hypotonic stress induces increases in $[Ca^{2+}]_i$ through TRPM3 and TRPV4 to regulate RANKL expression in primary cultured mouse osteoblasts.

References

1. Venkatachalam K, Montell C. TRP channels. Annu Rev Biochem 2007;76:387-417.
2. Weskamp M, Seidl W, Grissmer S. Characterization of the increase in $[Ca^{2+}]$i during hypotonic shock and the involvement of Ca^{2+}-activated K+ channels in the regulatory volume decrease in human osteoblast-like cells. J Membr Biol 2000;178:11-20.

Poster No. 36

Hypericum Perforatum (St. John's wort) modulate apoptosis and Ca^{2+} release values through TRPM2 and voltage gated Ca^{2+} channels in neutrophil of patients with multiple sclerosis

Mustafa Nazıroğlu[1], Süleyman Kutluhan[2], A Cihangir Uğuz[1], Mehmet Aykur[1], İ. Suat Örey[1], Vedat A. Yürekli[2]

[1] Department of Biophysics, Medical Faculty, Süleyman Demirel Universty, Isparta, TURKEY.
[2] Department of Neurology, Medical Faculty, Süleyman Demirel Universty, Isparta, TURKEY.

Ca^{2+} activates human neutrophils and it induces oxidative stress. Oxidative stress and apoptosis occur in various neurological disorders including multiple sclerosis (MS). *Hypericum perforatum* (HP) (St. John's wort) is one of the popular nutraceuticals for treating oxidative stress and inflammation. We investigated effects of *HP* on oxidative stress, apoptosis and cytosolic free Ca^{2+} $[Ca^{2+}]i$ release in neutrophil of patients with MS.

There were two groups i.e. control and patients with MS with 6 subjects in each. Blood samples were taken from both control and patient groups. Neutrophils were isolated from peripheral whole blood of healthy volunteers and patients with MS. The neutrophils were incubated by HP with/without TRPM2 channel blocker (2-APB) and voltage gated Ca^{2+} channels blocker (verapamil and diltiazem). Ca^{2+} signaling analysis were performed in a spectroflurometer by using Fura-2 although apoptosis values were measured in the spectroflurometer by commercial kit.

Apoptosis levels were higher in patients group than in control. The $[Ca^{2+}]i$ release in neutrophil were lower in HP+patients than in patients group. Oxidative stress and antioxidant values were also modulated in neutrophil of patients with MS by *HP*.

In conclusion, multiple sclerosis (MS) patients were found to have elevated Ca^{2+} influx and apoptosis in neutrophil which suggested disease activating free radical productions. However, Hypericum Perforatum modulated the values through TRPM2 and voltage gated Ca^{2+} channels in the neutrophils.

Poster No. 37

Usnic acid: an oxidant or antioxidant molecule?

Valéria A. Fernandes[1], Thássio R. Mesquita[1], Aline Lara[2], Luciano Capettini[2], José Evaldo R. Meneses-Filho[1], Michel Santana[1], André Talvani[2], Adriano Antunes[1], Silvia Guatimosim[2], Jader Cruz[2], Sandra Lauton-Santos[1]

[1] Department of Physiology, University of Sergipe, São Cristóvão, BRAZIL.

[2] Institute of Biological Science, University of Minas Gerais, Belo Horizonte, BRAZIL.

Usnic acid (UA) is a lichen secondary metabolite with potential therapeutic applications (1). UA-enriched extracts was recently characterized with redox activity in human neurons (2). The aim of this study was to evaluate a possible UA anti-oxidant role on heart mice.

Fifteen-week-old male mice were treated with UA (50mg/kg) by oral administration during 5 days. After, mice were anesthetized with sodium pentobarbital (100 mg/kg i.p.) and the heart quickly removed. Heart was homogenized in Buffer Phosphate Solution and catalase activity was measured in the supernatants and Lipid peroxidation products were quantified by thiobarbituric acid-reactive substances (3). NADPH and catalase expression was evaluated by western blot (3). For $O2^-$ and H_2O_2 measurements, ventricular cardiac cells were enzymatically isolated and loaded with 10 μM oxidant-sensitive fluorogenic probe dihydroethidium (DHE) and 0.1 μM 2,7-dichlorodihydrofluorescein-diacetate [H2DCFH-DA] (DCF), 30 min at 37°C. After, cardiomyocytes were incubated with UA (10nM), 15 min, at room temperature. Cells were analyzed in a LSM 510 META confocal system (Zeiss, Jena, Germany) with a ×63 oil immersion objective. ImageJ software was used for image analyses.

We found that UA treated mice exhibited a lower catalase activity than the control group. This decrease in activity could be related to the H_2O_2 depletion observed on isolated myocytes. The catalase expression was not altered. $O2^-$ amount also decreased and it could be associated with expression impairment of NADPH oxidase. UA didn't induce lipid peroxidation. In conclusion, these results suggest UA anti-oxidant role in heart mice, both *in vivo* treatment and *in vitro* exposure.

References

1. Cocchietto, M. Skert, N. Nimis, P and Sava, G. A review on usnic acid, an interesting natural compound. Naturwissenschaften. Vol. 89, no. 4: 137-146, 2002.
2. Rabelo, KT et, Al. Redox characterization of usnic acid and its cytotoxic effect on human neuron-like cells (SH-SY5Y). Toxicology in Vitro. Vol. 26, no. 2: 304-314, 2012.
3. Gioda, CR et. Al. Cardiac oxidative stress is involved in heart failure induced by thiamine deprivation in rats. American Journal of Physiology. Vol. 298 no. 6: 2039-2045, 2010.

Poster No. 38

TRPC6 is involved in lysophosphatidylcholine-induced increase in intracellular calcium concentration in human neutrophils

Taek-Keun Kim, Ashraful Hasan, Jun-Sub Jung and Dong-Keun Song

Department of Pharmacology, Institute of Natural Medicine, IDMRC, College of Medicine, Hallym University, Chunchon, S. KOREA.

Lysophosphatidylcholine (LPC), a major component of oxidized low-density lipoprotein, affects diverse biological functions in neutrophils,

including [Ca^{2+}]i. We recently reported that LPC induces increase in [Ca^{2+}]i via TRPM2 channel in human neutrophils (Hong et al. 2010). However, the mechanism of LPC-induced [Ca^{2+}]i changes in human neutrophils is not fully understood. TRPC6 is one of major TRP channels expressed in human neutrophils (Heiner et al. 2003). We observed that LPC-induced calcium increase has two peaks. Thus, we hypothesized that in addition to TRPM2, TRPC6 might be also involved in LPC-induced increase in [Ca^{2+}]i in human neutrophils. TRPC6 channel inhibitors (2-ABP 100 μM, gadolinium 100 μM and SKF 96365 10 μM) and TRPC6 siRNA blocked the first peak of LPC-induced calcium increase in neutrophils, whereas the second peak was not affected. LPC induced a rapid translocation of TRPC6. ML-7, an inhibitor of myosin light chain kinase, blocked the LPC-induced TRPC6-mediated calcium increase. These findings suggest that TRPC6 is involved in the first phase of LPC-induced increase of intracellular calcium in human neutrophils.

Poster No. 39

Effects of duloxetine on antioxidant system in rat brain tissues

Kadir Karakuş[1], Efkan Uz[2], Ramazan Özcankaya[3], H. Ramazan Yılmaz[4], Ayşe Yiğit[5], Kadir Demirci[6]

[1] Department of Psychiatry, Isparta State Hospital, Isparta, TURKEY.
[2] Department of Biochemistry, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.
[3] Department of Psychiatry, Medical Faculty, Suleyman Demirel University, Isparta, Turkey.
[4] Department of Medical Biology, Medical Faculty, Mevlana University, Konya, TURKEY.
[5] Department of Medical Biology, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.
[6] Department of Psychiatry, Tokat State Hospital, Tokat, TURKEY.

Antidepressant drugs are widely prescribed to treat the depression. Since the relationship between oxidative stress and depression has been shown, few studies have investigated the effect of these drugs on oxidant-antioxidant system and lipid peroxidation.

The aim of this study is to determine the effects of duloxetine, a new antidepressant drug that is an inhibitor of both serotonin and norepinephrine transporters, on the activities of the enzyme superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), and on malondialdehyde (MDA) levels in rat brain tissues.

Twenty male Sprague-Dawley rats were taken for the study and rats were divided into two equal groups. The first group was control group (n=10) and the second group was duloxetine group (n=10). Duloxetine was administered with a dose of 10 mg/kg intragastrically once daily, for 14 days in duloxetine group. Water was administered once daily intragastrically, for 14 days in control group. All rats were sacrificed at the end of the 14th day. Brain tissues were taken. Analyses were performed in rat brain tissues.

In this study our results showed that duloxetine increased the activity of SOD and decreased the activities of CAT significantly, increased the activity of GSH-Px and decreased MDA levels insignificantly compared to the control group.

In conclusion, the data obtained in this study thought that, duloxetine increased the resistance of brain to radicals which is sensitive to them via increasing the activity of antioxidant enzyme SOD, so this showed that duloxetine has an important role for the treatment of depression. Lack of a significant change in the level of lipid peroxidation product MDA in the rat brain suggests that duloxetine has a protective effect to brain against stress.

Poster No. 40

Designing of Nano Biosensor For Staphylococcus aureus Exotoxin Detection

[a]H. Ahari, [b]V. Razavilar, [d]B. Akbari, [b]AA. Motallebi, [b]A. Anvar, SH. Kakoolaki[c], Sima Moradi

[a] Member of Faculty, Food Science and Technology Science and Research Branch, Islamic Azad University, Tehran, IRAN.
[b] Faculty of Veterinary Medicine, Islamic Azad University, Food Hygiene Sciences, Tehran, I.R. Iran
[c] Iranian Fisheries Research Organization, No. 297, West Fatemi Ave., P.O. Box 14155–6116.
[d] Food & Drug Laboratories Research Center, Ministry of Health and Medical Education, P.O. Box 11136-15911, Tehran, IRAN.

Regarding to the growth of increasing the population and industrialize the evolution of human being, we are not be able to distinguish the toxin which has been gererated in the food productions with traditional ways.

Quarantine the food productions are not economical and even in many ways these operational ways by culture the bacteria could be mistaken by operator or compound consumption.

Therefore with improvment to the Nano Technologie and design the selective and intelligent sensors, there has been a great revolution in the food productions which could save the time and recongnize the germs and toxin in the bacteria.

In this research by using the Anti-body which connected to the Nano Particle has been pointed to recognize the toxin in Estafilococos bacteria as a Agzo Toxin in the food products.

Parchlorophnyl, Potassium Tetroxide, Nitrobanzene, Dibuthyl Phenulate, Benzyl Asetat, Nitrobenzene, PVC with high molecular weight and which have all been provided from the march company and used to make up the microsensor's membrane. For a better responsiveness, the electrode's body was made of graphite enclosed in the form of a micro wide pack. In all of the project's stages, ironless 2 time distilled water was used and for adjusting the PH, dissolved solutions of nitric acid and sodium hydroxide were applied. To make the microelectrode, we used a thin micro graphitic wire completely isolated at a soft glacial cap ill. Arica tube. Then the capillary containing the graphitic bar was vertically cut at the length and a very small cutting surface of the graphite was available to make up the membrane's cover. The electrical contact of the electrode in the outer circuit got prepared by the silver epoxy. Before doing the experiment, the electrode's surface was abraded and polished for 1 minute by a soft and.(Patange, S 2005).

After that, the electrode was exposed to ultrasonic waves in distilled water and finally dried on contact with the lab's air.

To prepare the membrane, we mixed a certain weight of the PVC Powder, 1 gr of ionophore 2 grs of KTPCIB and some NB plasty sizer and then solved the mixture in 10 ccs of THF inside a glacial beaker having a 2 cm Cutting surface. After wards, the solution was let vaporize the solvent to make up a thick and homogeneous oil solution. To speed up the solvent's evaporation, it should be mildly heated in a way that it doesn't reach the boiling temperature. (Norouzi, P 2007)

To make the thin polymer membrane on the electrode's surface, we use the momentous Deep method of the electrode's tip in the prepared thick solution, that is; the electrode prepared in the previous stage shall dip inside the solution so that the polymer membrane be made on its surface. The made membrane would be dried on contact with outer air within 24 hours. It will then be put in the 1×10^{-3} molar solution of staff Oreos A toxin so that the preparation of the electrode get done. To measure the upcoming potential from the sensor, we used the analysis pack at 250 mv and the PH- meter at the temperatures 25°C ± 1°C.(Norouzi, P 2006).

1 gr of nanosiver is taken and put on the. Antibody is then added to the nanoparticle drop by drop. After half an hour, they react together.

By the use of this device, we can detect the presence or absence of toxin inside distilled water. It is registered using distinct emitted wavelengths. At the end, by adding different saturations of toxin to the reacting distilled water, the signal changes would be registered and evaluated in the next step.

Reference

1. Norouzi, P., Ganjali, M. R., Labbafi, S., & Mohammadi, A. (2007). Subsecond FFTadsorptive,voltammetric technique as a novel method for subnano level monitoring of piroxicam in its tablets and bulk form at Au microelectrode in flowing solutions. Analytical Letters, 40, 747–762.
2. Norouzi, P., Ganjali, M. R., & Matloobi, P. (2005). Sub-second adsorption for subnanomolar,monitoring of metoclopramide by fast stripping continuous cyclic voltammetry. Electrochemistry Communications, 7, 333–338.
3. Norouzi, P., Ganjali, M. R., Shirvani-Arani, S., & Mohammadi, A. (2006). Novel method for the fast determination of ultra trace amount of nortriptyline in its pharmaceutical formulations by fast Fourier transform continuous cyclic voltammetric technique at Au microelectrode in flowing solutions. Journal of Pharmaceutical Sciences, 96, 893–904.
4. Norouzi, P., Nabi Bidhendi, G. R., Ganjali, M. R., Sepehri, A., & Ghorbani, M. (2005). Sub-second accumulation and stripping for pico-level monitoring of amikacin sulphate by fast Fourier transform cyclic voltammetry at a gold microelectrode in flow-injection systems. Microchimica Acta, 152, 123–129.
5. Patange, S. B., Mukundan, M. K., & Ashok Kumar, K. (2005). A simple and rapid method for colorimetric determination of histamine in fish flesh. Food Control, 16, 465–472.
6. Pihel, K., Hsieh, S., Jorgenson, J. W., & Wighman, R. M. (1995). Electrochemical detection of histamine and 5-hydroxytryptamine at isolated mast-cells. Analytical Chemistry, 67.

Poster No. 41

Effects of duloxetine on purine catabolism and nitric oxide levels in rat brain tissues

Kadir Karakuş[1], Efkan Uz[2], Ramazan Özcankaya[3], H. Ramazan Yılmaz[4], Ayşe Yiğit[5], Kadir Demirci[6]

[1] Department of Psychiatry, Isparta State Hospital, Isparta, TURKEY.
[2] Department of Biochemistry, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.
[3] Department of Psychiatry, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.
[4] Department of Medical Biology, Medical Faculty, Mevlana University, Konya, TURKEY.
[5] Department of Medical Biology, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.
[6] Department of Psychiatry, Tokat State Hospital, Tokat, TURKEY.

Antidepressant drugs are used in many psychiatric disorders, especially depression. Duloxetine is an antidepressant drug which inhibits both serotonin and norepinephrine reuptake. Major depression is associated with increased oxidative stress and lipid peroxidation.

The aim of this study is investigate the effects of duloxetine to purine catabolism enzyme activities

adenosine deaminase (ADA) and xanthine oxidase (XO) and nitric oxide (NO) levels in rat brain.

In this study twenty male Sprague-Dawley rats were taken and rats were divided into two equal groups. The first group was control group (n=10) and the second group was duloxetine group (n=10). Duloxetine was administered with a dose of 10 mg/kg intragastrically once daily, for 14 days in duloxetine group. Water was administered once daily intragastrically, for 14 days in control group. All rats were sacrificed at the end of the 14th day. Brain tissues were taken. Analyses were performed in rat brain tissues.

Duloxetine group compared with the control group; in the duloxetine group XO and ADA activities decreased significantly and NO levels decreased insignificantly.

As a result, decrease in the activity of XO and ADA shows that the purine catabolism decreased and radical formation is decreased. These decreases of XO and ADA enzym activities shows that duloxetine has a protective effect to brain against stress.

Poster No. 42

NCS-1 and Calcyon regulate Inositol 1,4,5-trisphosphate receptor Calcium release activity in PC-12 cells

Marc-Olivier Frégeau, Maxime Carrier and Gaétan Guillemette

Department of Pharmacology, Faculty of Medicine and Health Sciences, University of Sherbrooke, Sherbrooke, CANADA.

Intracellular Ca^{2+} levels are strictly regulated in the neuronal system. Loss of Ca^{2+} homeostasis is related to many neurological and neuropsychiatric disorders such as Parkinson, Alzheimer and Schizophrenia. In Schizophrenia, Calcyon and NCS-1 (Neuronal Calcium Sensor-1) are two upregulated Ca^{2+}-dependent proteins which are expected to play a central role in neuronal intracellular Ca^{2+} dysregulation. Therefore, our hypothesis is that Calycon and NCS-1 participate in the fine regulation of Inositol 1,4,5-trisphosphate receptors (IP_3R) Ca^{2+} release activity in PC-12 neuronal cells. We observed, by a co-immunoprecipitation approach, that both proteins interact with IP_3R-3 suggesting a modulating role on the IP_3R. With a spectrofluorimetric approach, we observed that siRNA targeting Calcyon diminished ATP-mediated IP_3R Ca^{2+} release by 23.05% and impaired subsequent Ca^{2+} oscillations. Calcyon siRNA also diminished ATP-mediated IP_3R Ca^{2+} release by 19.59% and impaired subsequent Ca^{2+} oscillations in free Ca^{2+} HBSS. At the opposit, siRNA targeting NCS-1 potentiated ATP-mediated IP_3R Ca^{2+} release by 79.33% and favorised Ca^{2+} oscillations. NCS-1 siRNA also potentiated ATP-mediated IP_3R Ca^{2+} release by 66.82% and favorised Ca^{2+} oscillations in free Ca^{2+} HBSS. In conclusion, these data suggest that in PC-12 cells, Calcyon acts as a potentiator of IP_3R Ca^{2+} release activity while NCS-1 acts as a moderator of IP_3R Ca^{2+} release. Taken together, these results suggest that in Schizophrenia, Calcyon upregulation is responsible for intracellular Ca^{2+} dysregulation that is counterbalanced by NCS-1 upregulation which moderates Ca^{2+} elevation.

Poster No. 43

Oxidative damage induced by aluminum and indium in the testicles

Maghraoui Samira[1], Ayadi Ahlem[1], Clichici Simona[2], Login Cezar[2], Moldovan Remus[2], Daicoviciu Doina[2], Decea Nicoleta[2], Mureşan Adriana[2], Tekaya Leila[1]

[1] Laboratory of Physiology. Faculty of Medicine of Tunis (University of Tunis El Manar). 15, Djebel Lakhdar Road, La Rabta 1007. Tunis, TUNISIA.

[2] Department of Physiology. University of Medicine and Pharmacy "Iuliu Hatieganu" of Cluj-Napoca. Clinicilor Street, Nb 01. 400006. Cluj-Napoca. ROMANIA.

Aluminum *(Al)* and indium *(In)* have embryotoxic, neurotoxic and genotoxic effects; oxidative stress is being one of the possible mechanisms involved in their cytotoxicity. We recently demonstrated that the intraperitoneal administration of indium determined histological disorganization of the testicular tissue. Our present work aimed to further investigate the systemic and testicular effects of the two III-A group elements, *Al* and *In,* on the oxidative stress equilibrium. Studies were performed on 24 Wistar rats, divided into 3 groups, intraperitoneally injected with *Al* or In soluble solution, for two weeks. Controls received physiological saline solution in identical conditions. Our results showed that In decreased significantly the testicles absolute weight. Measurements of lactate dehydrogenase (LDH) and paraoxonase (PON) activities showed that In induced significant augmentation in the first enzyme but no changes were observed in the second one. In the meantime, both *Al* and In determined oxidative stress in testicles by increase of malondialdehyde (MDA) and protein carbonyls (PC) production. At the same time, the antioxidant capacity, measured by thiol groups (-SH) and glutathione (GSH) in the testicles was enhanced. Finally, in the blood, while MDA was

not changed, GSH was significantly decreased in *Al* and *In* treated rats. Our results indicated that *Al* and *In* caused oxidative stress both in blood and testicles after their intraperitoneal administration but *In* had cytotoxic effect as well as negative impact on testicle's weight. These findings could explain the histological alterations found in testicles previously described after the intraperitoneal administration of these elements.

Poster No. 44

Multiple ligand binding site within intracellular C-terminus TRPV1

Lenka Grycova, Blanka Holakovska and Jan Teisinger

Institute of Physiology, Academy of Sciences of the CZECH REPUBLIC.

TRPV1 is a nonselective cation channel. However exact mechanism of TRPV1 regulation is still unclear. It has been suggested that its activity is regulated by intracellular ligands like PIP2 or calcium binding protein calmodulin (CaM). To analyze the PIP2/TRP interactions in detail, we used biochemical, biophysical approaches in combination with molecular modeling, focusing on TRPV1 – C terminus (CT).

Fusion protein of TRPV1- CT was expressed in bacteria E. coli, purified according to the two steps purification protocol using affinity chromatography and FPLC gel chromatography. Alanine substitution mutagenesis screening revealed the crucial amino acids for these interactions and the equilibrium dissociation constants were estimated using fluorescence anisotropy measurement. The surface plasmon resonance measurement with C-terminus of TRPV1 fusion protein and its mutants was employed as well.

We identified the PIP2-binding site and found mutations that decreased the affinity of the PIP2/TRPV1 interaction. Although this region overlaps with the previously described calmodulin binding site, we found that this interaction did not disrupt association of calmodulin with TRPV1 and different positively charged amino acids are employed. Moreover here we introduce S100A1 protein as another novel binding partner of TRPV1 receptor. Although there are no know physiological consequences this calcium binding protein associates with the same region of TRPV1 receptor.

This project was supported by grants GACR 301/10/1159, GACR P205/10/P308 and Research project No. AV0Z50110509.

Poster No. 45

Ca^{2+} binding proteins interact with two independent binding sites on TRPM3 N-terminus

Blanka Holakovska, Lenka Grycova and Jan Teisinger

Department of Protein Structures, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, Prague, 142 20, CZECH REPUBLIC.

Transient receptor potential melastatin 3 ion channel (TRPM3) belongs to the TRP family of cation channels involved in many important biological processes such as pain, thermo and mechanosensation. The channel was reported to play an important role in Ca^{2+} homeostasis but its gating mechanisms, functions and regulation are still elusive.

Two recombinant fusion proteins of TRPM3 N-terminus were expressed in *E.coli* and purified by affinity chromatography and gel filtration. Basic residues within these proteins were exchanged for alanine by site directed mutagenesis.

Using biophysical and biochemical methods we revealed two independent binding domains, A35-K124 and H291-G382 on the TRPM3 N-terminus, responsible for interactions with the Ca^{2+} binding proteins calmodulin (CaM) and S100A1. We identified several positively charged residues within these domains that have a crucial impact on CaM/S100A1 binding. The data also suggest that the interaction is calcium-dependent. We also performed competition assays, which suggested that CaM and S100A1 are able to compete for the same binding sites within the TRPM3 N-terminus. This is the first time that such an interaction has been shown for TRP family members.

This project was supported by grants GACR 301/10/1159, GACR P205/10/P308 and Research project No. AV0Z50110509.

Poster No. 46

Non-ionic contrast medium induces oxidative stress and apoptosis through Ca^{2+} influx in human neutrophils

Mustafa Kayan[1], Mustafa Nazıroğlu[2], İ. Suat Övey[2], Mehmet Aykur[2], A. Cihangir Uğuz[2], V. Ali Yürekli[4]

[1] Department of Radiology, Faculty of Medicine, Suleyman Demirel University, Isparta, TURKEY.
[2] Department of Biophysics, Faculty of Medicine, Süleyman Demirel University, Isparta TURKEY.
[3] Department Neurology, Faculty of Medicine, Süleyman Demirel University, Isparta TURKEY.

Non-ionic contrast media (CM) have shown to influence cellular functions such as secretion of leukotriens, activation of pahagocytic cells and oxidative stress (1). Neutrophils cells are primarily or secondarily involved in the pathogenesis of non-ionic CM (2). The CM can induce tissue kidney injury via activation of phagocytosis and oxidative stress although mechanisms of the injury via neutrophils are not clear. We aimed to investigate effects of the CM on oxidative stress and Ca^{2+} release in serum and neutrophils of human.

Ten migraine patients were used in the study. Serum and neutrophil samples from patients' peripheral blood obtained before (control) and 30 min after non-ionic (iopromide) CM injection. The neutrophils were also incubated 2-aminoethoxydiphenyl borate (2-APB), and verapamil+diltiazem.

Serum and neutrophil lipid peroxidation, apoptosis and intracellular Ca^{2+} release levels in the patients were higher in CM group than in control. The neutrophils reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) values, serum vitamin E and β-carotene concentrations were lower in CM group than in control. neutrophils lipid peroxidation levels were lower in CM+2-APB and CM+verapamil+diltiazem groups than in CM group although GSH, GSH-Px and intracellular Ca^{2+} values increased in CM+2-APB and CM+verapamil+diltiazem groups. However, caspase 3, caspase 9, vitamin A and vitamin C values did not change by CM treatment.

In conclusion, we observed that CM induced oxidative stress and Ca^{2+} influx by decreasing vitamin E, β-carotene and Ca^{2+} release levels in the human serum and neutrophils. However, we observed protective effects of Ca^{2+} channel blockers on the Ca^{2+} influx in the neutrophils.

References

1. Böhm I, Speck U, Schild H. 2008. Cell-dependent influence on the phagocytosis induced by non-ionic contrast medium injection. Br J Radiol. 81:199-203.
2.. Fanning NF, Manning BJ, Buckley J, Redmond HP. 2002. Iodinated contrast media induce neutrophil apoptosis through a mitochondrial and caspase mediated pathway. Br J Radiol. 75:861-73.

Poster No. 47

Capparis ovata modulates brain oxidative toxicity and epileptic seizures in pentylentetrazol-induced epileptic rats

Mustafa Nazıroğlu[1], Mehmet Berk Akay[2], Ömer Çelik[1], Muhammed İkbal Yıldırım[2], Erdinç Balcı[2]

[1] Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.
[2] Student, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.

It has been widely suggested that oxidative stress products play an important role in the pathophysiology of epilepsy. *Capparis ovata (C. ovata)* grows widely in TURKEY and it may useful treatment of epilepsy because it contains flavonoids which demonstrated as an antioxidant. Since it has not yet been clarified the modulator effects of *C. ovata* on the cellular survival and death in molecular pathways, we hence focused on the dual effect of *C. ovata* extracts in pentylentetrazol (PTZ)-induced epileptic rats by checking its role on lipid peroxidation, antioxidant levels and EEG values.

Thirty-two rats were randomly divided into four groups. First group was used as control although second group was PTZ group. Oral 100 and 200 mg/kg *C. ovata* were given to rats constituting the third and fourth groups for 7 days before PTZ administration. Second, third and forth groups received 60 mg/kg PTZ for induction of epilepsy. Three hours after administration of PTZ, EEG records, brain cortex and blood samples were taken all groups.

The lipid peroxidation levels of the brain cortex, number of spikes and epileptiform discharges of EEG were higher in PTZ group than in control and *C. ovata* group whereas they were decreased by *C. ovata* administration. Vitamin A, vitamin C, vitamin E and β-carotene concentrations of brain cortex and latency to first spike of EEG were decreased by the PTZ administration although the brain cortex and plasma vitamin concentrations, and brain cortex and erythrocyte glutathione and glutathione peroxidase values were increased in PTZ +100 and PTZ+200 mg *C. ovata* groups.

In conclusion, *C. ovata* administration caused protective effects on the PTZ-induced brain injury by inhibiting free radical, regulate of brain biopotential function and supporting antioxidant redox system.

Poster No. 48

Capparis ovata modulates oxidative stress and ADP ribose-induced TRPM2 channel currents in dorsal root ganglion of pentylentetrazol-induced epileptic rats

Mustafa Nazıroğlu[1], Cemil Özgül[1], Mehmet Berk Akay[2], İshak Suat Övey[1]

[1] Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.
[2] Student, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.

Transient Receptor Potential Melastatin-Like 2 (TRPM2) is a non-selective Ca^{2+} permeable cation channel and is known to be activated by H_2O_2 which is one of the most important indicators of intracellular oxidative stress. Recent reports exhibited that TRPM2 currents may be blocked by aminoethoxydiphenyl borate (2-APB) and anthralic acid (ACA) in a manner rapid and reversible (1). Neuronal hyperexcitability is a feature of epilepsy and both inflammatory and neuropathic pain. (2). *Capparis ovata (C. ovata)* is containing plenty of antioxidants and antinociceptive effects of *C. ovata* were recently reported (3). Hence, it may useful treatment of epilepsy-induced pain through modulation of TRPM2 channels because it contains flavonoids which demonstrated as an antioxidant. In this study we investigated effects of *C. ovata* on whole cell currents and Ca^{2+} influx arising from TRPM2 channels activated by H_2O_2 in dorsal root ganglion (DRG) of rats.

Thirty-two rats were randomly divided into four groups. First group was used as control although second group was PTZ group. Oral 100 and 200 mg/kg *C. ovata* were given to rats constituting the third and fourth groups for 7 days before PTZ administration. Second, third and forth groups received 60 mg/kg PTZ for induction of epilepsy. Three hours after administration of PTZ, DRG samples were taken all groups.

In the current study, In whole-cell patch clamp and Ca^{2+} signaling (Fura-2) experiments were made in the DRG neurons. TRPM2 currents and Ca^{2+} influx in the neurons were induced by H_2O_2. However, the current and influx were inhibited by 100 and 200 mg *C. ovata* supplementation. When extracellular *C. ovata* is introduced by patch-clamp chember TRPM2 channel currents were not activated by H_2O_2. The H_2O_2-induced Ca^{2+} gates and release were not blocked by the 2-APB and ACA.

In conclusion, we observed the modulator role of *C. ovata* on Ca^{2+} influx through a TRPM2 channel in the DRG neurons. Since cytosolic antioxidant depletion due to Ca^{2+} influx is a common feature of oxidative stress-induced pain in epilepsy, our findings are relevant to the etiology of epileptic pain in oxidative stress-induced epilepsy.

References

1. Nazıroğlu M. (2012) Molecular role of catalase on oxidative stress-induced $Ca^{(2+)}$ signaling and TRP cation channel activation in nervous system. J Recept Signal Transduct Res. 32(3):134-141.
2. Watanabe H, Nagata E, Kosakai A, Nakamura M, Yokoyama M, Tanaka K, SasaiH (2000) Disruption of the epilepsy KCNQ2 gene results in neural hyperexcitability. J Neurochem 75:28–33.
3. Arslan R, Bektas N (2010) Antinociceptive effect of methanol extract of Capparis ovata in mice. Pharm Biol 48:1185-1190.

Poster No. 49

The Anti Microbial Effect of Marine Alga *(Laurencia Snyderiae)*(20420) on Some Important Aquatic Pathogens (White Spot Syndrome Virus, Yersinia Ruckerii and Vibrio Harveyi)

Mohammad Afsharnasab[1]*, Khalil Pazir[2], Bhroze Derakhshesh[3] and Shapour Kakolaki[1]

[1] Aquatic Animal Health Department, Shrimp Division, Iranian Fisheries Research Organization, Tehran, IRAN.

[2] Shrimp Research Center, Bousher, IRAN.

[3] Persian Gulf Research Center, Persian Gulf University,Bousher, IRAN.

Our study was aimed to investigate the properties of the Marine alga, *Laurencia snyderiae against white Spot Virus and some selected bacteria.* In this study marine alga *L. snyderiae* were collected from the coastal area along the Persian Gulf and the powder alga extract consequently added with 1% of that to the shrimp diet. Shrimp *Litopenaeus vannamei* were infected by white spot syndrome virus experimentally and then were fed by the pellet with powder alga and the untreated group were not. The results showed that the Total Hemocyte Count (THC) and Total Protein Plasma (TPP) significantly increased in shrimp in which were fed with pellet with alga extract. The survival rate was higher than the control group in day 15_{th}. So, oral administration *L. snyderiae* extract was capable to prevent the infection via stimulation the specific immune system. For study the effect of alga on bacteria such as *Yersina ruckerii* and *Vibrio harveyi* different dosage of alga extracted and adhering using disc diffusion methods against the bacteria. The results showed disc with 8 mg extract had more sensitivity to *Yersina rucker* but were resistant to *Vibrio harveyi.*

Poster No. 50

Effects of cafeteria diet on serum oxidant/ antioxidant parameters in offspring of obese rats

Samira Bouanane[1], Hafida Merzouk[1], Fatima-Zohra BabaAhmed[1], Nassima Mokhtari[1], Sid-Ahmed Merzouk[2], Michel Narce[3]

[1] Laboratory of Physiology, Physiopathology and Biochemistry, Department of Biology, AbouBekr Belkaid University, Tlemcen, Algeria.

[2] Department of Physics, AbouBekr Belkaid University, Tlemcen, Algeria.

[3] INSERM UMR 866, Lipids Nutrition Cancer, Bourgogne University, Dijon, FRANCE.

Experimental obesity can be produced by dietary 'cafeteria', offering rats a variety of snack-type foods, normally consumed by humans (1). The aim of the present study was to test the hypothesis that obesity increases oxidative stress in the offspring of obese rats. Therefore several markers of oxidative stress were assessed by measuring the concentrations of plasma vitamins (A, E), hydroperoxides, carbonyl proteins and the activities of erythrocyte SOD and Catalase in pups from cafeteria-diet-fed at birth (day 0), weaning (day21) and 3months of age (day90). Plasma α-tocophérol (vitamin E) and retinol (vitamin A) were determined by reversed-phase HPLC. Hydroperoxides, markers of lipid peroxidation were measured by the FOX2, plasma carbonyl proteins were assayed by DNPH. Catalase activity was measured by spectrophotometric analysis of the rate of H_2O_2 decomposition at 240nm and SOD activity was measured by the NADPH oxidation procedure (2). These offspring had significantly lower Catalase activity and higher plasma hydroperoxide and carbonyl protein levels and SOD activity at birth, at days21 and 90 compared with control offspring. In conclusion, the oxidative stress occurred during intra-uterine life, persisted through adulthood in offspring of cafeteria-diet-fed rats.

References

1. Darimont C, Turini M, Epitaux M and al. β3-Adrenoceptor agonist prevents alterations of muscle diacylglyceroland adipose tissue phospholipids induced by a cafeteria diet. Nutr Metab 2004; 1: 4-13.
2. Bouanane S, Benkalfat NB, Baba Ahmed FZ, Merzouk H et al. Time course of changes in serum oxidant/antioxidant status in cafeteria fed obese rats and their offspring. Clin Sci 2009; 116: 669-80.

Poster No. 51

Ethyl pyruvate prevents ischemia reperfusion injury in isolated perfused rat heart

Hasan Akkoc[1], Selcuk Tunik[2], Ilker Kelle[1], Ahmet Gurkan[1], Zeynep Erdogmus[1], Zulfiye Gul[1] and Meral Erdinc[1]

[1] Department of Pharmacology, Medical Faculty, Dicle University, Diyarbakir, TURKEY.
[2] Department of Histology and Embryology, Medical Faculty, Dicle University, Diyarbakir, TURKEY.

Ethyl pyruvate (EP), a simple derivative of endogenous pyruvate, has an antioxidant and anti-inflammatory function. The aim of the present study was to investigate the protective effect of EP against ischemia reperfusion (IR) injury in isolated rat heart. Twenty four Male Sprague-Dawley rats, each weighing 250-300 g, were used. Rats were divided into three groups (n=8) ;Group 1:Sham-operated, Group 2: IR, Group 3: EP. In all groups, after hearts were isolated and mounted on Langendorff apparatus, they were perfused with constant flow (18 ml/min) of Krebs Henseleit Solution (aired with 95% O_2 + 5% CO_2) and maintained at 37^{0}C. Ischemia was produced for 30 min by blocking the perfusion with Krebs Henseleit Solution and it was followed by reperfusion for 60 min. In group 3 EP (2 mmol/L) was added into Krebs Henseleit Solution after stabilization period. In all groups, heart rate and coronary perfusion pressure were recorded. Lactat dehidrogenase (LDH) activity was determined in the samples of perfusate, which were taken at minutes 0. and 30. of reperfusion . At the end of the experiments, the extent of myocardial infarct size was estimated by triphenyltetrazolium chloride method. Coronary perfusion pressure in EP group was significantly lower as compared to the IR group ($p<0.05$). Treatment with EP significantly reduced IR induced increase in infarct size ($p<0.001$) and release of lactate dehydrogenase ($p<0.001$). These results show that ethyl pyruvate prevents ischemia reperfusion injury in isolated perfused rat heart.

Poster No. 52

The effects of Na^+- H^+ exchanger inhibitor – zoniporide – and poly(ADP-ribose) polymerase inhibitor – 5-aminoisoquinolinone – on ischemia-reperfusion injury in isolated perfused rat heart

Hasan Akkoc, Ahmet Gurkan, Ilker Kelle, Askın Tas Hekimoglu And Meral Erdin

Department of Pharmacology, Medical Faculty, Dicle University, Diyarbakir, TURKEY.

It is known that Na^+- H^+ Exchanger Inhibitor – Zoniporide – and Poly(ADP-ribose) Polymerase Inhibitor – 5-Aminoisoquinolinone (5-AIQ) have cardioprotective effects against iscemia-reperfusion (IR) injury. The present study was performed to investigate whether the use of Zoniporide and 5-AIQ together will provide an increase in protection against IR injury. Fourty Male Sprague-Dawley rats weighing each 250-350 g were used. Rats were divided into five groups (n=8); Group 1: Sham-opereted, Group 2: IR, Group 3: (5-AIQ), Group 4: Zoniporide and Group 5: Mix (Zoniporide + 5-AIQ). Isolated rat hearts were exposed to 30 minutes of global ischemia followed by 120 minutes of reperfusion using Langendorff's apparatus. In groups 3, 4 and 5, Zoniporide (50 nM/L) and 5-aminoisoquinolinone (7.5μM/L) were added into Tyrode Solution after stabilization period.

In all groups hemodynamic parameters (heart rate, coronary perfusion pressure, left ventricular developed pressure (LVDP), LV(dP/dt)max and LV(dP/dt)min were recorded. Myocardial injury was assessed in the terms of infarct size and release of lactate dehydrogenase (LDH) enzyme. Except heart rate all parameters were found significantly different in groups 3, 4 and 5 versus IR group ($p<0.05$). When compared to Zoniporide and 5-AIQ groups, in mix group there was no significant difference determined in LDH and hemodynamic parameters ($p>0.05$) but myocardial infarct size estimated by volume and weight method reduced significantly [($p<0.05$) and ($p<0.001$)]. These results show that use of Zoniporide and 5-AIQ together provides an increase in protection against IR injury especially by reducing the myocardial infarct size.

Poster No. 53

Caffeic acid phenethyl ester and oxidative stress

Ahmet Tomur[1], Sultan Yılmaz[2], Efkan Uz[3], H. Ramazan Yılmaz[2]

[1] Deparment of Physiology, Mevlana University, Faculty of Medicine, Konya, TURKEY.
[2] Deparment of Medical Biology, Mevlana University, Faculty of Medicine, Konya, TURKEY.
[3] Deparment of Biochemistry, Suleyman Demirel University, Faculty of Medicine, Isparta, TURKEY.

Caffeic acid phenethyl ester (CAPE) is a phenolic active component of honeybee propolis. It has been used as a folk medicine with no harmful effects on normal cells. CAPE has several biological and pharmacological properties such as free radical scavenging, antioxidant, anti-bacterial, antifungal, antiviral, anti-inflammatory, anti-mitogenic, anti-carcinogenic, antimutagenic, antiproliferative, antiatherosclerotic, neuroprotective, immunomodulatory and wound-healing acceleration activities. Reactive oxygen species (ROS) have been implicated in normal cells and tissue injuries. Oxidative stress plays a major role in many diseases. In this study, the protective effect of CAPE in various experimental animal models with different agents and in different cell cultures will be discussed.

Poster No. 54

Association between TRPM7 gene polymorphisms and colorectal cancer

Abdullah T. Demiryürek[1], Serdar Öztuzcu[2], Esma Özkara[2], Celaletdin Camcı[3]
Departments of [1]Medical Pharmacology, [2]Medical Biology, and [3]Medical Oncology, Faculty of Medicine, University of Gaziantep, Gaziantep, TURKEY.

TRPM7 is implicated in cellular Mg^{+2} homeostasis, but its function in CRC is unknown. The aim of this study was to investigate a possible association between TRPM7 gene polymorphisms and CRC development. A total of 83 patients operated due to CRC and 101 healthy controls with similar age and sex were included to this study. Polymorphisms were analyzed in genomic DNA using a BioMark 96.96 dynamic array system. There were significant associations between TRPM7 gene rs62021060, rs55924090 (Ile459Thr) and rs77165588 polymorphisms with CRC development. TT and CC genotypes and T allele frequencies in rs62021060 polymorphism were markedly low in patients. However, TC genotype and C allele frequencies were high in CRC group. GG genotype and G allele frequencies in rs55924090 polymorphism were markedly high in the patients group. Although CC genotype frequency in rs77165588 polymorphism were markedly low, CG genotype frequency were high in patients. CG and GG genotypes and G allele were not observed in the control group. GG genotype was absent in the patient group. These results are the first to demonstrate the contribution of TRPM7 channel in CRC development. Our data showed that the TRPM7 channel gene might be a risk factor for CRC, and suggested that genetic polymorphisms in the these genes modify individual susceptibility to CRC in the Turkish population. The presence of G allele in rs77165588 polymorphism may serve as a diagnostic marker for the CRC patients.

Poster No. 55

Association between TRPM2 gene polymorphisms and colorectal cancer

Celaletdin Camcı[1], Esma Özkara[2], Serdar Öztuzcu[2], Abdullah T. Demiryürek[3]

Departments of [1]Medical Oncology, [2]Medical Biology, [3]Medical Pharmacology, Faculty of Medicine, University of Gaziantep, Gaziantep, TURKEY.

Ion channels may have a critical role in cell proliferation and growth. The aim of this study was to investigate a possible association between TRPM2 gene polymorphisms and colorectal cancer (CRC) development. A total of 81 patients operated due to CRC and 161 healthy controls with similar age and sex were included to this study. Polymorphisms were analyzed in genomic DNA

using a BioMark 96.96 dynamic array system. There were significant associations between TRPM2 gene rs9978351 (Arg1189Gln), rs1618355 and rs1556314 (Asp543Glu) polymorphisms with CRC development. Frequencies of the AA genotype in rs9978351 polymorphism, AC genotype and C allele in rs1618355 polymorphism, and TG genotype and G allele in rs1556314 polymorphism were markedly high in CRC patients. These results are the first to demonstrate the contribution of TRPM2 channel in CRC development. Our data showed that the TRPM2 channel gene might be a risk factor for CRC, and suggested that genetic polymorphisms in the these genes modify individual susceptibility to CRC in the Turkish population.

Poster No. 56

Association between TRPM8 gene polymorphisms and colorectal cancer

Abdullah T. Demiryürek[1], Serdar Öztuzcu[2], Esma Özkara[2], Celaletdin Camcı[3]

Departments of [1]Medical Pharmacology, [2]Medical Biology [3]Medical Oncology, Faculty of Medicine, University of Gaziantep, Gaziantep, TURKEY.

TRPM8 is activated by cold temperatures and was present on nerve fibres throughout the wall of the colon. However, the role of TRPM8 in CRC is unknown. The aim of this study was to investigate a possible association between TRPM8 gene polymorphisms and CRC development. A total of 86 patients operated due to CRC and 177 healthy controls with similar age and sex were included to this study. Polymorphisms were analyzed in genomic DNA using a BioMark 96.96 dynamic array system. There were significant associations between TRPM8 gene rs10803666, rs10490018, rs12472151, rs2052029, rs2215173, rs6740118, rs4663995, rs1016062, and rs2362294 polymorphisms with CRC development. However, no associations were found between rs6431648 and rs2362295 polymorphisms with CRC. GC genotype and C allele in rs10803666, CT genotype and T allele in rs10490018, GA, AA genotypes and A allele in rs12472151, GC genotype and C allele in rs2052029, CT genotype and T allele in rs2215173, CT genotype and T allele in rs6740118, TT genotype and T allele in rs4663995, CT genotype and T allele in rs1016062, and GA genotype in rs2362294 polymorphism were markedly high in the patients group. These results are the first to demonstrate the contribution of TRPM8 channel in CRC development. Our data showed that the TRPM8 channel gene might be a risk factor for CRC, and suggested that genetic polymorphisms in the these genes modify individual susceptibility to CRC in the Turkish population.

Poster No. 57

Nitric Oxide (NO) and bacterial NOS proteins (bNOS) functions

Arzu Kart, Aynur Gül Karahan

Department of Food Engineering, Faculty of Engineering, Süleyman Demirel University, Isparta, TURKEY.

Nitric Oxide (NO) is generally known as a radical which has physiological and pathophysiological features in living organisms. The importance of this molecule in physiological functions was realized when it was identified as the endothelium–derived relaxing factor and as a neuronal messenger molecule twenty- five years ago.

Recently, NO has become a focus of scientific studies. At the same time, it is determined NO is synthesized from L-arginine in many organisms and called nitric oxide synthase enzyme which performs NO synthesis. Studies showing the functions of NOS enzymes are concentrated on the NOS enzymes in mammalian and plant cells. Besides there are limited number of studies which determine the functions of NOS enzymes found in bacteria. The full functions of these enzymes are still unknown. Nevertheless, there are some claims about their functions.

This review is focused on surveys and ideas about exact biological functions of the bNOS proteins which is found in bacteria. It is expressed that it is may be the functions of NO generated by bNOS proteins in signaling and defense mechanisms and biological reactions. It is thought that small molecules such as NO may contribute to quorum sensing in bacteria.

Despite all available data, it is seen that a limited numbers of studies reveal the full meaning of biologic functions of bNOS proteins. In addition, further studies which will reveal claimed and possible functions of bNOS proteins should be done.

References

1. Bird, LE., Ren, J., Zhang, J., Foxwell, N., Hawkins, AR., Charles, IG., Stammers, DK. "Crystal structure of SANOS, a bacterial nitric oxide synthase oxygenase protein from Staphylococcus aureus". Structure 2002; 10(12):1687-1696.
2. Buddha, MR., Crane, BR. Structures of Tryptophanyl-tRNA Synthetase II from *Deinococcus radiodurans* Bound to ATP and Tryptophan. The Journal of Biological Chemistry 2005a; 280(36): 31965–31973.

3. Buddha, MR., Keery, KM., Crane, BR. "An unusual tryptophanyl tRNA synthetase interacts with nitric oxide synthase in *Deinococcus radiodurans*". Proceedings of the National Academy of Sciences 2004b; 101(45): 15881-15886.
4. Karahan, AG., Çakmakçı, ML., Cicioğlu-Arıdoğan, B., Kart, A. Nitric oxide (NO) and lactic acid bacteria-contributions to health, food quality and safety. Food Reviews International 2005; 21 (3): 313-329.
5. Kart, A., Karahan, AG., Çakmakçı, ML. Production of nitric oxide (NO) by lactic acid bacteria isolated from fermented products. European Food Research and Technology 2006; 223: 35-38.
6. Wach, MJ., Kers, JA., Krasnoff, SB., Loria, R., Gibson, DM. Nitric oxide synthase inhibitors and nitric oxide donors modulate the biosynthesis of thaxtomin A, a nitrated phytotoxin produced by *Streptomyces* spp. Nitric Oxide 2005; 12: 46-53.
7. Waters, CM., Bassler, BL. "Quorum Sensing: Cell-To-Cell Communication in Bacteria". Annual Review of Cell and Developmental Biology 2005; 21; 319-346.

Poster No. 58

Serum ADMA and apelin levels in type 2 diabetics with and without vascular complications

Akar Karakoç[1], Arzu Sahin[2], Elif Şimsek Polat[1], Elvin Aliyev[1], AbdulkadirYıldırım[1], Nuri Bakan[1]

[1] Department of Biochemistry, Faculty of Medicine, Atatürk University.
[2] Department of Biochemistry, School of Health, Artvin Coruh University.

Type 2 diabetes mellitus (T2DM), one of the most prevalent diseases accompanied by oxidative stress, is a metabolic disorder characterized by insulin resistance, associated with obesity. Asymmetrical dimethylarginine (ADMA), formed by the hydrolysis of proteins containing methylated arginine residues, is an endogenous inhibitor of nitric oxide synthase (NOS), which oxidize L-arginine to citruline and nitric oxide (NO), related to hyperinsulinaemia and hyperlipidaemia. Apelin is a recently discovered peptide, present in a number of tissues and also produced by adipocytes, and play role in insulin sensitivity improvement. In this study, it was purposed the investigation of relationshıp between serum ADMA and apelin levels and vascular complications of T2DM.

This study included (a total of) 59 diabetic patients. Of the patients, 30 were diabetic with complications, and 29 without complications. In serum samples obtained from the patients, serum ADMA and apelin levels were measured spectrophotometrically and by ELISA.

In this study, no statistically significant difference could be found between the groups with and without complications with respect to the ADMA and apelin levels. ADMA levels in groups with and without complications were meanly 0.86±0.51 and 0.93±0.56 (p=0,62), respectively. Apelin levels of group with complications were lower than those of group without complications, however this was not significiant (meanly 1.50±0.41 and 1.59±0.51, respectively, p=0.45). Besides, there was not a correlation between ADMA and apelin levels of patients (p=0.47, r=-0.097)

The results of this study have been showed no statistically significant relationship present between ADMA-apelin levels and complications of T2DM.

Poster No. 59

Serum Paraoxonase and Arylesterase Activities in Patients withThyroid Dysfunction

Arzu Şahin[1], Serap Yıldırım[2], Musa Düdükçü[3], Engin Şebin[3], Abdulkadir Yıldırım[3], Şenol Dane[4]

[1] Department of Physiology, School of health, Artvin Coruh University Artvin- TURKEY.
[2] Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum -TURKEY.
[3] Department of Biochemistry, Faculty of Medicine, Atatürk University,Erzurum-TURKEY.
[4] Department of Physiology, Faculty of Medicine, Fatih University, Ankara-TURKEY.

Thyroid hormones are associated with the oxidative and antioxidative status in the cells and the extracellular environment. Serum paraoxonase-1 (PON-1) is an antioxidant enzyme that has both paraoxonase (PON) and arylesterase (ARE) activities. The purpose of this study is to investigate whether there are any changes in serum PON-1 activities in patient with hypo- and hyper-thyroid.

For this purpose, we measured the activities of PON and ARE in serum samples of 75 patients with hypothyroid, 75 patients with hyperthyroid and 50 healthy control. For PON-1 measurements, diethyl-p-nitrophenyl phosphate was used as the substrate and for ARE activity phenyl acetate.

PON-1 activities were found to be significantly higher in the patients with thyroid dysfunction compared to the healthy controls (controls: 118.7±89.8 U/mL; hyperthyroid: 135.5±101.7U/mL; hypothyroid:164.4±101.3 U/mL, p<0.05 ANOVA). Similarly, the patients with thyroid dysfunction had significantly higheractivities of serum arylesterase than healthy controls (controls: 40.9±10.9 U/mL; hyperthyroid: 53.3±15.8 U/mL; hypothyroid:60.6±17.9 U/mL, p<0.0001 ANOVA).

These results indicate thatPON-1 activities increasein the patients with thyroid dysfunction, which may associate with altered oxidative status.

References

1. Yildirim S,Karadeniz A, Karakoc A, Yildirim A, Kalkan Y, Şimşek N. Effects of Royaljelly on liverparaoxonaseactivity in ratstreatedwithcisplatin. Turk J MedSci 2012; 42: 367-375.
2. YıldırımS, Akar S, Kuyucu M, Yıldırım A, DaneŞ, Aygül R. Paraoxonase 1 gene polymorphisms, paraoxonase/arylesteraseactivitiesandoxidizedlow-densitylipoproteinlevels in patientswithmigraine. Cell BiochemFunct 2011; 29: 549–554.
3. Coria MJ, Pastrán AI, Gimenez MS. Serum oxidativestressparameters of womenwithhypothyroidism. ActaBiomed 2009;80:135-9.

Poster No. 60

Effects of Magnetic Fields on Blood Fields on Blood Biochemistry of Diabetic Rats

Ayşe Demirkazık[1], S. Gültürk[2], E.Özdemir[2], T. Demir[2]

[1] Department of Biophysics, Medical Faculty, Cumhuriyet University, Sivas, TURKEY.
[2] Department of Physiology, Medical Faculty, Cumhuriyet University, Sivas, TURKEY.

We investigated the effects of a long-term treatment with insulin, or/and magnetic field on the blood biochemistry parameters in streptozotocin (STZ)-induced diabetic rats. Fifty three adult Wistar albino male rats (Cumhuriyet University Animal Center, Sivas, Turkey), weighing 250-300 g, were used. Before study procedure, rats were randomly assigned in four groups: sham, exposed to no MF; MF, exposed to MF; DM, induced with streptozotocin; DMMF, induced with streptozotocin and exposed to MF. Then, five mL blood was collected from intracardiac non-fasting rats final experiment before the rats were sacrificed by decapitation. Pre- and post- glucose levels, TG, cholesterol, HDL, LDH, Na, K, Ca, Mg and Fe electrolits were elevated analyzer devices. There were statically differences on the mean values of pre- and post- glucose levels, TG, cholesterol, HDL, LDH between sham and DMMF. In conclusion, the states of improved glucose metabolism may be prevented blood biochemistry parameters and the hypoglycemic effect of magnetic field on the function of β cells may be able to help increase in insulin concentration and sensitivity to glucose metabolism.

References

1. Nazıroğlu M. Role of selenium on calcium signaling and oxidative stress-induced molecular pathways in epilepsy. Neurochem Res 2009; 34:2181–2191.
2. Uğuz AC, Nazıroğlu M, Espino J, Bejarano I, González D, Rodríguez AB, Pariente JA. Selenium modulates oxidative stress-induced cell apoptosis in human myeloid HL-60 cells via regulation of caspase-3, -9 and calcium influx. J Memb Biol 2009; 232:15–23.

Poster No. 61

Effects of Nigella sativa L. on NO production and DNA fragmentation in acute inflammation

C. Özbayer[1], N. Tekin[2], B. Yangi[1], E. Entok [3], F. Akyüz [2], H. Kurt [1], İ. Değirmenci [1], Hv. Güneş [1], Mc. Üstüner [1]

[1]Department of Medical Biology, [2] Department of Biochemistry, [3]Department of Nuclear Medicine, Medical Faculty, Eskisehir Osmangazi University, Eskisehir, TURKEY.

Inflammation has an important role in many diseases including several cancer types. Nitric oxide (NO) is often produced under inflammatory conditions, induction of NOS in epithelial cells by inflammatory cytokines. NO produced in inflamed tissues may cause to DNA damage in this way contribute to cell carcinogenesis (1). *Nigella sativa* L. is herbaceous plant known as the black seed and is commonly used as a natural food and a traditional medicine. It has many pharmacological effects including antibacterial, antifungal, antitumor, analgesic, antipyretic activity (2). The aim of this study was to investigate the effects of *N.sativa* on NO production and DNA fragmentation in acute inflammation.

We used the experimental lipopolysaccharides (LPS) induced model. Intraperitonal LPS (E. coli, serotype055-B5) 1mg/kg was administered to the three group. *Nigella sativa* extract(500 mg/kg) and essential oil(5ml/kg) were given orally to treatment groups, after 24-hours of intraperitoneal LPS injection. To determine the liver inflammation status via FDG-PET, ^{18}F-fluoro-deoxy-D-glucose (0,8ml/kg) was administrated under the anesthesia by intracardiac injection before the 1h of PET scanning. After the FDG-PET analyses, blood and tissue samples were collected. Liver ^{18}F-FDG uptake was calculated. Liver and lung NO and DNA fragmentation levels were determined.

When compare the control 18F-FDG uptake of liver in inflammation groups were increased. NO levels and DNA fragmentation were decreased in treated inflammation groups whereas increased in untreated inflammation group. We conclude that, in lps-induced acute inflammation, *N.sativa* have a therapeutic and protective effects. It has been indicate that the use of black cumin seeds for the treatment of various inflammatory diseases seems quite useful and reasonable.

References

1. Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. Cancer Res. 2000 Jan 1;60(1):184-90.

2. Mahmoud MR, El-Abhar HS, Saleh S. The effect of Nigella sativa oil against the liver damage induced by Schistosoma mansoni infection in mice. J Ethnopharmacol. 2002 Jan;79(1):1-11. PubMed PMID: 11744288.

Poster No. 62

The Effects of NOS Isoforms In Human Normospermia, Asthenospermia And Oligospermia Cases

H. Karakaya, E. Ünsal, A. Kandil*, Y. E. Canıllıoğlu, Canan Hürdağ

Department of Histology and Embryology, Medical Faculty, İstanbul Bilim University, İstanbul-Turkey
*Department of Biology, Science Faculty, İstanbul University,İstanbul-Turkey

Male factor infertility accounts for up to half of all case of infertility and affects 1 man in 20 in the general population. Evidence now suggests that reactive oxygen species (ROS)-mediated damages to sperm is a significant contributing pathology, 30-80% of all cases (1). The highly biologically active free radical is NO (2). NO is known to play important functional roles in a variety of physiological system as well as the male reproductive system. In this study we aimed to examine both iNOS and eNOS isoforms by using immunohistochemistry methods in human normospermia, asthenospermia and oligospermia groups and also determine in seminal plasma nitrite/nitrate concentrations. For this study we obtained samples from the infertile laboratory. Based on our findings, eNOS reactions decreased in postacrosomal and equatorial regions for both asthenospermia and oligospermia groups when compared to normospermia groups. iNOS reactions were observed in the mid-piece region of asthenospermia and oligospermia groups. Furthermore, the acrosomal staining was also prominent in the normospermia. In conclusion, the increased level of NO concentration in seminal plasma leads to decreased sperm motility and viability. Our immunohistochemistry results confirm NO plays a key role in sperm functions. Physiological and pathological processes depend on the relative level of nitric oxide and oxidative stress.

References

1. K. Oxidative Stress and Male Infertility-a Clinical Perspective. Human Reproduction Update 2008.14 3:243-258.
2. Aitken J.R. 2006. Reactive Oxygen Species:Friend or foe. In: Jonge C. and Barratt C,editor. Sperm Cambridge University Press: Edinburgh, UK.,pp170-194.

Poster No. 63

A Comparison between the effects of N-Acetylcysteine and amifostine on radiation-induced bone deterioration using 3-point bending test

Can Demirel, Berrin Tiryaki

Department of Biophysics, Faculty of Medicine, Gaziantep University, Gaziantep, TURKEY.

In this study, the protective effects of Amifostine and *N-Acetylcysteine* (NAC) on radiation-induced bone deterioration were compared with biomechanical methods (1).

For this purpose, 32 rats were divided into Control (C; n=8), Radiation (R; n=8), Radiation+NAC (R+NAC; 100 mg/kg, i.p; n=8) and Radiation+Amifostine (R+WR; 200 mg/kg, i.p; n=8) groups and used. R, R+NAC and R+WR groups were exposed to a single dose of 50 Gy of left femur radiation by using a Cobalt-60 apparatus. 6 weeks after the application, the left femurs of all groups were isolated and 3-point bending test was applied (2).

As a result of biomechanical analyses, the decreases in displacement, breaking force, stiffness, work to failure and strain parameters of the femurs in R group were found significant as compared to C group ($p<0.05$), while the changes in stress, Young's modulus and toughness values of R group were not statistically significant as compared to C group. A comparison of displacement, work to failure, strain and toughness values of R+NAC group with those of C group yielded no statistically different results. Breaking force and stress parameters of R+NAC group were statistically different from those of C group ($p<0.05$). The increases in stiffness and toughness values of R+NAC group were statistically different from those of R group ($p<0.05$). The difference resulting from a comparison of stiffness and stress parameters of R+WR group with those of C group was significant ($p<0.05$).

When these results are considered, it might be stated that the protective effect of a low dose of NAC application on radiation-induced bone deterioration is similar to that of Amifostine.

References

1. Demirel C, Kilciksiz S, Gürgül S, Erdal N, Yildiz N. N-acetylcysteine ameliorates gamma radiation-induced deterioration of bone quality in the rat femur Journal of International Medical Research 2011; 39(6): 2393-2401.
2. Turner CH and Burr DB. Basic Biomechanical Measurements of Bone: A Tutorial. Bone 1993; 14: 595-606.

Poster No. 64

Monitoring of the water pollution in Uzuncayir dam lake (Tunceli, TURKEY) by using some oxidative stress biomarkers

Cemil Ergin[1], Nuran Cikcikoğlu Yildirim[1], Durali Danabaş[2], Numan Yildirim[1], Ayten Öztüfekçi Önal[3], Gülşad Uslu[4], Erhan Ünlü

[1] Tunceli University, Faculty of Engineering, Department of Environmental Engineering, TR62000, Tunceli, TURKEY.
[2] Tunceli University, Fisheries Faculty, TR62000, Tunceli, TURKEY.
[3] Tunceli University, Faculty of Engineering, Department of Geology Engineering, TR62000, Tunceli, TURKEY.
[4] Fırat University, Faculty of Engineering, Department of Environmental Engineering, TR23119, Elazığ, TURKEY.
[5] Dicle University, Faculty of Science and Art, Department of Biology, Diyarbakır, TURKEY.

Uzuncayir Dam is built approximately 25 km away from the junction of Munzur and Pülümür Watercourses of Tunceli province and started to retain water since October, 2009. Along with going into operation of Uzuncayir Dam, pollution caused by contaminants given into the dam lake (domestic liquid waste, water seepage arising from irregular solid waste area of the city, elements washed up from rocks etc.) have started a discussion about the interaction between parameters of water quality of dam lake and potential physiological changes of fishes that exposed to this pollution. In this study, it is aimed to evaluate the results of oxidative stress parameters first 2 sampling periods of our project which was made seasonal and for a longer time (TUBITAK-CAYDAG 110Y118).

In this study, 10 sampling stations are determined and in these stations, *Capoeta umbla* (Heckel, 1843) was used as the indicator organism. The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (CAT) were determined in samples of the liver and gill tissues by using ELISA kit.

As a result of the change caused by the pollution, in the physicochemical and biologic structure of water, changes in antioxidant defense system of fish have been observed and differences between stations have been seen on measured parameter.

Poster No. 65

TRPC1 silencing suppresses proliferation of HuH-7 human hepatoma cell line

Cigdem Selli[1], Yasemin Erac[1], Buket Kosova[2], Esra Erdal[3], Metiner Tosun[1]

Departments of Pharmacology, Faculty of Pharmacy[1], Medical Biology, Faculty of Medicine[2], Ege University and Medical Biology and Genetic, Faculty of Medicine, Dokuz Eylul University[3], Izmir/ TURKEY.

We previously showed that transient receptor potential canonical 1 (TRPC1) and TRPC6 expression levels were reciprocally altered in aging rat thoracic aorta (1). In TRPC1-silenced A7r5 embryonic rat aortic smooth muscle cell line, TRPC6 expression and store-operated Ca^{2+} (SOC) entry were up-regulated (2). Our previous studies on HuH-7, human hepatoma cell line, showed that TRPC1 expression was significantly decreased in shTRPC1-expressing vector transfected HuH-7 cells (3). Furthermore, Ca^{2+} released from endoplasmic reticulum and entered via SOC channels were significantly increased (3). The purpose of this study was to investigate the role of TRPC1 on proliferation and migration of HuH-7 cells. For this purpose, cells were cultured in DMEM and transiently transfected with shTRPC1-expressing vector (pSUPERIOR). Real-time cellular analyzer (RTCA-MP/DP, Roche Applied Science) was used to determine the proliferation and migration rates. In order to determine the real-time changes in proliferation rate, cells were seeded on E-plate 96 (10000-1250 cells/well) 24 hours after transfection and monitored for 120 hours. 72 hour after transfection, cells were seeded on CIM-plate 16 (5000 cells/well) and migration monitored real time for 12 hours. Proliferation rate was significantly suppressed in TRPC1-silenced HuH-7 cells (P<.01). TRPC1 silencing did not significantly affect the migration of HuH-7 cells. These results suggest that TRPC1 may possibly take part in HuH-7 cell proliferation. Others also showed an association between TRPC6 and HuH-7 proliferation (4). Delineation of antiproliferative mechanism associated with TRPC1 down regulation awaits for further studies. This study was supported in part by The Scientific and Technological Research Council of TURKEY (TUBITAK, SBAG-108S072 to MT; BIDEB-2211 to CS) and Ege University (BAP 08ECZ009 to MT).

References

1. Erac Y, Selli C, Kosova B, Akcali KC, Tosun M. Expression levels of TRPC1 and TRPC6 ion channels are reciprocally altered in aging rat aorta: implications for age-related vasospastic disorders. Age 2010; 32:223-230.

2. Selli C, Erac Y, Kosova B, Tosun M. Post-transcriptional silencing of TRPC1 ion channel gene by RNA interference upregulates TRPC6 expression and store-operated Ca2+ entry in A7r5 vascular smooth muscle cells. Vascul Pharmacol 2009; 51:96-100.
3. Selli C, Erac Y, Kosova B, Tosun M. Store-operated calcium entry increases in shTRPC1-expressing vector-transfected hepatocellular carcinoma cell line. 3rd International Congress on Cell membranes and Oxidative Stress: Focus on Calcium signaling and TRPchannels. Cell membranes and Free Radical Research 2010; 2(1):27.
4. El Boustany C, Bidaux G, Enfissi A, Delcourt P, Prevarskaya N, and Capiod T. Capacitative calcium entry and transient receptor potential canonical 6 expression control human hepatoma cell proliferation. Hepatology 2008; 47:2068-77.

Poster No. 66

The Effects of Zinc and Metallothionein in Protection Against Diabetic Nephropathy in STZ-Diabetic Rats

D. Özçelik[1], M. Tunçdemir [2], M. Nazıroglu [3], Ö. Çelik [3], M. Öztürk [2]

[1] Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, TURKEY.
[2] Department of Medical Biology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, TURKEY.
[3] Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.

Zinc is the major metal that binds to MT under physiological conditions and is a potent inducer of MT. The present study therefore explored whether zinc supplementation can protect against diabetic nephropathy through kidney MT induction. For this purpose, the effects of zinc against STZ-induced diabetic kindney damage in rats were investigated, morphologically and biochemically.

The present study, four groups were set each has 8 Wistar Albino male rats. Group 1: Control, Group2: Untreated Streptozotocin-diabetic (STZ,60mg/kg,i.p.,single doses), Group3:Zn-treated group after STZ-application (Zn:30mg/kg/day,i.g), Group 4:Zn-treated control group. During the experimental period blood glucose levels, microalbuminuria levels, body weight and kidney weight were measured.

At the end of the experiment, diabetes caused degenerative morphological changes, a decrease in metallothionein immunreactivity; an increase in Lipid Peroxidation (LPO) in kidney tissue. Immunoreactivity of MT were observed to be higher in tubules of Zn treated diabetic group compared to the untreated diabetic group. Kidney tissue zinc and MT concentrations were significantly higher in the Zn-treated diabetic group compared to the untreated diabetic group. On the other hand, zinc administration to STZ-diabetic rats caused a significant a decrease in LPO levels in kidney tissues.

As a result, the present study indicates that zinc has a protective effect against STZ-induced diabetic damage in kidney tissue both by stimulation of metallothionein synthesis and through regulation of the lipid peroxidation.

Poster No. 67

The influence of treatment with bevacizumab on oxidant/antioxidant balance in alkali injury related corneal neovascularization

Ebru Beytut[1], Metin Ekinci[2], Elif Erdem[3], Osman İbiş[1], Hamit Uslu[1], Gözde Atila[1], H. Avni Eroğlu[1]

[1] Department of Phisyology, Faculty of Veterinary Medicine, Kafkas University, Kars-TURKEY.
[2] Department of Ophthalmology, Faculty of Medicine, Kafkas University, Kars-TURKEY.
[3] Department of Ophthalmology, State Hospital, Iskenderun-TURKEY.

The purpose of this study was to evaluate the effects of the subconjunctival uses of bevacizumab on oxidant/antioxidant balance in alkali injury related corneal neovascularization. Corneas of 12 New Zealand Rabbits were cauterized with silver nitrate crystal. Animals were divided in three groups: control group that received 0.02ml of 0.9% saline solution, alkali injury with $AgNO_3$-KOH group; group bevacizumab that received 0.5 mg of bevacizumab subconjunctivally at the 24th h after the lesion was formed. Animals corneas were extracted on the 14th day under general anesthesia. The corneas were homogenized and blood samples were centrifugation at 2000×g for 15 minutes at 4°C. Corneal TAS and TOS levels incresead significantly in group 2,3 with the control group ($p<0.001$) and blood TAS and TOS levels of groups 2,3 showed a significantly decrease with the control group ($p<0.001$). However, there was no significant difference plasma TAS levels of all groups. Plasma TOS levels of exxperimental groups decreased significantly with control group ($p<0.001$). This study suggest that bevacizumab and decreased TAS levels may contribute to the pathogenesis of alkali injury related corneal neovasculaization.

Poster No. 68

The influence of treatment with ranibizumab on oxidant/antioxidant balance in alkali injury related corneal neovascularization

Ebru Beytut[1], Metin Ekinci[2], Elif Erdem[3], Osman İbiş[1], Gözde Atila[1], Hamit Uslu[1], H. Avni Eroğlu[1], Birkan Topçu

[1] Department of Phisyology, Faculty of Veterinary Medicine, Kafkas University, Kars-TURKEY.
[2] Department of Ophthalmology, Faculty of Medicine, Kafkas University, Kars-TURKEY.
[3] Department of Ophthalmology, State Hospital, Iskenderun-TURKEY.

The purpose of this study was to evaluate the effects of the subconjunctival uses of ranibizumab on oxidant/antioxidant balance in alkali injury related corneal neovascularization. Corneas of 12 New Zealand Rabbits were cauterized with silver nitrate crystal. Animals were divided in three groups: control group that received 0.02ml of 0.9% saline solution, alkali injury with $AgNO_3$-KOH group; group ranibizumab that received 0.5 mg of ranibizumab subconjunctivally at the 24th h after the lesion was formed. Animals corneas were extracted on the 14th day under general anesthesia. The corneas were homogenized and blood samples were centrifugation at 2000×g for 15 minutes at 4˚C. Corneal TAS and TOS levels incresead significantly in group 2,3 with the control group ($p<0.001$) and blood TAS levels of groups 2,3 showed a significantly decrease with the control group ($p<0.001$). However, there was no significant difference in the blood TOS and plasma TAS levels of all groups. Plasma TOS levels of all groups increased significantly with control group ($p<0.001$). This finding suggest that alkali injury related corneal neovasculaization leads to oxidative stres in the cornea and that ranibizumab may prevent oxidative effects in the cornea.

Poster No. 69

Evaluation of the effects of novel nafimıdon derivatives on thermal hypoalgesia in mice with diabetic neuropathy

Kadir Batcioglu, Suat Kamisli, Arzu Karakurt, Ayse B. Uyumlu, Basri Satilmis, Abdullah Alagoz, Metin F. Genc
Inonu University, Faculty of Pharmacy, Division of Biochemistry & Pharmaceutical Chemistry, Malatya, TR Inonu University, Faculty of Medicine, Division of Neurology, Malatya, TR.

Diabetic neuropathy (DN) is a common complication in diabetes mellitus (DM).The streptozotocin (STZ)-induced diabetic rodent is the most commonly used animal model of diabetes and increased sodium channel expression and activity was revealed in this models. The role of altered Na channel activity in the pathogenesis of DN is still unknown. Several recent studies have implicated the investigation of the activity of the novel anticonvulsant compounds for the other pathophysiological issues such as neuropathic disorders. Nafimidone is an example of (arylalkyl) azoles, which possess a profile of activity similar to that of phenytoin or carbamazepine but distinct from barbiturates or valproic acid. At this study, we evaluated the effect of three different nafimidone derived (arylalkyl)azole compounds on disorders of thermal pain sensation in diabetic mice. We used hot and cold plate, and tail-immersion tests for assesment of thermal nociceptive responses. We found that Valproic acid derivatives among the nafimidon oxim esters which we have used was the most effective on loss of sensation in terms of three tests.

References

1. Vincent AM, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. Endocr Rev 2004; 25: 612-628.
2. Obrosova IG. Diabetic Painful and Insensate Neuropathy: Pathogenesis and Potential Treatments. Neurotherapeutics 2009; 6: 638-647.
3. Early Painful Diabetic Neuropathy Is Associated with Differential Changes in Tetrodotoxin-sensitive and -resistant Sodium Channels in Dorsal Root Ganglion Neurons in the Rat. The Journal of Biological Chemistry 2004; 279(28): 29341–29350.
4. Karakurt A, Ozalp M, Işık Ş, Stables JP, Dalkara S. Synthesis, anticonvulsant and antimicrobial activities of some new 2-acetylnaphthalene derivatives. Bioorganic & Medicinal Chemistry 2010; 18: 2902-2911.

Poster No. 70

Rose oil (Rosa damascena Mill) vapor attenuates depression-induced oxidative toxicity in brain of rat

Mustafa Nazıroğlu[1,2], Süleyman Kozlu[3], Emre Yorgancıgil[3], Abdülhadi Cihangir Uğuz[1], Kadir Karakuş[4]

[1] Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.
[2] Neurosscience Research Center, Suleyman Demirel University, Isparta, TURKEY.
[3] Student, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.
[4] Department of Psychiatry, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.

Oxidative stress is a critical way of damage in various physiological stress-induced disorders including depression. Rose oil may be a useful treatment of depression because it contains flavonoids which demonstrated a free radical antioxidant compounds such as rutin or quercetin. We investigated the effects of absolute rose oil (*Rosa damascena* Mill) and experimental depression on lipid peroxidation and antioxidant levels in cerebral cortex of rats.

Thirty-two male rats were randomly divided into four groups. First group was used as control although second group was chronic mild stress (CMS) depression-induced group. Oral (1.5 ml/kg) and vapor (0.15 ml/kg) rose oil were given to CMS depression-induced rats constituting the third and fourth groups for 28 days, respectively. Sucrose preference test was weekly used to identify depression-like phenotypes during the experiment. End of the experiment, cerebral cortex samples were taken from all groups.

The lipid peroxidation levels in the cerebral cortex in CMS group were higher than in control whereas their levels were decreased by the rose oil vapor exposure. The vitamin A, vitamin E, vitamin C and β-carotene concentrations in the cerebral cortex were lower in CMS group than in control group whereas their concentrations were higher in the rose oil vapor plus CMS group than in CMS group. The CMS-induced antioxidant vitamin changes were not modulated by oral treatment. The glutathione peroxidase activity and reduced glutathione did not change in the five groups statistically by CMS or both treatments.

In conclusion, the experimental depression is associated with elevated oxidative stress although treatment with the rose oil vapor induced protective effects on the oxidative stress in the depression.

Poster No. 71

Melatonin's effect on balance of oxidative/antioxidative system in hepatocellular carcinoma cell line HepG2

M. Betul Yerer Aycan, Eren Demirpolat, Bilge Odabasi, Merve Inanir

Department of Pharmacology, Faculty of Pharmacy, Erciyes University, Kayseri, TURKEY.

Melatonin is released by pineal gland in body and regulates biologic rhytm (1). Also, it acts whether antioxidant or prooxidant depending on free radical status of the cell. (2).

In our study, we investigate melatonin's effect on balance of oxidant/antioxidant system in hepatocellular HepG2 cell line without triggering oxidative stress externally. We used Cayman Chem SOD and TBARS kits to evaluate the levels of SOD and TBARs both in and out of the cell. Multifunctional microplate reader was used to measure the parameters.

According to our findings, between 0.5mM and 0.01mM Mel concentrations, melatonin acts like a prooxidant in sonicated cells; because the level of TBARs are higher than the control. Increase of TBARs indicates that there are higher amount of free radicals compared to control. The level of SOD is higher to compensate oxidative stress of melatonin at 0.01mM mel, but at the other concentrations it is not significantly different from the control.

In supernatant, when we consider TBARs, melatonin is prooxidant just like in sonicated cells at the same concentration range and the levels are higher than control. Level of glutathione peroxidase is the highest in the presence of 0.5mM mel and the highest level is achieved with 0.01mM mel for SOD.

As a result, we showed that melatonin increases TBARs levels compared to the control significiantly and acts as a prooxidant. For further research, we plan to assess anticancerogen effect of melatonin which is dependent on its prooxidant effect by using MTT or trypan blue assays.

References

1. Aydogan S, Yerer M. B, Goktas A, Melatonin and Nitric Oxide. Journal of Endocrinol. Invest. 2006; 29 (3): 281-287.
2. Rouf A, Osseni, Patrice R, Andre B, Jean Michel W, Yvan T, Evidence of prooxidant and antioxidant of melatonin on human liver cell line HepG2 Life Sciences 2000; 68: 387-399.

Poster No. 72

Antioxidant Activity and α- Glucosidase Enzyme Inhibition Potentials of Some Marine Macroalgae from the Coasts of Urla

Fatma Gül Çelenk[1,2], Atakan Sukatar[2]

[1] Department of Clinical Pharmacology, Faculty of Medicine, Ege University, İzmir, TURKEY.
[2] Department of Biology, Faculty of Science, Ege University, İzmir, TURKEY.

Active substances derived from macroalgal extracts currently receive great attention and many researches includes activity studies such as antioxidant, hypoglycemic, hypolipidemic, antibiotic, anticoagulant, anti-inflammatory, anticoagulant and antitumoural activities (1,2,3,4,5). Because of their wide biological potential, methanol extracts of some macroalgae collected from the coasts of Urla were subjected to antioxidant activity assays and α- glucosidase enzyme inhibiton test. While *Gracilaria gracilis* showed the highest gallic acid equivalence in total phenol assay, *Laurencia*

pinnatifida, Padina pavonica and *Scytosiphon lomentaria* showed the higher DPPH free radical scavenger activities than 0.010mg ml^{-1} of ascorbic acid (70.9, 70.1 and 57.6, respectively). When results evaluated in order to taxonomical grouping of macroalgae, the brown algae was the group which had the highest mean for total phenolic content and DPPH free radical scavenger activity. However, the α- glucosidase enzyme inhibition activities of the macroalgae highly varied within the test set, *Scytosiphon lomentaria* and *Padina pavonica* exhibited alpha- glucosidase inhibitor effect similar to acarbose.

References

1. Kim, K. Y., K. A. Nam, H. Kurihara & S. M. Kim (2008) Potent α-glucosidase inhibitors purified from the red alga Grateloupia elliptica. *Phytochemistry,* 69, 2820-2825.
2. Mayer, A. M. S. & K. R. Gustafson (2008) Marine pharmacology in 2005–2006: Antitumour and cytotoxic compounds. *European Journal of Cancer,* 44, 2357-2387.
3. Mayer, A. M. S., A. D. Rodríguez, R. G. S. Berlinck & N. Fusetani (2011) Marine pharmacology in 2007–8: Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 153, 191-222.
4. Nwosu, F., J. Morris, V. A. Lund, D. Stewart, H. A. Ross & G. J. McDougall (2011) Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. *Food Chemistry,* 126, 1006-1012.
5. Guinea, M., V. Franco, L. Araujo-Bazán, I. Rodríguez-Martín & S. González (2012) In vivo UVB-photoprotective activity of extracts from commercial marine macroalgae. *Food and Chemical Toxicology,* 50, 1109-1117.

Poster No. 73

The Effects of Tannic Acid on Heart Tissue and Blood in Experimental Hypertension

Doğaner Fulya[1], Turgut Coşan Didem[1], Özbayer Cansu[1], Saydam Faruk[1], Soyocak Ahu[1], Değirmenci İrfan[1], Güneş Hasan Veysi[1], Kurt Hülyam[1], Üstüner M. Cengiz[1], Bal Cengiz[2]

[1]Department of Medical Biology, [2]Department of Biostatistics and Medical Informatics, Medical Faculty, Eskisehir Osmangazi University, Eskisehir, TURKEY.

Studies have been shown to decrease cardiovascular disease in a variety of foods that contain polyphenol (1-3). In this study, we purposed to determine the effects of superoxide dismutase, malondialdehit and catalase in experimental hypertension model of a polyphenol tannic acid in rats.

Sprague Dawley rats were divided into 4 groups. Blood pressures of all rats were measured by tail-cuff method before the application of N (omega)-nitro-L-arginine (L-NNA). The first group was identified as a control group. Tannic acid (50 mg/kg) was given intraperitonally (ip) to the tannic acid control group. N (omega)-nitro-L-arginine (0,5 gr/l) was given orally with drinking water to the hypertension control group. The fourth group was identified as an experiment group (L-NNA+tannic acid) that was given orally L-NNA and intraperitonally (ip) tannic acid. Blood pressures were measured on 15, 20 and 30 days of application. At the end of the 30th day, superoxide dismutase, malondialdehid and catalase levels were determined in heart tissue. Hemoglobin and hematocrit levels were also determined in the blood.

Superoxide dismutase levels in the other groups were not statistically different than control group. In L-NNA + tannic acid group, Malondialdehid was decreased according to the other groups. Catalase activity statistically significant decreased in tannic acid group compared the control group. The results of this study show that the tannic acid, may have beneficial effects in the treatment of hypertension via lipid peroxidation.

References

1. Dohadwala MM, Vita JA. Grapes and cardiovascular disease. The Journal of Nutrition 2009; 139: 1788–1793.
2. Manach C, Mazur A, Scalbert A. Polyphenols and prevention of cardiovascular diseases. Curr Opin Lipidol. 2005 16(1):77-84
3. Mursu J. 2007. Polyphenols and cardiovascular health. In: Alhava E, Sulkava R, Tammi M, editors. The role of polyphenols in cardiovascular diseases. Kuopio University Publications D. Medical Sciences. Finland pp 31- 44.

Poster No. 74

The Determination of Oxidative Stress and Homocysteine And Hepcidin Status In Iron And Vitamin B12 Deficiency Anemia In Childhood

Tünay Kontaş Aşkar[1], Olga Büyükleblebici[2], Adil Hişmioğulları[3]

[1] Department Of Biochemistry ,College Of Science Çankırı Karatekin University, Çankırı, TURKEY.
[2] Aksaray Vocational School, Aksaray University, Aksaray, TURKEY.
[3] Department Of Medical Biochemistry, Balıkesir University, Balıkesir, TURKEY.

In children, the most frequent nutritional deficiency is iron deficiency. Iron, is an element that is required for oxidative metabolism and cellular immune response. In growing children megablastic

anemia is developed in the deficiency of vitamin B12 due to the insufficient intake of animal protein in meals. Many free radicals and reactive oxygen species (ROS) are produced during metabolic and physiological processes in human cells. Oxidative stress is a condition that is characterized by impairment in the antioxidant mechanisms and/or increase of ROS. Aim of this study is to determine the thiobarbituric acid derivatives of oxidative stress (TBARS), total antioxidant capacity, serum homocysteine, and hepcidin levels in iron and vitamin B12 deficiency anemia in childhood.

Study was executed in 15 children who have iron deficiency anemia,15 children with vitamin B12 deficiency anemia and 15 children who is healthy (control group). The iron deficiency group was constituted with children who have <11,5 gr/dL Hb values, <30 μg/dL serum iron levels and <12 ng/mL serum ferritine levels. The vitamin B12 deficiency group was constituted with children who have <11,5 gr/dL Hb values and <200 pg/dL serum vitamin B12 levels. TBARS, total antioxidant capacity, serum homocysteine, and hepcidin levels were evaluated using commercial kits with ELISA in serum samples which were taken from children.

TBARS levels were significantly higher than control group in children who have iron and vitamin B12 deficiency ($p<0.001$), total antioxidant capacity levels were lower ($p<0.05$). Altough serum hepcidin levels were significantly lower than control group in children who have iron deficiency, there wasn't statistically significant difference in children who have vitamin B12 deficiency. Altough serum homocysteine levels in children who have vitamin B12 deficiency were higher than control group ($p<0.001$), there wasn't statistically significant difference in children who have iron deficiency when compared to the control group.

In conclusion, it is indicated that with the determination of high TBARS levels and low antioxidant capacity levels, oxidative stress occurs in children who have iron and vitamin B12 deficiency. Also, it is stated that determination of serum hepcidin levels may be helpfull for differential diagnosis in iron deficiency and homocysteine levels may be helpfull for differential diagnosis in B12 deficiency.

References

1. Coghetto Baccin A, Lauerman Lazzaretti L, Duarter Martins Brandao V, et al. Oxidative stress in older patients with iron deficiency anaemia. J Nutr Health Aging. 2009 Aug;13(8):666-70
2. Aslan M., Horoz M., Koçyğit A., et al. Lymphocyte DNA damage and oxidative stress in patients with iron deficiency anemia. Mutation Research 601 (2006) 144-149.
3. Gropper S.S., Kerr S., Barksdalr M., Non-anemic iron deficiency, oral iron supplementation, and oxidative damage in college-aged females, Journal of Nutritional Biochemistry 14 (2003) 409-415.
4. Nordberg J, Arner ES. (2001). Reactive oxygen species, antioxidants and mammalian thierodoxin systems. Free Radical Biol Med 31: 1287-1312.

Poster No. 75

Protective effect of curcumin against renal injury induced by carbon tetrachloride in rats

A.Adil Hişmioğulları, Ş. Ege Hişmioğulları, Ömür Karaca, F. Bahar Sunay, Serpil Paksoy, Mehmet Can, Ilter Kuş, Kamil Seyrek, Özlem Yavuz

Department of Medical Biochemistry, School of Medicine, Balıkesir University, Balıkesir, TURKEY.

Attempt was made to investigate the effect of curcumin administration on carbon tetrachloride (CCl_4) - induced nephrotoxicity in rats. Thirty male Wistar-Albino rats weighing 250-300 g were used and randomly divided into three group of 10. Nephrotoxicity was induced by CCl_4 (0.5 mg/dl in olive oil s.c.) every other day for three weeks. One group of rats received curcumin (200 mg/kg/day, by gavage for three weeks) plus CCl_4 (0.5 mg/dl in olive oil s.c.) every other day while the control group received olive oil at the same amounts and duration. Administration of CCl_4 significantly ($p<0.05$) increased the levels of renal function test such as creatinine, urea and protein. Furthermore, treatment of CCl_4 significantly elevated the oxidant status of renal tissues while decreased the anti-oxidant status of renal tissues. Histopathological studies of kidney also showed protective effect of curcumin as well. Our results suggest that curcumin might be a protective agent against renal injury induced by CCl_4 most likely through its antioxidant and free radical scavenger effects.

Poster No. 76

Evaluation of oxidant/antioxidant effects of Omega-3-fatty acids and Kefir on isoproterenol-induced myocardial infarction in rats

G. Unver[1], G. Kanbak[1], E. Soztutar[2], A. Oglakcı[1], C. Bal[3], M. Inal[1]

[1]Department of Medical Biochemistry, [2]Deparment of Anatomy, [3]Department of Biostatistics and Medical Informatics Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, TURKEY.

In the present study, the protective effects of Omega-3 fatty acids and kefir against Isoproterenol (IPH)-induced myocardial infarction (MI) was studied in male Spraque-Dawley rats. These rats were divided into 5 groups; group I was the control, group II rats were subjected to MI, group III rats were given 10% kefir in drinking water for 30 days and then subjected to MI, group IV rats were administered omega-3 fatty acids orally at

dose levels of 300 mg/kg daily for 30 days and then subjected MI, group V were given both kefir and omega-3, and subjected to MI. At the end of the experimental period, IPH (100 mg/kg s.c.) were injected twice at an interval for 24hrs to induce MI (1), and then blood samples collected from rats, subjected for diagnostic cardiac enzymes. The heart tissue specimens were used for determination of superoxide dismutase (SOD), nitric oxide (NO), and malondialdehyde (MDA) levels. When compared to group II; group III, IV and V had decreased NO levels but only the difference between groups II and V showed statistically significance. MDA levels of group III, IV and V were lower compared to groups I and II, but these decreases weren't statistically significant. SOD levels were higher in all groups than the control group, however only the difference between group I and II showed statistically significance. These evidence shows that adding Kefir and omega-3 in daily diet can help to improve cardiovascular health and they may have protective effects from MI. But further studies are needed to reveal the underlying biochemical mechanisms.

1. Antman EM, Braunwald E. (2001). Acute myocardial infarction Braunwald E, Zipes D, Libby P. (Eds) Braunwald's Heart Disease (6th edition), Saunders Company: 1184-1288.
2.. Myocardial NOS activity and connexin-43 expression in untreated and omega-3 fatty acids-treated spontaneously hypertensive and hereditary hypertriglyceridemic rats. Jana Radosinska, Barbara Bacova, Iveta Bernatova, Jana Ravanora, Anna Zhukovska, AngelaSyysh. Mol Cell Biochem (2011) 347:163-173.

Poster No. 77

Effect of calcium channel blockers on paraoxonase-1 (PON1) activity and oxidative stress

Hakan Söyüt[a], C. Türkeş[b], Ş. Beydemir[b]

[a] Department of Primary Education, Faculty of Education, Bayburt University, Bayburt, TURKEY.
[b] Department of Chemistry, Faculty of Science and Arts, Atatürk University, Erzurum, TURKEY.

Oxidative stress is a disorder occurring with the increase in favor of the production of oxygen free radicals of balance between the production of free oxygen radicals and antioxidant defense system. PON1 is one of the antioxidants in the human body. Paraoxonases are cleaning systems of free oxygen radicals. Paraoxonase protects low-density lipoprotein (LDL) from oxidation induced of Cu ions and free radicals. Paraoxonase (PON1; EC 3.1.8.1) is an enzyme belongs to A-esterases group that has ability to hydrolyze paraoxon to an active metabolite parathion which is an organophosphorus insecticide (1). PON1 is an important liver enzyme that causes hydrolysis on organophosphate agents which bound to high-density lipoprotein (HDL) and nerve gases. It has also protection role on low-density lipoprotein (LDL) oxidation, lipid peroxidation formation and bacteria endotoxins (2). In this study, PON1 enzyme purified from human serum using ammonium sulfate precipitation, DEAE-Sephadex A-50 ion-exchange chromatography and Sephadex G-200 gel-filtration chromatography that was purified approximately 230 fold with 34.2% yield. For purity determination of the enzyme SDS polyacrylamide gel electrophoresis was used and molecular mass was approximately determined 43 kDa in this method. In addition, we were investigated the effects of calcium channel blockers (amlodipine besylate, isradipine, nitrendipine and nifedipine) on PON1 enzyme activity from human serum in *in vitro* conditions. The results show that calcium channel blockers exhibit inhibitory effects on hPON1 at low concentrations with IC_{50} values and K_i constants. IC_{50} values for nifedipine, nitrendipine, isradipine, and amlodipine besylate were determined to be 0.121 mM, 0.130 mM, 0.255 mM and 0.304 mM, respectively. K_i constants were calculated to be 0.222 ± 0.049 mM, 0.151 ± 0.067 mM, 0.286 ± 0.137 mM and 0.321 ± 0.002 mM, respectively. Amlodipine besylate showed a noncompetitive inhibition, while others was inhibited in competitive manner.

References

1. İşgör MM, Beydemir Ş. Some cardiovascular therapeutics inhibit paraoxonase 1 (PON1) from human serum. Eur. J. Pharmacol 2010; 645:135-142.
2. Ekinci D, Beydemir Ş. Effect of some analgesics on paraoxonase-1 purified from human serum. J. Enzyme Inhib. Med. Chem 2009; 24: 1034–1039.

Poster No. 78

Effect of carnosine on hemorhological parameters, blood glucose and insulin levels in diabetic rats

Hande Yapislar[1], Sami Aydogan[2]

[1] Istanbul Bilim University, Medical Faculty, Physiology Department, Istanbul-TURKEY.
[2] Erciyes University Medical Faculty Physiology Department, Kayseri-TURKEY.

Much researche underlies that oxidative stress is a common mediator of micro and macrovascular complications of DM. Membrane lipids can get into the reaction with free radicals as a result of oxidative

stress and cause peroxidation. RBC membranes are vulnerable to lipid peroxiadation because of the lipid components of their membranes. The endothelium plays a major role in maintaining normal vascular tone and perfusion through the release of various vasorelaxing factors like nitric oxide (NO). Oxidative stress impairs NO signaling pathways in endothelial cells and contributes to endothelial dysfunction. Carnosine is a dipeptide having strong antioxidant effects. Aim of this study was to investigate the effect of carnosine on blood glucose and insuline levels and hemorheological factors in diabetic rats.

Wistar albino rats were used in the study. Rats were devided into 4 groups consisting of 8 rats each: I.Group: Control; II. Group: Diabetic; III. Group: Carnosine; IV. Group: Diabetic+Carnosine. Glucose, insuline, MDA(Malondialdehyde) ,NO levels were measured and red blood cell deformability indexes were calculated in groups.

In Diabetic group, insulin, NO and RBC deformability levels were decreased, blood glucose and MDA levels were increased significantly compared to control group ($p<0,05$). In Diabetic+Carnosine group it was found that glucose and MDA levels were significantly decreased, ability of RBC to deform was improved and insulin and NO levels were elevated significantly when compared to Diabetic group ($p<0,05$).

Carnosine can reduce blood glucose levels and increase insulin levels in diabetic rats which are main parameters in diabetes. Also carnosine can protect cells and tissues in diabetes against harmful effect of oxidative stress by decreasing lipid peroxidation. We can conclude that carnosine can recover vascular and microvascular circulation problems and reduce some of the risk of vascular complications by improving RBC deformability and NO levels.

References

1. World Health Organization: Diabetes Mellitus: Report of a WHO Study Group.Geneva, World Health Org, 1985 (Tech. Rep. Ser., no. 727).
2. Tavazzi B, Pierro D, Amorini AM, Fezzina G, Tuttobene M, Giardina B. Energy metabolism and lipid peroxidation of human erythrocytes as a function of increased oxidative stress. Eur J Biochem 2000 ; 267 : 684-689
3. Quinn PJ, Boldyrev AA, Formazuyk VE. Carnosine : its properties, function and potential therapeutic applications. Mol Aspects Med 1992 ; 13 : 379-444.
4. Aydogan S, Yapislar H, Artis SA, Aydogan S. Impaired erythrocytes deformability in H2O2 induced oxidative stress:protective effect of L-carnosne.Clin Hemorheol Microcirc 2008;39(1-4):93-8.

▶ Poster No. 79

Effect of coffee extracts on intracellular calcium levels in glial cells

Demet Akin*, Uzay Gormus**, Hande Yapislar***, Adriana Farah****

*Dept. of Pharmacology, School of Medicine, Istanbul Bilim University; **Dept. of Physiology, School of Medicine, Istanbul Bilim University; ***Dept. of Biochemistry, School of Medicine, Istanbul Bilim University; **** Lab. Química e Bioatividade de Alimentos & Núcleo de Pesquisa em Café Prof. Luiz C.Trugo, Instituto de Nutrição, Universidade Federal do Rio de Janeiro.

Widely used antidepressant drugs such as fluoxetine exert additional blocking effects on voltage gated Ca^{+2} channels. Differences in intracellular calcium levels may be involved in the release of monoamines, which play important role in the pathogenesis of depression[1,2,3]. Coffee is one of the most widely used beverages and is potentially beneficial to various psychiatric disorders, including depression and psychosis. In a recent study, it was suggested that caffeine intake was associated with a decreased risk of clinical depression[4]. Nevertheless, caffeine intake has been also reported to increase depressive symptoms in cross-sectional studies[5,6,7]. Therefore, the role of coffee in the promotion or prevention of depression is still unclear. In the present study, we examined the effect of coffee extracts with distinct chemical compositions, on intracellular calcium levels in glial cells, using ratiometric fura-2-based spectrofluorometric measuring. Glial cells were incubated for 2h with six coffee extracts at 0.01% (w/v), obtained from decaffeinated and non-decaffeinated green and roasted (light medium and dark roasting degrees) *C. canephora* beans. The regular green coffee extract, containing the highest levels of chlorogenic acids, and caffeine and trigonelline, among other compounds, increased intracellular calcium levels in glial cells after 2h incubation. This increase was significantly higher comparing to the effect of decaffeinated green coffee extract. Additionally, as roasting intensity increased, the effect was also weaker and results from dark roasted coffees were not different from control. Considering the thermostability of caffeine, our findings indicate that the association of caffeine with other compounds present in green coffee can promote differences in the intracellular levels of calcium and that the consumption of green coffee may be related to depressant or antidepressant effects. Further behavioral and *in vitro* studies with individual compounds are required to clarify these findings.

References

1. Majid et al., 1992. *Biochim Biophys Acta* 1136, 283-289.
2. Nakazawa & Inoue, 1992. *J. Neurophysiology* 68, 2026-2032.
3. Suh et al., 1995. *J Neurochem* 64, 1071-1079.
4. Crystal et al., 2010 *Cancer Epidemiol Biomarkers Prev*, 19,39-47
5. Greden et al, 1978. *Am. J. Psychiatry* 135, 963-966.
6. Gilliland & Andrews, 1981. *Am. J. Psychiatry* 138, 512-514.
7. Smith. Caffeine, cognitive failures and health in a non-working community sample. Hum Psychopharmacol Clin Exp 24, 29-34. 2009.

Poster No. 80

The Protective effect of curcumin on carbon tetrachloride-induced liver fibrosis in rats

Ş. Ege Hişmioğulları, A.Adil Hişmioğulları, F. Bahar Sunay, Serpil Paksoy, Mehmet Can, Hasan Akşit, Ömür Karaca, M. Tevfik Yavuz, Kamil Seyrek, Özlem Yavuz

Department of Medical Biochemistry, School of Medicine, Balıkesir University, Balıkesir, TURKEY.

Curcumin, a polyphenolic compound of turmeric has been reported to reduce hepatic fibrosis and oxidative stress in rats. The aim of the present study was to examine the protective effectiveness of curcumin on carbon tetrachloride (CCl_4) - induced fibrosis and to understand the detailed mechanisms of curcumin involved in preventing fibrosis. Thirty male Wistar-Albino rats weighing 250-300 g were used and randomly divided into three group of 10. Liver fibrosis was induced by CCl_4 (0.5 mg/dl in olive oil s.c.) every other day for three weeks. One group of rats received curcumin (200 mg/kg/day, by gavage for three weeks) plus CCl4 (0.5 mg/dl in olive oil s.c.) every other day while the control group received olive oil at the same amounts and duration. Curcumin used in this study resulted in hepatoprotective effect as shown by significantly decreased in collagen deposition in histopathological examination. In addition, curcumin administration decreased the serum levels of alanine aminotransferase and aspartate aminotransferase. Furthermore, curcumin significantly activated the hepatic total antioxidant status whereas decreased hepatic the total oxidant status. These results may indicate that curcumin could be a protective agent against the CCl_4 toxicity through its antioxidant, anti-inflammatory and free radical scavenger effects.

Poster No. 81

The impact of antibiotic drugs on PON1 activity, oxidative stress and cardiovascular diseases

İmdat Aygül[a], H. Söyüt[b], Ş. Beydemir[c], E. Köksal[d]

[a] Department of Nutrition and Dietetics, Health School, Gümüşhane University, Gümüşhane, TURKEY.
[b] Department of Primary Education, Faculty of Education, Bayburt University, Bayburt, TURKEY.
[c] Department of Chemistry, Faculty of Science and Arts, Atatürk University, Erzurum, TURKEY.
[d] Department of Chemistry, Faculty of Science and Arts, Erzincan University, Erzincan, TURKEY.

The face of death from cardiovascular diseases worldwide, the emphasis is put into cardiovascular diseases. Oxidative stress and / or weak antioxidant defense systems is considered to be major players cardiovascular diseases. PON1 is one of the antioxidants in the human body. Paraoxonases are cleaning systems of free oxygen radicals. Paraoxonase protects low-density lipoprotein (LDL) from oxidation induced of Cu ions and free radicals. Human serum PON1 enzyme (arylesterase, EC 3.1.8.1, hPON1) which is synthesized in the liver is an ester hydrolase (1). PON1 is has a anti-oxidative stress role to play by hydrolyzing lipid peroxides and preventing the oxidation of LDH. PON1 is not only the oxidation of LDL, but also prevents the oxidation of HDL (2). In this study, PON1 enzyme purified from human serum using ammonium sulfate precipitation, DEAE-Sephadex A-50 ion-exchange chromatography and Sephadex G-200 gel-filtration chromatography that was purified approximately 290.9 fold with 53.3% yield. For purity determination of the enzyme SDS polyacrylamide gel electrophoresis was used and molecular mass was approximately determined 43 kDa in this method. In addition, we were investigated the effects of some antibiotic drugs (ampicillin sodium, ofloxacin hydrochloride and acycloviron) on PON1 enzyme activity from human serum in in vitro conditions. The results show that some antibiotic drugs exhibit inhibitory effects on hPON1 at low concentrations with IC_{50} values. IC_{50} values for ampicillin sodium, ofloxacin hydrochloride and acycloviron were determined to be 41.254 mM, 76.558 mM and 89.767 mM respectively.

References

1. Alıcı HA, Ekinci D, Beydemir Ş. Intravenous anesthetics inhibit human paraoxonase-1 (PON1) activity in vitro and in vivo. Clin Bioch 2008; 41: 1384-1390.
2. Ekinci D, Beydemir Ş. Evaluation of the impacts of antibiotic drugs on PON 1; a major bioscavenger against cardiovascular diseases. Eur J Pharmacol 2009; 617: 84–89.

Poster No. 82

Protective role of melatonin on 2.45 GHz (wireless)-induced oxidative stress in lens of rats

Mustafa Nazıroğlu[1], Salih Doğan[2], Dilek Ozkaya[3], Mehmet Cemal Kahya[4], Levent Tök[3]

[1] Department of Biophysics, Medical Faculty, Süleyman Demirel University, Isparta, TURKEY.
[2] Ministry of Agriculture, Malatya, TURKEY.
[3] Department of Ophthalmology, Medical Faculty, Süleyman Demirel University, Isparta, TURKEY.

[4] Department of Biophysics, Medical Faculty, İzmir Katip Çelebi University, İzmir, TURKEY.

It has been recently reported protective effects of essential antioxidant element melatonin on electromagnetic radiation (EMR)-induced oxidative stress in different tissues (Taysi et al., 2008; Nazıroğlu et al., 2009 and 2012) although the effects of melatonin on wireless (2.45 GHz)-induced oxidative stress in lens have not been understood. We aimed to investigate the protective effects of melatonin and 2.45 GHz electromagnetic radiation (EMR) on lens antioxidant redox system in rat.

Thirty two rats were equally divided into four different groups namely Group A1: Cage control, Group A2: Sham control, group B: 2.45 GHz EMR, group C: 2.45 GHz EMR + melatonin. Groups B and C were exposed to 2.45 GHz EMR during 60 minutes/day for 30 days. End of the experiments, lens samples were taken. Lipid peroxidation levels in the lens were higher in group B than in group A1 and A2 although their concentrations were decreased by melatonin supplementation (in group C). Glutathione peroxidase and reduced glutathione values were lower in group B than in group A1 and A2 although their values were increased by melatonin supplementation.

In conclusion, melatonin supplementation in lens seems to have protective effects on the 2.45 GHz-induced increase glutathione and glutathione peroxidase.

References

1. Nazıroğlu M, Gümral N. Modulator effects of selenium and L-carnitine on wireless devices (2.45 GHz) induced oxidative stress and electroencephalography records in brain of rat. Internat J Radiat Biol 2009; 85: 680-9.
2. Nazıroğlu M, Çelik Ö, Özgül C, Çiğ B, Doğan S, Bal R, Gümral N, Rodríguez AB, Pariente JA. Melatonin modulates wireless (2.45 GHz)-induced oxidative injury through TRPM2 and voltage gated $Ca^{(2+)}$ channels in brain and dorsal root ganglion in rat. Physiol Behav. 2012;105:683-692.
3. Taysi S, Memisogullari R, Koc M, Yazici AT, Aslankurt M, Gumustekin K, Al B, Ozabacigil F, Yilmaz A, Tahsin Ozder H. Melatonin reduces oxidative stress in the rat lens due to radiation-induced oxidative injury. Int J Radiat Biol. 2008;84:803-808.

Poster No. 83

The Oxidative Stress Index may be an Important Predictive Biomarker for Successful Cardiopulmonary Resuscitation; Fancy or Fact?

Kenan Ahmet Turkdogan[1], Fatma Mutlu Kukul Guven[2], Huseyin Aydin[3], Oguz Karahan[4], Ali Zorlu[5]

[1]Isparta State Hospital Department of Emergency Medicine, TURKEY, Isparta Cumhuriyet University Medical School Department of [2]Emergency and [3]Biochemistry, TURKEY, Sivas [4]Dicle University Medical School Department of Cardiovascular Surgery, TURKEY, Diyarbakir [5]Bulanik State Hospital Department of Cardiology, TURKEY.

Oxidative stress acts a mechanism with a systemic role in the pathogenesis of atherosclerosis, cancer, and other chronic diseases. Oxidative stress during cardiac arrest may violate myocardial enzyme activities and thereby deteriorate myocardial functions in hypoxic ischemic process. The aim of this study was to investigate the relation between oxidative stress index levels and the success rate of the cardiopulmonary resuscitation (CPR) action.

A total of 90 in-hospital or out-of-hospital cardiac arrest patients and 40 age- and sex-matched healthy volunteers as the control group were evaluated prospectively. The patients were classified according to the CPR response into a successful group (n = 46) and a failed group (n = 44). 10 cc of venous blood samples were taken from all subjects, total antioxidant capacity and total level of oxidative stress were studied. TAS, TOS rate obtained from the oxidative stress index and all parameters were compared statistically.

Each group was statistically similar in terms of demographic findings (such as age, sex, hypertension, diabetes, coronary artery disease). Also the CPR durations and specialties were compared. OSI levels were significantly lower in successful CPR group ($p<0,001$). Additionally other ventricular specialties were slightly higher in failed CPR group (Table 1).

The oxidative stress may affect the response of myocardial tissue negatively and it may determine the success of the CPR. It may presents important tricks to detect the OSI levels for the fallow up of the determinants of success, such as the reversibility, additional electrophysiological disorders and hospitalization.

Poster No. 84

Effect of High Fructose Diet on Visfatin and IL-6 Levels in Liver and Fat Tissues

Meltem Özgöçmen[1], Alpaslan Gökçimen[2], Hakan Darici, İ. Aydın Candan[1], Meral Öncü[1]

[1] Department of Histology and Embryology, Süleyman Demirel University, Isparta, TURKEY.
[2] Department of Histology and Embryology, Adnan Menderes University, Aydin, TURKEY.

Nonalcoholic Fatty Liver Disease (NAFLD) has recently gained increasing importance as the most common of liver disorders. NAFLD comprises a spectrum of chronic liver diseases, starting from a simple fatty liver which may progress to steatohepatitis, fibrosis and cirrhosis. The link between obesity, insulin resistance and NAFLD and its more severe from called nonalcoholic steatohepatitis (NASH) remain as the major focus of investigation to enlighten the pathogenesis and the treatment of the disease. Adipokines which are secreted from adipose tissues increases with the body mass.

IL-6 is an inflammatory cytokine, secreted from adipose tissue. IL-6 causes insulin resistance which is related with advanced Type II diabetes. Similar to Leptin, IL-6 is also known to increase endothelial adhesion molecules, inhibits CRP and inflammation. Visfatin (aka PBEF) was found in the inflammatory cells and vascular fat tissues. Visfatin acts like insulin via binding insulin receptors in tissues such as liver, muscle and fat therefor protects against insulin resistance.

Most adipokines increased in the fatty liver but changes of the levels of Visfatin and IL-6 remains controversial. We aimed to investigate role of Visfatin and IL-6 in NAFLD and compear with adipose tissues.

29 Swiss mice used in experiment divided as one control (8 mice) and one experiment (21 mice) groups. All animal had free access to food. Control animals were fed with tap water while experiment groups were fed with 30% fructose solution for 4, 5 and 6 weeks respectively. At the end of feeding period animals were sacrificed, liver, fat and blood tissues were taken for histological and biochemical analysis.

Statistically meaningful differences were found between control and experiment groups in histological scores, blood ALP, AST, T.Chol and glucose levels. Increased visfatin and IL-6 staining observed in IHC sections of fat and liver tissues so it is concluded that visfatin and IL-6 levels were increased with fat gain.

It could be concluded that high fructose diet caused serious damage and lipogenesis accompanied by visfatin and IL-6 increase in mice. Our study may be helpful to further studies related with liver lipogenesis and adipokines.

Poster No. 85

Antioxidant effects of alpha-lipoic acid and n-acetyl cysteine on acrylamide-induced gonotoxicity in male rats

Meral Yüksel[1], A.Özer Şehirli[2], Enise Çavuşoğlu-Dalgıçdir[3], Olgu Enis Tok[4], Can Erzik[3], Feriha Ercan[4], Goncagül Haklar[5]

[1] Department of Medical Laboratory, Vocational School of Health Related Professions.
[2] Department of Pharmacology, School of Pharmacy.
[3] Department of Medical Biology.
[4] Department of Histology and Embryology.
[5] Department of Biochemistry, School of Medicine, Marmara University, Istanbul, TURKEY.

Acrylamide (ACR) is a water-soluble, vinyl monomer used in preparing polymers and copolymers containing polar functional groups. Exposure to monomeric ACR has the potential for adversely affecting male reproductive system, whereas females seems resistant. The aim of this study is to determine the effects on oxidative stress parameters of alpha-lipoic acid (LA) and n-acetyl cysteine (NAC) on acrylamide-induced male gonotoxicity. Male Sprague-Dawley rats were included in the study. ACR was given at a dose of 45 mg/kg/day. LA group received additionally 35 mg/kg/day LA, and NAC group received 150 mg/kg/day NAC. Controls were injected saline at the same dose. After 10 days their testes were removed and the degree of oxidative stress was measured by glutathione (GSH), malondialdehyde (MDA), luminol and lucigenin enhanced chemiluminescence (CL) methods. Additionally DNA fragmentation and histopathological evaluation was also determined. MDA levels in ACR group was significantly higher than controls (22,3±8,4 vs. 9,4±2,6 nmol/mg tissue; p<0,001). LA and NAC reduced MDA levels in ACR toxicity (8,8±2,2 vs. 8,5±3,2 nmol/mg tissue; p<0,001). Because GSH levels are significantly lower in ACR group with respect to the control group (1,0±0,2 vs. 2,4±0,5 μmol/g tissue; p<0,001). LA and NAC increases GSH levels in testes after ACR toxicity significantly (2,2±0,7 vs. 2,0±0,3 μmol/g tissue; p<0,001 and p<0,01, respectively). Luminol (which is specific for .OH, H_2O_2, HOCl) and lucigenin (for O_2.-) enhanced CL measurements were higher in ACR group than controls (Lum: 16,8±6,3 vs. 10,1±2,5 rlu/mg tissue; p<0,05; 19,4±7,7 vs. 9,1±2,7 rlu/mg tissue; p<0,001). Because LA and NAC has no significantly effects on luminol CL. Only LA has a significantly reducing effect on lucigenin enhanced CL in ACR induced male rat testes (11,9±1,6; p<0,05). DNA fragmentation, which is a non-specific marker for apoptosis was not significantly changed between groups. Histopathological observations show cellular degeneration in testes in ACR group. In conclusion, ACR induced toxicity in male rats increases oxidative stress which results with increased MDA, reduced antioxidative capacity (GSH), and increased free radical release. LA infusion was more protective against oxidative stress testicular tissues after ACR toxicity than NAC.

References

1. Tyl R.W., Marr M.C., Myers C.B., Ross W.P., Friedman M.A.: Relationship between acrylamide reproductive and neurotoxicity in male rats. Reprod Tox. 2000; 14: 147-157.

2. Alturfan A.A., Tozan-Beceren A., Şehirli A.Ö., Demiralp E., Şener G., Omurtag G.Z.: Resveratrol ameliorates oxidative DNA damage and protects against acrylamide-induced oxidative stress in rats. Mol Biol Rep 2012; 39: 4589-4596.

Poster No. 86

Efficacy of the St John's Wort oil on oxidative stress induced by indomethacin on mucosal damage

Moheb Saadat S, Kurt H, Özbayer C, Özdemir Z, Tuncel T, Değirmenci İ, Üstüner Mc, Güneş Hv

Department of Medical Biology, Medical Faculty, Eskisehir Osmangazi University, Eskisehir, TURKEY.

Even though non-steroidal antiinflammatory drugs (i.e: indomethacin) are used for the treatment of pain, these drugs may cause side-effects, especially in the gastrointestinal tract, and these are induced development of reactive oxygen species (ROS). Antioxidants may also play an important role in the prevention of mucosal damage. In recent years, the antioxidant properties of numerous plant compounds have been widely reported (1). *Hypericum perforatum* L., commonly known as St John's wort is a traditional herb that has been used to treat many diseases. St John's wort extract has also effects as an antiinflammatory, antimicrobial, wound healing, antioxidant, antineoplastic, antianxiety, antidepressant and antiulcerative effects (2, 3).

In this study, male Sprague-Dawley rats were used. 1 hour before mucosal damage was induced, St John's wort oil (2 ml/kg) was added into the groups. Mucosal damage is induced by intragastrically applying 30 mg/kg indomethacin. From samples of stomach, gastric pH, analysis of gastric mucus and ulcer index were calculated. In stomach homogenates SOD, MDA and CAT levels were determined.

Ulcerative control group showed higher scores than control group which was treated with St John's wort oil; when ulcer scores, gastric mucus and pH level of stomach are compared. While SOD, MDA and CAT were high in ulcerative group, St John's wort oil treatment decrease lipid peroxidation and increase antioxidant enzyme production.

Consequently, it was defined that St John's wort oil has an antioxidative effect and may help prevention of mucosal damage.

References

1. Halici M, Odabasoglu F, Suleyman H, Cakir A, Aslan A, Bayir Y. Effects of water extract of Usnea longissima on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. Phytomedicine. 2005 Sep;12(9):656-62.
2. Zdunić G, Godevac D, Milenković M, Vucićević D, Savikin K, Menković N, Petrović S.: Evaluation of Hypericum perforatum Oil Extracts for an Antiinflammatory and Gastroprotective Activity in Rats. Phytother Res. 2009 Nov;23(11):1559-64.
3. Abdel-Salam OM. Anti-inflammatory, antinociceptive, and gastric effects of Hypericum perforatum in rats. ScientificWorldJournal. 2005 Aug 8;5:586-95.

Poster No. 87

Evaluation of Lymphocyte DNA Damage and Oxidative Status in Silver Jewelry Workers

Necmettin Aktepe[1], Abdurrahim Kocyigit[2], Abdullah Taskin[2], Yunus Yükselten[1], Cumali Keskin[1], Hakim Çelik[2]

[1] Mardin Artuklu Universty School of Health.
[2]Harran Universty Medical Faculty Biochemistry Department

Silver has long been valued as a *precious metal,* and it is used to make ornaments, jewelry, high-value tableware, utensils and currency coins. Human exposure to silver and silver compounds can ocur orally, dermally, or by inhalation. It has been known that chronic inhalation exposure of workers to silver dusts resulted in some detrimental effects. In this study, we investigated genotoxic and oxidative effects of silver exposure in silver jewelry workers. DNA damage in mononuclear leukocytes was measured using the alkaline

comet assay. Serum total antioxidative status (TAS), total oxidative status (TOS), total thiol contents and ceruloplasmin levels were measured using colorimetric methods in silver jewelry workers, oxidative stress index (OSI) was calculated using a novel automated measurement method and, findings compared with non-exposed healthy subjects. Serum TOS, OSI and ceruloplasmin levels were found significantly higher in silver exposed group than those of non-exposed group ($p<0.001$, $p<0.001$, $p<0.01$ respectively). However, serum TAS levels and total thiol contents of silver exposed group were found significantly lower ($p<0.01$, $p<0.001$ respectively). The mean values of mononuclear leukocyte DNA damage were higher than control subjects but, it was not statistically significant. Thus, the findings of the present study reveal that, exposure of silver in silver jewelry workers cause oxidative stress but not an accumulation of DNA damage

Key words

Silver, DNA damage, oxidative stress, comet assay.

Poster No. 88

The curative effects of Olea europaea L. leaf extract on carbon tetrachloride induced liver injury and the oxidative stress

Üstüner MC.[1], Çolak E.[1], Tekin N.[2], Üstüner D.[3], Çolak E.[4], Yangi B.[1], Burukoğlu D.[5], Özbayer C.[1], Akyüz F.[2], Güneş Hv.[1], Değirmenci İ[1]

[1]Department of Medical Biology, [2]Department of Biochemistry, [4]Department of Biostatistics and Medical Informatics, [5]Department of Histology and Embryology, Medical Faculty, [3]Vocational School of Health Eskisehir Osmangazi University, Eskisehir, TURKEY.

We investigated the curative effects of *Olea europaea* L. leaf extract; the most popular plant materials used in traditional medicine in Mediterranean countries (1), on carbon tetrachloride induced liver injury and oxidative stress.

All experiments were carried out on 3-4 month old 42 male Wistar albino rats. CCl_4, CCl_4 natural recovery and curative groups received CCl_4 (0.2 ml/kg) dissolved in olive oil (1:1) intraperitoneally for the first 10 day to generate liver injury. The following 14 days, the curative group was intragastrically received the *Olea europaea* L (80 mg/kg) (2) leaf extract dissolved in distilled water by gavage technique and meanwhile CCl_4 natural recovery group was left to recover itself. Heart blood and liver tissue samples were collected from each rat in all the groups under anesthesia following the ethics regulation. To determine the liver injury, serum levels of AST, ALT, ALP and LDH were measured. The antioxidant enzymes activities were determined by measuring SOD and CAT both in liver tissue and in blood. Also, MDA levels, a marker of lipid peroxidation, were measured. DNA fragmentation was analyzed from liver tissue samples.

The significant differences were found between curative and natural recovery groups according to ALT, MDA in liver and blood, CAT in blood. DNA fragmentation was increased by CCl_4 application and the fragmentation was decreased in the curative group.

We concluded that *Olea europaea* L leaf extract has curative effect on decreasing the oxidative stress and liver damage induced by CCl_4.

References

1. Scheffler A, Rauwald HW, Kampa B, Mann U., Mohr FW, Dhein S, 2008, *Olea europaea* leaf extract exerts L-type Ca^{2+} channel antagonistic effects, Journal of Ethnopharmacology, 120:233-240.
2. Dekanski D, Ristić S, Mitrović DM, 2009, Antioxidant effect of dry olive (Olea europaea L.) leaf extract on ethanol-induced gastric lesions in rats, Mediterr J Nutr Metab,2:205-211.

Poster No. 89

The effects of S-allyl cysteine on lung tissue in Rat Model of Lipopolysaccharide (LPS) - Induced Sepsis

Tekin N.[1], Aydın O.[1], Bayraktar O.[1], Akyuz F.[1]

[1] Department of Medical Biochemistry, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, TURKEY.

The generation of reactive oxygen species (ROS) triggered by bacterial endotoxin lipopolysaccharide (LPS) plays a key role during the pathogenesis of sepsis (1). The present study examined the hypothesis that S-allyl cysteine (SAC), organosulfur compounds found in garlic extract has been shown to posses several antioxidant properties (2), would reduce oxidative stress-associated sepsis. To test this hypothesis, 32 male Wistar rats were divided into 4 groups of 8 rats in each. These were group 1, untreated (standart diet); group 2, SAC control (50 mg/kg SAC administered orally twice a day); group 3, sepsis control (single dose of 5 mg/kg of LPS i.p.); and group 4, Sepsis+SAC (5 mg/kg LPS i.p.+ 50 mg/kg SAC administered orally twice a day) groups. Animals were sacrificed 2days after LPS application. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured in serum. Myeloperoxidase (MPO), nitric oxide (NO) and DNA fragmentation were measured from homogenates isolated from the lung. Sepsis significantly increased ALT, AST and ALP levels. In lung tissue MPO and NO levels were elevated in group 3 compared with group 1. MPO activity and NO levels were decrease by SAC application in group 4 compared with group 3. Application of SAC to septic rats wasn't had significant effect on DNA fragmentation. The increase in NO levels and MPO activity in sepsis group demonstrate the role of oxidative mechanisms in sepsis-induced tissue damage. In conclusion, SAC abrogates LPS-induced markers of liver injury and suppresses the release of NO and MPO in lung tissue via its antioxidant properties.

Referance

1. Singhb N, Li L. 2012, Reduced oxidative tissue damage during endotoxemia in IRAK-1 deficient mice, Molecular Immunology 50: 244–252.

2. Garcia E, Villeda-Hernandez J, Pedraza-Chaverri J, Maldonado PD, Santamaria A. 2010, S-allylcysteine reduces the MPTP-induced striatal cell damage via inhibition of pro-inflammatory cytokine tumor necrosis factor-_ and inducible nitric oxide synthase expressions in mice, Phytomedicine 18: 65–73.

Poster No. 90

Investigation some oxidant/antioxidant parameters during the estrous cycle in cows

Nurettin Aydilek[1], Ö. Varışlı[2], Ş. Selek[3], Ö. Korkmaz[4], M.O. Atlı[5], A. Taşkın[3]

[1] Department of Physiology, Harran University, Faculty of Veterinary Medicine, Sanliurfa, TURKEY.
[2] Department of Reproduction and Artificial Insemination, Harran University, Faculty of Veterinary Medicine, Sanliurfa, TURKEY.
[3] Department of Biochemistry, Harran University, Faculty of Medicine, Sanliurfa, TURKEY.
[4] Department of Obstetrics and Gynecology, Harran University, Faculty of Veterinary Medicine, Sanliurfa, TURKEY.
[5] Department of Obstetrics and Gynecology, Dicle University, Faculty of Veterinary Medicine, Diyarbakır, TURKEY.

Reactive oxygen species (ROS) have multifunctional roles in reproductive functions. To comprehensively evaluate the relation between ROS and fertility problems, it has to be known physiological variations during the normal estrous cycle in healthy cows. In the present study, 25 healthy multiparous Holstein dairy cows having regular estrous cycles were used. Cows were synchronized by ovsynch protocol (by using buserelin acetate and cloprostenol sodium). Oxidant [lipid hydroperoxide (LOOH), total oxidant status (TOS), oxidative stress index (OSI)] and antioxidant parameters [total antioxidant status (TAS), total free sulfhydryl groups (SH), ceruloplasmin (CP), paraoxonase-1 (PON), arylesterase (ARE), uric acid (UA)], lipid profile [triglyceride (TG), total cholesterol, high density lipoprotein (HDL)] and hormones [estradiol (E), progesterone (P)] levels in plasma were assayed on estrous, metestrous, diestrous and proestrous periods of the estrous cycle . Oxidative parameters (TOS, OSI, LOOH) and antioxidant parameters (TAS, SH, UA) significantly decreased during metestrous and diestrous periods ($p<0.05$). There were positive correlations among TOS, OSI, LOOH, TAS, SH and UA. On the other hand, cholesterol and HDL-C values significantly decreased during metestrous and diestrous periods. Levels of CP and ARE activity did not change during the estrous cycle. Activity of PON decreased in metestrous ($p<0.05$) while it increased in diestrous ($p<0.05$) compared to other periods. In conclusion, these results demonstrated that especially TAS, SH and UA showed adaptive response to oxidative parameters (TOS, OSI, LOOH) and there was a dynamic balance between oxidant and antioxidant status in healthy cows during the estrous cycle.

Poster No. 91

Comparison of plasma levels of hypoxanthine, xanthine, uric acid, nitrite, allantoin and xanthine oxidase activity in normal and preeclamptic pregnancy

Oguz Elmas[1], Yakup Aliciguzel, Onur Elmas, Tayyup Simsek

[1] Korkuteli State Hospital, Biochemistry Laboratory, Antalya, 07800, TURKEY.

Preeclampsia is a syndrome that is found about 1-5 % percent in all pregnancies, which is characterized with hypertension and proteinuria. Especially in last fifteen years, lots of studies are experienced related with free radical theory, for explaining the preeclampsia. In our study, we detected the roles of hypoxanthine, xanthine, uric acid, nitrite, allantoin and xanthine oxidase activity which are related to endothelial dysfunction, in normal and preeclamptic pregnants. Nitrite was measured via an electrochemical method and other parameters were detected via HPLC method. Hypoxanthine, xanthine, uric acid, allantoin and xanthine oxidase activity has been found with higher levels in preeclampsia than normal pregnants levels (Table 1). Other parameters are not changed in both groups. According to our results, we concluded increased xanthine oxidase activity may play a role for endothelial dysfunction in preeclampsia, and higher levels of hypoxanthine, uric acid and allantoin are strong evidences of free radical induced injury in preeclampsia.

	Normal (n=20)	*Preeclamptic (n=20)*	*% Variation*	*P Value*
Hypoxanthine (mM/l)	1.902 ± 0.475	2.306 ± 0.384	21.24	<0.05*
Xanthine (mM/l)	11.069 ± 6.155	8.400 ± 4.399	24.11	>0.05
Uric acid (mM/l)	233.004 ± 50.58	378.567 ± 63.838	62.47	<0.05*
Allantoin (mM/l)	23.461 ± 5.874	35.530 ± 12.810	51.44	<0.05*
Nitrite (mM/l)	3.716 ± 1.581	4.142 ± 1.746	11.46	>0.05
Xanthine Oxidase Activity (mM/min/l)	0.253 ± 0.125	0.488 ± 0.217	92.88	<0.05*

Table 1: Parameters in the study, (µM = micromole, l=liter, min=minute). The results were given as arithmetic average ± standard deviation.

Poster No. 92

Renovascular hypertension and nitrogen (II) oxide level in case of usage of the different antihypertensive drugs (experimental research)

Lyashchenko Ol'ga[1], Krutikov Ye.S.[1], Kubyishkin A.V.[1]

[1] Crimean state medical university named after S.I. Georgievskiy Ukraine, 95006, Simferopol, 5/7, Lenin Avenue.

Nitrogen (II) oxide (NO) is an essential agent which provides a connection in between different types of cells that are the part of the cardiovascular system NO is able to regulate renal and cardiovascular homeostasis. In physiological condition NO is involved in adjustment of the cardiovascular system to the increased metabolic load. Insufficient amount of NO can lead to different diseases such as arterial hypertension, ischemic heart disease and atherosclerosis.

The aim of our research was to determine the level of the NO in the peripheral blood of the rats in case of the artificially induced renovascular hypertension.

Our research was made on 40 Wistar rats (males and females); weight 74-110g (the age of the rats – 1 month old). Ten rats served as a control. For solving this problem we created an experiment. We induced renovascular hypertension on all of the rats that were under experiment. The treatment started after three month of proved hypertension condition in rats. Pharmacological correction was used during 3 month. In the first experimental group we used angiotensin-converting enzyme inhibitors, in the second group – calcium channel blockers, in third – combined action of the above mentioned drugs. The level of the NO was determines according to the Griss method.

Concentration of the NO reached its maximum in the second experimental group. The minimal concentration of the NO was noticed in the first experimental group.

The results received proved that monotherapy (usage of the angiotensin-converting enzyme inhibitors only) is not effective in treating renovascular hypertension.

Poster No. 93

The effect of trehalose on oxidative stress parameters, DNA damage and some sperm parameters of Angora Buck frozen/thawed semen

Pürhan Barbaros Tuncer[1], Umut Taşdemir[1], Serhat Büyükleblebici[1], Taner Özgürtaş[2], Erdem Coşkun[3], Halil Erol[1], Fevzi Nuri Aydın[2], İsmail Safa Gürcan[4]

[1] Livestock Central Research Institute, Lalahan, Ankara, TURKEY.
[2] Biochemistry and Clinical Biochemistry Laboratory, Gulhane Military Medical Academy, Ankara, TURKEY.
[3] Department of Toxicology, Faculty of Pharmacy, Gazi University, Ankara,TURKEY.
[4] Department of Biostatistics, Faculty of Veterinary, Ankara University, Ankara,TURKEY.

Few studies have been done on the effects of trehalose supplementation in the cryopreservation of Angora buck semen. The objective was to determine the effects of different doses of trehalose in Tris extender supplemented on sperm motility, plasma membrane integrity, oxidative stress parameters and comet test after post freezing-thawing process. Ejaculates collected from 5 Angora goats were evaluated and pooled at 37^{0}C. Semen samples, which were diluted with a Tris base extender containing the 6 different doses of trehalose (12.5, 25, 50, 75, 100 and 150 mM) and a base extender with no additives (control) for a total of 7 experimental groups, were cooled to 5^{0}C and frozen at a programmed rate of 3^{0}C /min from +4 to –10^{0}C; 40^{0}C/min from –10 to –100^{0}C; 20^{0}C/min from –100 to –140^{0}C in a digital freezing machine. Frozen straws were thawed individually at 37^{0}C for 30 s in a water bath for subjective and CASA motility and, HOS test evaluation. Biochemical assays were performed in a spectrophotometer and commercial kits were used. DNA damage analysis was performed by Comet Image Analysis (COMET III) programme. The freezing extender supplemented with 50 mM trehalose led to higher percentage of computer assisted semen analyzer (CASA) sperm motility (53.6±4.7%) when compared to the other groups and control ($P<0.05$). Moreover 25 and 50 mM trehalose treatment resulted in greater sperm subjective motility with an improvement over the control and other groups (61.9±3.40% and 61.9±4.11%, respectively, $P<0.001$). Additionally, 25 and 50 mM trehalose dose (56.1±2.6% and 53.9±1.89%, respectively) gave higher percentages of membrane integrity assessed by HOST than those of the other groups ($P<0.01$). The freezing extender supplemented with 50 mM, 100 mM and 150 mM trehalose led to higher GSH, LPO and CAT (mU/ml-10^9 cell/ml) values than other groups (108.1±11.1, 21.3±3.9 and 36.2±11.4, respectively). However, the cryoprotectans did not provide difference on the level of LPO, GPx, GSH, CAT and total antioxidant activities ($P>0.05$). DNA damage was measured for tail length (μm), tail intensity (%) and tail movement by COMET test, which were gave the lowest value for trehalose 50 mM (50.9±8.3, 8.6±1.7 and 2.8±0.7, respectively). While all groups of trehalose did not any significantly affect the DNA damage ($P>0.05$). Our results indicate that the optimum trehalose

concentration had been determined to be 25, 50 and 75 mM in Angora goat semen. When the trehalose dose is increased, sperm motility and plasma membrane integritiy is decreased.

References

1. Pürhan Barbaros Tuncer, Serpil Sarıözkan, Mustafa Numan Bucak, Pınar Alkım Ulutaş, Pınar Peker Akalın, Serhat Büyükleblebici, Fazile Cantürk. Effect of glutamine and sugars after bull spermatozoa cryopreservation. Theriogenology, 2011; 75: 1459-1465.
2. Pürhan Barbaros Tuncer, Mustafa Numan Bucak, Serpil Sarıözkan, Fatih Sakin, Deniz Yeni, İbrahim Hakkı Ciğerci, Ahmet Ateşşahin, Fatih Avdatek, Mustafa Gündoğan. The effect of raffinose and methionine on frozen/thawed Angora buck (Capra hircus ancryrensis) semen quality, lipid peroxidation and antioxidant enzyme activities. Cryobiology, 2010; 61: 89-93.
3. Mustafa Numan Bucak, Serpil Sarıözkan, Pürhan Barbaros Tuncer, Pınar Alkım Ulutaş, Halil İbrahim Akçadağ. Effects of antioxidants on microscopic semen parameters, lipid peroxidation and antioxidant activities in angora goat semen following cryopreservation. Small Rum. Res., 2009; 81: 90-95

This study was supported by the Republic of TURKEY, Ministry of Food, Agriculture and Livestock, General

Directorate of Agricultural Research and Policy (GDAR) Project number: 09/08/04/01.

Poster No. 94

The effects of carvedilol on isolated perfused rat kidney during cisplatin-induced nephrotoxicity

Meral Erdinc[1], Levent Erdinc[2], Yusuf Nergiz[3], Ilker Kelle[1],Hasan Akkoc1 And Zeynep Erdogmus[1]

[1] Department of Pharmacology, Medical Faculty, Dicle University, Diyarbakir, TURKEY.

[2] Department of Biochemistry, Medical Faculty, Dicle University, Diyarbakir, TURKEY.

Department of Histology and Embryology, Medical Faculty, Dicle University, Diyarbakir, TURKEY.

Cisplatin is an important chemotherapeutic agent widely used in treatment of several cancers, with a severe nephrotoxic side effect. It has been known that different mechanisms such as oxidative stress play an important role in cisplatin induced nephrotoxicity .This study was performed to investigate the effect of carvedilol, an antihypertensive drug(β-adrenoceptor blocking agent) with antioxidative potential, on cisplatin-induced renal oxidative stress and nephrotoxicity in rats

Male wistar albino rats were divided into 3 groups (n=8):1-Control group 2-Cisplatin group (a single dose of cisplatin(5 mg/kg, i.p) 3- A single dose of cisplatin (5 mg/kg, i.p) + Carvedilol (2 mg/kg/day, i.p)for five days. . In all groups one of the kidneys was isolated and perfused with warmed (37 ^{0}C) and aerated (5% CO_2 in O_2) Krebs'-Henseleit solution . The other kidneys were separated and used for histopathological examinations and tissue malondialdehyde (MDA) measurements Serum urea and creatinine levels were determined . In group 2 perfusion pressures, serum urea, creatinine and tissue MDA levels were found significantly high ($p<0.001$)and widespread tubular necrosis and dilatation were observed versus other groups. In group 3, treatment with carvedilol significantly decreased ($p<0.05$) perfusion pressures, serum urea , creatinine and tissue MDA levels and diminished tubular dilatation and necrosis.

We concluded that carvedilol has protective effects against renal oxidative stress with altered renal haemodynamics during cisplatin nephrotoxicity.

Poster No. 95

Does ovariectomy have any impact on spatial memory performance in morris water maze?

Serap Yalın[1], Bora Reşitoğlu[2], Rezan Hatungil[2], Ülkü Çömelekoğlu[3], Nefise Özlen Şahin[4], Mehmet Berköz[4], Pelin Eroğlu[1], Hüseyin Beydağı[2]

[1] Mersin University, Faculty of Pharmacy, Department of Biochemistry.

[2] Medical Faculty, Department of Physiology.

[3] Medical Faculty, Department of Biophysics.

[4] Faculty of Pharmacy, Division of Biopharmaceutics, Yenisehir Campus, Mersin 33169, TURKEY.

In this study, it was aimed to investigate whether ovariectomy has any impact on spatial memory performance in Morris water maze or not. 19 Wistar Albino rats were used. They were divided into two groups as control (n=12) and ovariectomy (n=7). Memory performance of the rats were evaluated employing long-term memory experiment in a Morris water maze apparatus. In the first four days of the experiment, the mean latency to reach platform was recorded as well as time spent in quadrants in one minute of the fifth day. In order to test the compliance of latency data to normal distribution, Shapiro-Wilk's test for normality was applied. On the other hand, the comparison of the control and ovariectomy groups in terms of daily latency data was carried out using Mann Whitney U test. Furthermore, the comparison of days regarding the latency data was performed with Repeated Measures ANOVA test and then, pairwise comparisons of this test were carried out with LSD test. In the control group, a significant difference was detected between the 1. day throw and the 3.-4. days' throws, regarding the latency to reach the platform ($p=0.001$). In the ovariectomy group,

latency to reach platform was found significantly different upon comparison of the 1. day to all days in which the experiments were conducted (for ovariectomy groups: p=0.007, p=0.027, p=0,0005, respectively). No significant difference was found among different days when groups were compared with each others (p>0,05). In the throw of the fifth day, a significant difference was determined in both control and ovariectomy groups, regarding the time spent in quadrants (p=0.003, p=0.001, respectively). Based on the data, it can state that ovariectomy does not have any impact on latency to reach the platform in the first four days of throws and the time spent in the east quadrant following the throw of the rats into Morris water maze on the fifth day.

Poster No. 96

Effects of the Egonol on some xenobiotic-metabolizing enzymes in rat liver

Serap Şahin Başak[a], Safiye Emirdağ Öztürk[b], Yavuz Siliğ[c]

Department of Biochemistry[a], Faculty of Science, Cumhuriyet University, 58140 Sivas, TURKEY.
Department of Chemistry[b], Faculty of Science, Ege University, 35100 Izmir, TURKEY.
Department of Biochemistry[c], Faculty of Medicine, Cumhuriyet University, 58140 Sivas, TURKEY.

Cytochrome P450 (CYP)-dependent monooxygenaseis the primary oxidation enzyme system involved in detoxification and bioactivation of a number of drugs, environmental pollutants and chemical carcinogens. Cytosolic GST is an important enzyme for conjugation reactions, particularly glutathione conjugate formation. CYP and GST are responsible for both inductive, as well as inhibitory effects of many exogenous factors including herbal medicine (1). *Styrax officinalis* L. is a member of the Styracaceae family, which is constituted of small trees and shrubs. It has been found from in Palestine to Italy. In Turkey, it grows mainly in north, south and western Anatolia (2,3). Egonol, a natural 2-aryl benzofuran, is known to be an effective pyrethrum synergist (4). Egonol and its derivatives attracted the attention of synthetic chemists due to their antibacterial and antifungal (5), anti-complement (6) activities besides their considerable cytotoxic activities against human leukaemic HL-60 cells (7).

Adult Wistar albino rats weighing about 150-200 g each and nourished under normal conditions were used in this study. Rats were divided into three groups each of which contains eight animals. Egonol were administered to rats by gavage at dose levels of 10 mg/kg (Group 1) and 20 mg/kg (Group 2) for consecutive 3 days. Animals in the control group administered water by gavage (2.0 ml/kg body) for consecutive 3 days. The effect of Egonol isolated from seeds of *Styrax officinalis* L. on drug-metabolizing enzymes, such as N-nitrosodimethylamine demethylase (cytochrome P4502E1), NADPH cytochrome c reductase, NADH cytochrome b5 reductase, glutathione-S-transferase and glutathione level were studied in Wistar albino rat liver. Whereas a significant decrease was observed in glutathione level in both groups, a significant increase was observed cytochrome P4502E1 only in group 1 ($p<0.05$). GST, NADPH cytochrome c reductase and NADH-cytochrome b5 reductase activities were unaltered in both groups as compared with controls($p>0.05$). The results demonstrated that egonol triggers induction of cytochrome P4502E1 but decrease of glutathione level in liver.

References

1. Guengerich FP. Cytochromes P-450. Comp Biochem Physiol C. 1988;89(1):1-4. Review.
2. Davis, P. H.. Edinburg, 1972; Vol. 4, p 144.
3. Vardar, Y.; Oflas, S. Qual. Plant. Mater. Veg. 1973, XXII, 145–148.
4. Takanashi, M.; Takizawa, Y. Phytochemistry 1988, 27, 1224.
5. Pauletti, P. M.; Araujo, A. R.; at al. Phytochemistry 2000, 55, 597.
6. Min, B. S.; Oh, S. R.; at al.Planta Med. 2004, 70, 1210.
7. Hirano, T.; Gotoh, M.; Oka, K. Life Sci. 1994, 55, 1061.

Poster No. 97

Investigation of influence of chronic sertraline therapy on oxidative stress parameters in rats

Dilek Battal[1], Nefise Özlen Şahin[2], Marcelina Cebo[2,4], Mehmet Berköz[2], Ebru DERİCİ Eker[2], Serap Yalın[3], Ayça Aktaş[1]

Mersin University, Faculty of Pharmacy, [1]Department of Pharmaceutical Toxicology,[2] Division of Biopharmaceutics,[3] Department of Biochemistry, Yenisehir Campus, Mersin 33169, TURKEY. [4]Jagellonian University, Faculty of Pharmacy, Krakow, Poland.

Depression is a widely seen, devastating disorder causing serious physical anomalies and psychosocial disturbances. It is characterized with long term attacks, Depresyon sık görülen, uzun süreli atakları olan, high chronicity, relapse and recurrance rates. Depression can occur with different symptoms at any age. Potential to overcome biological, psychological, and sociological stress sometimes becomes very low. Antidepressants takes third

place among all drugs currently used in medicine. Selective serotoninw re-uptake inhibitors (SSRI) are 80% of the antidepressants. Sertraline, a leading antidepressant in SSRI group of medicine, is the most frequently prescribed drug. It's well-known fact that central nervous system medication, in particular, triggers oxidative stress, causing oxidation in the lipid structure of brain tissue. Also, oxidative stress plays an improtant role in the pathogenesis of psychiatric diseases such as depression. Therefore, oxidative stress in the brain tissues of depression patients using antidepressants dramaticaly increasing. Consequently, dose adjustment in antidepressant therapy might significantly reduce potential oxidative damage.

In this study, the influence of the chronic therapy with the different doses of sertraline on oxidative stress was investigated. Rats were divided into four group: control (n=6), low dose (10 mg/kg/day), moderate dose (40 mg/kg/day), and high dose (80 mg/kg/day). Each group had 6 animals. All three groups were administered sertraline with above mentioned doses by gavage throughout 28 days of the study. Control group received tap water of the same volume by gavage during the same period. In the end of the animal study, all rats were anesthezied with ketamine and their blood were withdrawn via cardiac puncture. Serum of all collected blood samples were separated and used for the determination of paroxanase (PON), catalase (CAT), and superoxyde dismutase (SOD) activities as well as malondialdehyde (MDA) for free radical levels. Statistical analysis of the data was carried out using SPSS v 16.0 program and ANOVA test. "p" value lower then 0.05 was accepted as significantly different.

In this study, it was determined that low dose sertraline administration didn't cause significant difference in serum MDA levels whereas moderate and high doses of administration results in increase. Serum SOD activities showed increase in all groups. However, the largest increase was caused by low and moderate sertraline administration. Serum catalase activities decreased in all groups. This increase was statistically significant in moderate and high dose groups. Serum paroxanase activity showed decrease in all groups, but the decrease in moderate and low dose groups were statistically significant.

Based on the data, it can be stated that moderate and high dose sertralin administration enhances oxidative stress. Therefore, dose adjustment in depression patients seems significant as it may help prevention of further progrnosis of the diseases. Furthermore, it may be suggested that the administration of nutraceuticals with antioxidant properties may play an important role in prevention of potential oxidative damage in the depression patients under the treatment of antidepressants

Poster No. 98

Effect of the crude saponin extract on DNA from ovarian cancer tissue

Hacer Cesur[a], Sercan Ozbek Yazici[b], Ismail Ozmen[a], Mehmet Okan Ozkaya[c]

[a] Faculty of Arts and Sciences, Süleyman Demirel University, Isparta, TURKEY.
[b] Health School, Mehmet Akif Ersoy University, Burdur, TURKEY.
[c] Faculty of Medicine, Süleyman Demirel University, Isparta, TURKEY.

Gypsophila species are distributed all over Turkey mostly in steppe regions (1). Several triterpenoid saponins have been isolated and identified in the genus *Gypsophila* (2). The physicochemical (surfactant) properties of saponins and their biological activities (such as anticancer, antioxidant) have led to the emergence of saponins as commercially significant compounds in food, and pharmaceutical sectors. In this study, effect of the crude saponin obtained from *Gypsophila arrostii Var. Nebulosa* on DNA from healthy and tumor tissues was investigated.

G. arrosti was collected from Atabey/Isparta regions of Turkey. The plant was first extracted with petroleum ether and then 70% ethanol in a Soxhlet apparatus. The obtained extract was precipitated with acetone (-20˚C) to get crude saponins. A group of 4 patients suffering from ovarian cancer was utilized. Two specimens were taken from each individual patient: one from the tumor mass and one from the healthy ovarian tissue. The genomic DNA was extracted from the tissues. The crude saponin and DNA were interacted and visualized by agarose gel electrophoresis method.

The differences between the effect of extract on DNA from healthy tissue and tumor tissue were observed. The bands of DNA from tumor tissues were more intense than healthy tissues. It is suggested that saponin exracts may be useful for treatment of ovarian cancer-patient. Further detailed studies of the extract are required to determine which of its effects. Some studies have indicated that the saponins in plants are associated with a reduced risk of cancer (3).

References

1. Yücekutlu AN, Bildacı I, 2008. Determination of Plant Saponins and Some of *Gypsophila* Species: A review of the literature. Hacettepe Journal of Biology and Chemistry, 36 (2), 129-135
2. Elagamal MHA, Soliman HSM, Karawya MS, Mikhova B, Duddeck H, 1995. Phytochemistry 38:1481-1485.
3. Xiao, JX, Huang GQ, Zhu CP, Ren DD, Zhang SH, 2007. Morphological study on apoptosis Hela cells induced by soysaponins. Toxicol. In Vitro 21:820–826.

Poster No. 99

DNA cleavage abilities of metal complexes

Cansu Tas[a], Sercan Ozbek Yazici[b], Ismail Ozmen[a]

[a] Faculty of Arts and Sciences, Süleyman Demirel University, Isparta, TURKEY.
[b] Health School, Mehmet Akif Ersoy University, Burdur, TURKEY.

Metal complexes with their efficient DNA binding and cleavage properties are the subject of intense investigation in the fields of chemistry, biology and medicine. Especially this kind of study has become a very important field in the development of DNA molecule probes and chemotherapeutics in recent years (1). In the study, the interactions of complexes with DNA in the absence or presence of H_2O_2 as were electrophoretically investigated.

To test cleavage of pBR 322 DNA by agarose gel electrophoresis experiments, supercoiled pBR322 DNA was treated with complexes. After incubation at 37oC for 2h, the mixed solution was loaded on 1% agarose gel. Gel was photographed under UV light. The efficiency of the DNA cleavage was measured by determining the ability of the complex to form linked circular or nicked circular DNA from its supercoiled form.

we found that complexes can cleave supercoiled pBR322 DNA to linear DNA (form III) compared with the control. In the presence of H_2O_2 (lanes 1, 2 and 4), the complexes resulted in formation of more intense circular supercoiled DNA (form I) and a new form. Also, the circular supercoiled DNA (form II) band was found to disappear completely while linear DNA (form III) band apparently increases for lanes 4, 7 and 8. These results are similar to that observed for some Cu (II) and Co(II) complexes used as chemical nucleases (2). In conclusion, the pBR 322 DNA treated with the complexes showed important changes in the form levels. Our findings indicate that the examined complexes induce conformational changes on supercoiled DNA. Further studies are underway to clarify the cleavage mechanism.

References

1. Dede B, Özmen I, Karipcin F, Cengiz M, 2009. Homo- and Heteropolynuclear Copper(II) Complexes Containing a New Diimine-Dioxime ligand and 1,10-phenanthroline: Synthesis, Characterization, Solvent-extraction Studies, Catalase-like Functions and DNA Cleavage Abilities. Appl. Organometal. Chem, 23(12): 512-519.
2. Lu LP, Zhu ML, Yang P, 2003.C rystal structure and nuclease activity of mono(1,10-phenanthroline) copper complex. Journal of Inorganic Biochemistry 95:31–36.

Poster No. 100

The effect of different doses of some cryoprotectans on post-thawed Angora goat sperm motility, plasma membrane integrity, oxidative stress parameters and DNA damage

Serhat Büyükleblebici[1], Pürhan Barbaros Tuncer[1], Umut Taşdemir[1], Erdem Coşkun[2], Taner Özgürtaş[3], Halil Erol[1], Emre İspir[3], İsmail Safa Gürcan[4]

[1] Livestock Central Research Institute, Lalahan, Ankara, TURKEY.
[2] Department of Toxicology, Faculty of Pharmacy, Gazi University, Ankara,TURKEY.
[3] Biochemistry and Clinical Biochemistry Laboratory, Gulhane Military Medical Academy, Ankara, TURKEY.
[4] Department of Biostatistics, Faculty of Veterinary, Ankara University, Ankara,TURKEY.

This study was conducted to determine the effects of glycerol, ethylene glycol (EG) and dimethylsulphoxide (DMSO) on sperm motility, plasma membrane integrity, oxidative stress parameters and comet test. Ejaculates collected from 5 Angora goats were evaluated and pooled at 37^{0}C. Semen samples, which were diluted with a Tris base extender containing the 3 differents cryoprotectans (glycerol, EG and DMSO) and 2 doses 3% or 6%, were cooled to 50C and frozen at a programmed rate of 3^{0}C /min from +4 to –10^{0}C; 40^{0}C/min from –10 to –100^{0}C; 20^{0}C/min from –100 to –140^{0}C in a digital freezing machine. Frozen straws were thawed individually at 37^{0}C for 30 s in a water bath for subjective and CASA motility and, HOS test evaluation. Biochemical assays were performed in a spectrophotometer and GPx for Gpx-340TM Oxis research kit, GSH for GSH Oxis research-420TM kit, Catalase for CAT520TM Oxis research kit and antioxidant capasity for Sigma-Aldrich Antioxidant assay CS 0790 kit were used. DNA damage analysis was performed by Comet Image Analysis (COMET III) programme. The freezing extender supplemented with 6% glycerol led to higher percentage of subjective and computer assisted semen analyzer (CASA) sperm motility (58.8±2.3% and 36.9±4.6%, respectively) when compared to the others espacially DMSO groups ($P<0.001$ and $P<0.01$, respectively). However, EG 6% dose (56.0±2.8%) gave rise to higher percentages of membrane integrity assessed by HOST than those of the other groups ($P<0.001$). The extender supplemented with 6% and 3% glycerol led to higher GSH (mU/ml-10^9 cell/ml) and CAT (mU/ml-10^9 cell/ml) values than other groups (37.2±4.0 and 23.4±5.1,

respectively). However, the cryoprotectans did not show any effectiveness on the maintenance of GPx, GSH, CAT and total antioxidant activities, when compared to the others (P>0.05). DNA damage was measured for tail length (μm), tail intensity (%) and tail movement by COMET test, which gave the lowest value for EG 3% 88.0±11.8, for glycerol 6% 16.3±2.7 and 9.4±2.2, respectively. While all groups of cryoprotectans did not affect the DNA damage significantly (P>0.05). Our results indicate that when DMSO was used as a cryoprotectant, sperm motility and plasma membrane integrity were supressed.

References

1. Pürhan Barbaros Tuncer, Serpil Sarıözkan, Mustafa Numan Bucak, Pınar Alkım Ulutaş, Pınar Peker Akalın, Serhat Büyükleblebici, Fazile Cantürk. Effect of glutamine and sugars after bull spermatozoa cryopreservation. Theriogenology, 2011; 75: 1459-1465.
2. Pürhan Barbaros Tuncer, Mustafa Numan Bucak, Serpil Sarıözkan, Fatih Sakin, Deniz Yeni, İbrahim Hakkı Ciğerci, Ahmet Ateşşahin, Fatih Avdatek, Mustafa Gündoğan. The effect of raffinose and methionine on frozen/thawed Angora buck (Capra hircus ancryrensis) semen quality, lipid peroxidation and antioxidant enzyme activities. Cryobiology, 2010; 61: 89-93.
3. Mustafa Numan Bucak, Pürhan Barbaros Tuncer, Serpil Sarıözkan, Pınar Alkım Ulutaş, Kenan Çoyan, Nuri Başpınar, Birol Özkalp. Effects of hypotaurine, cysteamine and aminoacids solution on post-thaw microscopic and oxidative stress patameters of Angora goat semen. Research in Veterinary Science, 2009; 87: 468-472.

This study was supported by the Republic of Turkey, Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Policy (GDAR) Project number: 09/08/04/01.

Poster No. 101

Effects of inactivated parapoxvirus ovis/zylexis® in equine polymorphonuclear leukocytes

Sinem Ülgen[1], , Çağla Parkan Yaramış[2], Erkan Rayaman[3],Ümran Soyoğul Gürer[3], Erman Or[1], Ahmet Özer Şehirli[4], Tamercan Morkaç[5]

[1] Istanbul University, Faculty of Veterinary, Department of Internal Medicine,Istanbul, TURKEY.
[2] Istanbul University, Vocational School of Veterinary Faculty, Equine and Equine Training Programme,Istanbul, TURKEY.
[3] Marmara University, Faculty of Pharmacy,Department of Pharmaceutical Microbiology, Istanbul, TURKEY.
[4] Marmara University, Faculty of Pharmacy, Deparment of Pharmacology, İstanbul, TURKEY.
[5] Interhas Limited Company, Ankara, TURKEY.

Inactivated parapoxvirus ovis (iPPVO)/Zylexis® shows strong immunomodulatory activities in several species and is used in veterinary medicine as an immunostimulatory biological for the prevention and/or treatment of infectious diseases (1). There is limited researches on the immunomodulatory activity of inactivated parapoxvirus ovis (iPPVO)/Zylexis® in horses. It was recently shown that İPPVO/Zylexis® effectively stimulates canine blood phagocytes by Schütze et al (1) In this study, it is aimed to research the effect of iPPVO/Zylexis® on polymorphonuclear leukocytes (PMNL) functions (phagocytosis and intracellular killing activity) and myeloperoxidase (MPO) level of PMNLs in horses. With this aim, 24 healthy horses with an average age of 11 years were included in the study. 10 ml venous blood samples were taken before and after administration of iPPVO/Zylexis® three times a week. PMNLs (1x10^7 cell/ml) were isolated by ficoll-hypaque gradient centrifugation method from venous blood with EDTA (0,1g/ml). Pre and after administration of iPPVO/Zylexis ®, phagocytosis and intracellular killing activity were assayed by modifying Alexander's method and MPO level of PMNLs were assayed by modifying O-diasinidin method (2,3). As a result, administration of iPPVO/Zylexis ® significantly increased the phagocytic and intracellular killing activities and level of MPO of PMNLs from equine.(p=0.0058,p=0.0050,paired t test; p=0.0070,student t testi). Prominence of the correlation between amount of MPO and functions of PMNL was shown in this study. It was concluded that iPPVO/Zylexis ® administration on horses had a supportive effect on cellular immunity and immunomodulatory effect on equine viral infections.

References

1. Schütze N, Raue R, Büttner M, Alber G. 2009. Inactivated parapoxvirus ovis activates canine blood phagocytes and T lymphocytes. Vet Microbiol.,12;137(3-4):260-7.
2. Alexander JW, Windhorst DB, Good RA. 1968.Improved tests for the evaluation of neutrophil function in human disease. J. Lab. Clin. Med.,72(1):136-48.
3. Hartert M, Bourgeois E, Grulke S, Dupont G,Caudron I, Deby C, Lamy M and Didier S.1998.Purification of Myeloperoxidase from Equine Polymorphonuclear Leucocytes.Can.J.Ver. Res.,62:127-132..

Poster No. 102

The relationship between the inhibition of Glutathione S-conjugate transport and oxidative damage in K562 cells

Sule Özdaş[1], İlhan Onaran[2], Gönül Kanıgür[2]

[1] Department of Medical Biology and Genetic, Medical Faculty, Istanbul Bilim University, İstanbul, TURKEY.
[2] Department of Medical Biology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, TURKEY.

Reactive oxygen species (ROS) damage all molecules especially on DNA molecule. It is known that, most oxidants produce ROS therefore aggravate DNA damage (1). Mainly, induced cellular DNA damage by hydrogen peroxide, which is an effective exogen agent, clearly understood (2). Also we know that, elimination of the products of xenobiotic metabolism is an important step in cellular detoxification and involves a specific transport system (3). The aim of this study is to investigate the cytotoxic and genotoxic effects of the GSH S-conjugate transport inhibitors on K562 cell line that already has an oxidative stress induced with hydrogen peroxide. Our purpose is to induce apoptosis on K562 erytroleukemic cell line, by this way. We also search the effect of the high glucose levels on that cell line. Lymphocytes are used as control group. In our study, the transport of the GSH S-conjugate in K562 cell line and lymphocytes was inhibited by several different inhibitors, such as S-hexyl GSH, Etacrinic Asid and NaF. It is found that lipid peroxidation and DNA damage are significantly increased on K562 cell line. Otherwise we as a result, apoptosis is induced by inhibition of the GSH S-conjugate transport on K562 cells, with pretreatment with high glucose. In conclusion, by the inhibition of GHSH-conjugate transport, K562 cells become more susceptible to oxidative stres that leads apoptosis.

References

1. Ziech D, Franco R, Pappa A, Panayiotidis MI. Reactive oxygen species (ROS)--induced genetic and epigenetic alterations in human carcinogenesis. Mutat Res. 2011 Jun 3;711(1-2):167-73.
2. Sang-Oh Yoon, Chang-Hyun Yun, An-Sik Chung. Dose effect of oxidative stress on signal transduction in aging. Mechanisms of Ageing and Development 123 (2002) 1597 /1604
3. Wen-Hsiung Chan. Effect of Resveratrol on High Glucose-Induced Stress in Human Leukemia K562 Cells. Journal of Cellular Biochemistry 94:1267–1279 (2005).

Poster No. 103

Investigation of The Effectiveness of Oleum *Cinnamomum Zeylanicum* on Alcohol Induced Liver Injury

T Tuncel, H Kurt, C Özbayer, S Moheb Saadat, Z Özdemir, D Burukoğlu, Değirmenci İ, Güneş H V.

The liver is the major target organs effected by chronic alcohol intake. Alcohol-induced liver injury cause the accumulation of toxic substances generated during alcohol metabolism, which in turn generates reactive oxygen species (ROS) and other free radicals. Production of ROS leads to modification of cellular macromolecules, morphological changes thus tissue damage and aberrant biochemistry may occur in the liver (1). *Cinnamomum zeylanicum* essential oil exhibits a significant antioxidant activity also cinnamon has hepatoprotective effects (2). We aimed to investigate the protective and antioxidant effects of Oleum *Cinnamomum Zeylanicum* (OCZ) on the alcohol-induced liver injury.

In our experiment, liver damage is induced by applying 70% ethanol intragastrically. OCZ (2,5 ml/kg, ig) was given 1 hour before administration of ethanol . After the experiment, rats were dissected. In the collected blood samples, AST, ALT and LDH levels were determined. MDA, SOD and CAT levels were measured in liver tissues. Liver histology was evaluated by hematoxylin and eosin staining method.

As a result of our study, in alcohol induced injury groups, AST, ALT and LDH levels were high compared to control. Liver MDA and CAT levels in injury group were significantly increase compared the control group while SOD levels were significantly decreased. As a result of the histological examination, hepatocytes of injury groups, vacuolization, sinusoidal dilatation and a partial bleeding were seen.

We demonstrated that *Oleum Cinnamomum* Zeylanicum has antioxidant effect and protective effect against alcohol induced liver injury.

References

1. Kundu R, Dasgupta S, Biswas A, Bhattacharya A, Pal BC, Bandyopadhyay D, Bhattacharya S, Bhattacharya S. Cajanus cajan Linn. (Leguminosae) prevents alcohol-induced rat liver damage and augments cytoprotective function. J Ethnopharmacol. 2008 Aug 13;118(3):440-7. Epub 2008 May 15.
2. Faix Š, Faixová Z, Plachá I, Koppel J.: Effect of Cinnamomum zeylanicum Essential Oil on Antioxidative Status in Broiler Chickens. Acta Vet. Brno 2009, 78: 411-417; doi:10.2754/avb200978030411.

Poster No. 104

Effects of various cryoprotectans on bull sperm quality and oxidative stress parameters

Umut Taşdemir[1] Serhat Büyükleblebici[1], Pürhan Barbaros Tuncer[1], Erdem Coşkun[2], Taner Özgürtaş[3], Fevzi Nuri Aydın[3], Olga Büyükleblebici[4], İsmail Safa Gürcan[5]

[1] Livestock Central Research Institute, Lalahan, Ankara, TURKEY.
[2] Department of Toxicology, Faculty of Pharmacy, Gazi University, Ankara,TURKEY.
[3] Biochemistry and Clinical Biochemistry Laboratory, Gulhane Military Medical Academy, Ankara, TURKEY.
[4] Aksaray Vocational Scholl, Aksaray University, Aksaray,TURKEY.
[5] Department of Biostatistics, Faculty of Veterinary, Ankara University, Ankara,TURKEY.

The objectives of this study were to compare glycerol (G), ethilene glycol (EG) and dimethile sulfoxide (DMSO) at different concentrations as cryoprotectans for bull sperm and to compare standard sperm parameters, plasma membrane integrity, DNA integrity as well as antioxidant activity. Three Eastern Anatolian Red bulls aging 2-3 years were used with quality semen characteristics were selected to be the source of semen. Ejaculates were collected using an artificial vagina for twice a week. The ejaculates containing spermatozoa with >80% forward progressive motility and concentrations higher than $1.0x10^9$ spermatozoa/ml were used in this study. A Tris-based extender (Tris 30.7 g, citric acid 16.4 g, fructose 12.6 g, egg yolk 20% (v/v), 1000 ml distilled water, pH 6.8, with no cryoprotectant) was used as the base extender. Based extender (T) was divided into seven parts and modified as folows; G6, T+6% G (control); EG6, T+6% EG; DMSO6, T+6% DMSO; GEG3, T+3% G+3% EG; GDMSO3, T+3% G+3% DMSO; EGDMSO3, T+3% EG+3% DMSO; GEGDMSO2, T+2% G+2% EG+2% DMSO. Pooled ejaculates were split into seven equal experimental groups and diluted to a final concentration of $15x10^6$/ml spermatozoa with the modified base extender, then cooled slowly to 4^0C equilibrated for 4 h. Diluted semen samples were loaded into 0.25 ml French straws after equilibrium and frozen in a programmable digital freezing machine (Digitcool 5300 ZB 250, IMV, France). Thereafter, the straws were plunged into liquid nitrogen at -196^0C. Frozen straws were thawed individually at 37^0C for 30 seconds in a water bath to analyse progressive motility and sperm motion characteristics as well as to assess HOS test. Biochemical assays were performed in a spectrophotometer using commercial kits. DNA damage was evaluated by Comet Assay using Image Analysis System (COMET III by Perceptive Instruments). Using EG and DMSO to replace G as a cryoprotectant did not give better result on the persentages of post-thaw sperm subjective, CASA and progressive motilities as well as sperm motility charasteristics (VAP, VSL, VCL, ALH and BCF). Spermatozoa frozen in a Tris based extender which containing 6% glycerol exibited the highest percentages of subjective (58±2.13%), CASA (43.7±2.92%) and progressive (26.4±2.64%) motilities compared to other groups (P<0.001). G6 and EG6 had higher values for membrane integrity assessed by HOS test than the other groups (P<0.001). There were no significant differences in the percentages of total abnormalities among treatment groups (P>0.05). DNA integrity was affected by type of cryoprotectant; DMSO6 and EGDMSO3 resulted in higher chromatin damage than the other groups, increasing the DNA damage. As regards antioxidant activity GPx, GSH, CAT and total antioxidant activity were affected by type of cryoprotectant, notably group GEGDMSO2 to the lowest results in comparison to the other groups (P<0.001). In conclusion, no advantages were found in using EG or DMSO to replace G in bull sperm cryopreservation. G provided the best sperm characteristics for bull spermatozoa post freezing and thawing.

References

1. Guthrie HD, Liu J, Critser JK. Osmotic tolerance limits and effects of cryoprotectants on motility of bovine spermatozoa. Biol Reprod, 2002;67:1811–1816.
2. Watson PF. The causes of reduced fertility with cryopreserved semen. Anim Reprod Sci, 2000;60–61: 481–492.

This study was supported by the Scientific and Technological Research Council of TURKEY (TUBITAK) (project no. 106G005, TURKHAYGEN-1)

Poster No. 105

The effect of zonisamide on Ca^{2+} signaling, oxidative stress, cell viability and caspase activity in experimental model of parkinson's disease in PC12 cells

Semih Gürler[1], Vedat Ali Yürekli[1], Mustafa Naziroğlu[2], Abdülhadi Cihangir Uğuz[2], Hasan Rifat Koyuncuoğlu[1]

[1] Department of Neurology, Faculty of Medicine, Suleyman Demirel University, Isparta, TURKEY.

[2] Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, TURKEY.

Parkinson's disease(PD) is a common progressive neurodegenerative condition. Randomized control studies demonstrated that zonisamide (ZNS) is effective in PD; however, the detailed mechanism of antiparkinsonian effects remains to be clarified.

We investigated the effect of zonisamide on the oxidative stress, cell viability, Ca^{2+} Signaling and caspase activitiy in the experimental model of Parkinson disease that induced by the MPP+, in neuronal PC12 cells.

PC12 cells divided into 4 groups; Group 1 (Control group), Group 2 (ZNS group), Group 3 (MPP^+), Group 4 (ZNS+MPP^+ group). In PC12 cells, glutathione (GSH), glutathione peroxidase (GSH-Px), changes in intracellular free Ca^{2+} ion, lipid peroxidation levels and caspase-3 activity were determined.

Lipid peroxidation levels were significantly higher in the MPP^+ group, but they were significantly lower in ZNS and ZNS+MPP^+ groups($p < 0.05$). GSH and GSH-Px levels were significantly lower in the MPP+ group ($p < 0.05$) but in ZNS and ZNS+MPP+ groups, GSH and GSH-Px levels were significantly higher($p < 0.01$). Cytosolic Ca^{+2} release was found to be significantly increased in the MPP^+ ($P < 0.001$) and ZNS+MPP^+ ($p < 0.01$) groups than the ZNS ($p < 0.01$) group. And also according to the control group cytosolic Ca^{+2} release was found to be significantly decreased in ZNS ($p < 0.01$) group.

Caspase-3 activity was found to be significantly lower in the ZNS ($p < 0.001$) group than the MPP^+ ($p < 0.001$) group.

In conclusion, in this study, zonisamide has a neuroprotective effect against MPP^+ neurotoxicity in experimental model of PD in PC12 cells.

Poster No. 106

The Effect of Planned Education given in Preeclamptic Pregnant Women on Oxidative Stress and Anxiety Levels

Emel Taşçı Duran[1], Serdal Öğüt[2], Mehmet Okan Özkaya[3]

[1] Süleyman Demirel University, Health Sciences Faculty, Nursing Department Gynecology and Obstetrics Nursing, 32260, Isparta/TURKEY.
[2] Süleyman Demirel University, Faculty of Medicine, Blood Center, 32260, Isparta/TURKEY.
[3] Suleyman Demirel University, Faculty of Medicine, Department of Gynecology and Obstetrics , 32260, Isparta/TURKEY.

This research was planned to examine of effect of planned education in preeclamptic pregnant women on Oxidative Stress and Anxiety Levels.

The research was an experimental study. The study population was created all the patients treated with the diagnosis of preeclampsia at SDU research and practice hospital, obstetrics and gynecology department. The sample was created that selected with purposeful sampling technique according to the criteria, patients with preeclampsia who had been treated Obstetrics and Gynecology department. Data were collected, a questionnaire and used to measure levels of anxiety and Spielberger et al (1970) developed by the State-Trait Anxiety (Anxiety) Inventory will be used. Laboratory assessments and continuity state anxiety scale will apply before and after the education about preeclampsia.

In the study sample according to the criteria were included in the group 18 cases of preeclampsia patients. Also the sampling criteria were considered as the control group 12 patients with preeclampsia.

Pregnant women that are in case group was provided education the disease. In blood samples before and after the education, in the total antioxidant capacity and total antioxidant capacity measurements carried out spectrophotometrically. The case group and control group, the total antioxidant capacity and oxidative stress index values calculated from the total antioxidant capacity compared to the values determined by the effectiveness of education. (WBC, PLT, RDW, MPV, PDW, PCT,HGB, HCT, MCV, MCH, MCHC, NE, LY, MO, EO, BA, RBC).

Data were analyzed using Statistical Package for the Social Sciences (SPSS for Windows, Client Version 16.0). The obtained data were analyzed for numerical and percentage distribution, average, and standard deviation, and a combination of one-way analysis of variance (ANOVA), t-test.

Women's average age was 30.03±4.21 years. The 53.3 % of the mothers had not information relating to the disease. The mean gestational week of women was 30.80±4.03. The 90% of women do not have high blood pressure problem before pregnancy. The 80% of women think a negative impact on itself of the disease. There are significant differences between groups (case-control) in terms means of state anxiety scale ($F=3.061$ $p=0.02$). Significant difference were found between TOS measurements that before and after education in case group ($t=6.56$ $p=0.00$). Significant difference were not found between TOS measurements of case and study groups ($t=-0.33$ $p=0.74$) Significant difference were found between TAS measurements that before and after education in case group ($t=-9.71$ $p=0.00$).

This study is important that is the first study that is patients with preeclampsia, the planned exploration of the effect of education on levels of oxidative stress. According to the results, education has an important effect on reduction of oxidative stress and anxiety.

References

1. Spielberger CD. *The effects of anxiety on computer-assisted learning.* CAI Center Technical Report 1970; (No. 14, pg. 47). Florida State University, Tallahassee.
2. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. Proc. Society for experimental Biology and Medicine 1999; 222(3): 222-35.
3. Erel O. A novel automated method to measure total antioxidant response against potent free radicals reactions. Clin Biochem 2004;37:112–9.
4. Erel O (2005) A novel automated colorimetric method for measuring total oxidant status. Clin Bioche 2005;38:1103–1111.

Poster No. 107

Effects of altered TRPC1 expression in primary human aortic smooth muscle cells

Yasemin Erac[1], Cigdem Selli[1], Petek Ballar[2], Buket Kosova[3], Metiner Tosun[1]

Departments of Pharmacology[1] and Biochemistry[2], Faculty of Pharmacy and Medical Biology, Faculty of Medicine[3], Ege University, 35100, Izmir, TURKEY.

We previously showed that transient receptor potential canonical 1 (TRPC1) and TRPC6 expression levels were reciprocally altered in aging rat thoracic

aorta (1). TRPC1 downregulation appeared to be causative as TRPC6 was upregulated likewise in TRPC1-silenced A7r5 embryonic rat aortic smooth muscle cell line (2). Therefore, we investigated whether this regulation is operational in primary human aortic smooth muscle cells (HASMCs). For this purpose, overexpression- and shRNA-vector-transfected HASMCs were used in quantitative PCR and functional analyses. Based on RT-PCR analyses, TRPC1 mRNA levels were decreased by 54% in shTRPC1-transfected HASMCs while mRNA levels of TRPC1-overexpressed cells were drastically increased by 2000 fold. In fura-2-loaded TRPC1 downregulated HASMCs, store-operated Ca^{2+} entry was elevated two fold. Although not statistically significant, store-operated Ca^{2+} entry decreased in TRPC1-overexpressed cells. These results demonstrate that TRPC1 might be regulatory in store-operated Ca^{2+} entry in HASMCs as we observed in Huh7 and A7r5 cell lines. In addition, whole genome expression analysis on total mRNA isolated from TRPC1-overexpressed HASMCs showed two fold changes in 380 transcripts involved in different signaling pathways. This study was supported in part by The Scientific and Technological Research Council of TURKEY (TUBITAK, SBAG-110S096 to MT) and by Ege University (EBİLTEM 11BİL004 to MT).

References

1. Erac Y, Selli C, Kosova B, Akcali KC and Tosun M. Expression levels of TRPC1 and TRPC6 ion channels are reciprocally altered in aging rat aorta: implications for age-related vasospastic disorders. AGE 2010; 32:223-230
2. Selli C, Erac Y, Kosova B, Tosun M "Post-transcriptional silencing of TRPC ion channel gene by RNA interference upregulates TRPC6 expression and store-operated Ca^{2+} entry in A7r5 vascular smooth muscle cells." Vascul Pharmacol 2009; 51:96-100.

Poster No. 108

Effects of the Egonol on antioxidant defense system in rat liver

Yavuz Siliğ[a], Serap Şahin Başak[b], Safiye Emirdağ Öztürk[c]

Department of Biochemistry[a], Faculty of Medicine, Cumhuriyet University, 58140 Sivas, TURKEY.
Department of Biochemistry[b], Faculty of Science, Cumhuriyet University, 58140 Sivas, TURKEY.
Department of Chemistry[c], Faculty of Science, Ege University, 35100 Izmir, TURKEY.

Styrax officinalis L. is a member of the Styracaceae family, which is constituted of small trees and shrubs. It has been found from in Palestine to Italy. In Turkey, it grows mainly in north, south and western Anatolia (1,2). Egonol, a natural 2-aryl benzofuran, is known to be an effective pyrethrum synergist (3). Egonol and its derivatives attracted the attention of synthetic chemists due to their antibacterial and antifungal (4), anti-complement (5) activities besides their considerable cytotoxic activities against human leukaemic HL-60 cells (6). It was also reported that significant activities were observed for egonol against C6 (rat glioma) and Hep-2 (larynx epidermoid carcinoma) cell lines (7).

The aim of this study was to investigate the effects on antioxidant defense system in liver of rats exposed to Egonol, that isolated from seeds of *Styrax officinalis L.* Adult Wistar albino rats weighing about 150-200 g each and nourished under normal conditions were used in this study. Rats were divided into three groups each of which contains eight animals. Egonol were administered to rats by gavage at dose levels of 10 mg/kg (Group 1) and 20 mg/kg (Group 2) for consecutive 3 days. Animals in the control group administered water by gavage (2.0 ml/kg body) for consecutive 3 days. At the last day of experiment, liver tissues were taken and homogenized. It has been determined that SOD, CAT, GR, GPx, G6PDH, XO and AO enzyme activities and MDA level in rat liver. It has been found out SOD, CAT, G6PDH, and XO enzyme activities and MDA levels were unaltered in two groups as compared with controls ($p>0.05$). While AO activity was decreased both two groups ($p<0.05$), significantly decrease was shown regarding GR and GPx activities only in 2. group according to the control group ($p<0.05$).

As a result, egonol were observed different effects on antioxidant enzyme activities in rat liver. However, not exhibiting lipid peroxidation, suggests that egonol was not effective in the formation of oxidative stress.

References

1. Davis, P. H.. Edinburg, 1972; Vol. 4, p 144.
2. Vardar, Y.; Oflas, S. Qual. Plant. Mater. Veg. 1973, XXII, 145–148.
3. Takanashi, M.; Takizawa, Y. Phytochemistry 1988, 27, 1224.
4. Pauletti, P. M.; Araujo, A. R.; at al. Phytochemistry 2000, 55, 597.
5. Min, B. S.; Oh, S. R.; at al.Planta Med. 2004, 70, 1210.
6. Hirano, T.; Gotoh, M.; Oka, K. Life Sci. 1994, 55, 1061.
7. Teles, H. L.; Hemerly, J. P.; at al.. Nat. Prod. Res. 2005, 19, 319.

Poster No. 109

Molecular pathways involved in the apoptosis of smooth muscle cells isolated from thoracic abdominal aneurysms in response to oxidized sterols

Zelal Adiguzel[1], Muge Serhatli[1], Omer Kacar[1], Altug Tuncer[2], Kemal Baysal[3], Nazli Arda[4], Ceyda Acilan[1]

[1] TÜBİTAK, Marmara Research Center, Genetic Engineering and Biotechnology Institute, 41470, Gebze, Kocaeli / TURKEY.
[2] T.C. Ministry of Health, Kartal Koşuyolu Advanced Training and Research Hospital 38846 Kartal, İstanbul / TURKEY.

[3] Dokuz Eylül University, Medical Faculty, Department of Biochemistry, 35340, İnciraltı, İzmir / TURKEY.
[4] Istanbul University, Department of Molecular Biology and Genetics, 34134, Vezneciler, Istanbul / TURKEY.

Oxidative stress is associated with several cardiovascular diseases including aortic aneurysms (AA), which is characterized by the dilatation and weakening of the aorta. Reactive oxygen species (ROS) are shown to be produced above physiological levels in both thoracic (TAA) and abdominal AAs. Reduction in ROS formation is also shown to result in decreased AAs in mice suggesting a pivotal role for ROS in aneurysm development. Oxysterols, oxygenated derivatives of cholesterol, are found abundantly in the plasma and atherosclerotic plaques. Among the oxysterols, namely 7-ketocholesterol and 25-hydroxycholesterol, lead both to induction of ROS in cells and to apoptosis in SMCs probably due to increased oxidative stress. In this study, these agents were used as a mean of ROS induction in smooth muscle cells (SMCs) to test whether cells isolated from TAAs are more prone to apoptosis and which molecules participate in the ROS induced apoptosis of TAAs through silencing of candidate genes. Our results confirmed that SMCs die mainly as a result of apoptosis as judged by cellular shrinkage, blebbing, DNA condensation/ fragmentation in response to oxysterol treatment. Cell death can be prevented by the addition of resveratrol, indicating that ROS plays a role in the apoptosis of these cells. Interestingly, there was no significant difference in cell death between TAA and control SMCs. Efficient silencing of p53, Akt1 and Akt2 showed that while apoptosis can occur through p53 independent pathways, the antiapoptotic Akt pathway appears to be important as silencing of both Akts leads to a significant increase in cell death.

Poster No. 110

Protective effects of propolis on oxidative stress caused by L-NAME administration in testis tissues of rats

Zeliha Selamoglu Talas

Department of Biology, Faculty of Arts and Science, Nigde University, Nigde, 51200 TURKEY.

Inhibition in the synthesis or bioavailability of nitric oxide (NO) plays a important function in progress of hypertension (1,2). The blocking of nitric oxide synthase (NOS) activity may reason vasoconstriction with reactive oxygen species (ROS) formation (1,2). Propolis is a resinous substance collected by honey bees from various plants (3,4). The aim of this study was to examine the effect of propolis on catalase (CAT) activity, malondialdehyde (MDA) and NO levels in the testis tissues of hypertensive rats by NG-nitro-L-arginine methyl ester (L-NAME). Rats have been received NOS inhibitor (L-NAME, 40 mg/kg, intraperitoneally) for 15 days to produce hypertension and propolis (200mg/kg, by gavage) the lastest 5 of 15 days. MDA level in L-NAME-treated group significantly increased compared with control group ($P<0.01$). MDA level of L-NAME + propolis-treated rats significantly reduced ($P<0.01$) compared to L-NAME-treated group. CAT activity and NO level significantly reduced ($P<0.01$) in L-NAME group compared to control group. There were not statistically significant increases in CAT activity and NO level of L-NAME + propolis group compared with L-NAME-treated group ($P>0.01$). These results suggest that propolis changes CAT activity, NO and MDA levels in testis of L-NAME treated rat and there by may modulate the antioxidant system.

References

1. Harrison DG, Gongora MC, Guzik TJ, Widder J. Oxidative stress and hypertension. J Am Soc Hypertens 2007; 1(1): 30-44.
2. Kanematsu Y, Tsuchiya K, Ohnishi H, Motobayashi Y, Izawa Y, Ishihara M, Ishizawa K, Abe S, Kawazoe K, Tamaki T. Effects of angiotensin II type 1 receptor blockade on the systemic blood nitric oxide dynamics in Nω-nitro-L-arginine methyl ester-treated rats. Hypertens Res 2006; 29: 369-374.
3. Maruyama H, Sumitou Y, Sakamoto T, Araki Y, Hara H. Antihypertensive effects of flavonoids isolated from brazilian green propolis in spontaneously hypertensive rats. Biol Pharm Bull 2009; 32(7): 1244-50.
4. Bhadauria M, Nirala SK. Reversal of acetaminophen induced subchronic hepatorenal injury by propolis extract in rats. Environ Toxicol Phar 2009; 27: 17-25.

Poster No. 111

The effects of L-NAME and glibenclamide on ethyl pyruvate induced cardioprotection in isolated perfused rat heart during ischemia reperfusion injury

Hasan Akkoc[1], Selcuk Tunik[2], Ilker Kelle[1], Ahmet Gurkan[1], Zeynep Erdogmus[1], Zulfiye Gul[1] and Meral Erdinc[1]

[1] Department of Pharmacology, Medical Faculty, Dicle University, Diyarbakir, TURKEY.
[2] Department of Histology and Embryology, Medical Faculty, Dicle University, Diyarbakir, TURKEY.

In this study, we investigated the effects of nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) and ATP-sensitive K(+) channel

antagonist glibenclamide on ethyl pyruvate (EP) induced cardioprotection in isolated perfused rat heart during ischemia reperfusion injury.

Fourty Male Sprague-Dawley rats, each weighing 250-300 g, were used. Rats were divided into five groups (n=8) ;Group 1:Sham-opereted, Group 2: IR, Group 3: EP, Group 4: EP+L-NAME, Group 5: EP+Glibenclamide. In all groups, after hearts were isolated and mounted on Langendorff apparatus, they were perfused with constant flow (18 ml/min) of Krebs Henseleit Solution (aired with 95% O_2 + 5% CO_2) and maintained at 37°C. Ischemia was produced for 30 min by blocking the perfusion with Krebs Henseleit Solution, and it was followed by reperfusion for 60 min. In groups 3, 4 and 5, EP (2 mM/L), L-NAME (100 μM/L) and glibenclamide (10 μM/L) were added into Krebs Henseleit Solution after stabilization period. In all groups, heart rate and coronary perfusion pressure were recorded. Myocardial injury was assessed in terms of infarct size and release of lactate dehydrogenase (LDH) enzyme. Coronary perfusion pressure in EP and EP+L-NAME groups was significantly lower as compared to the IR group ($p<0.05$). Myocardial infarct size and LDH parameters were found to be significantly different in groups 3, 4 and 5 versus IR group ($p<0.001$).

These results suggest that L-NAME and glibenclamide did not abolish ethyl pyruvate induced cardioprotection completely in isolated perfused rat heart.

Poster No. 112

The Protective and Antioxidant Effect of Hypericum perforatum on Indomethacin-Induced Renal Damage

Z. Özdemir[1], C. Özbayer[1], H. Kurt[1], S. Moheb Saadat[1], T. Tuncel[1], Mc. Üstüner[1], D. Burukoğlu[2], İ. Değirmenci[1], HV. Güneş[1]

[1] Department of Medical Biology, [2]Department of Histology and Embriyology, Medical Faculty, Eskisehir Osmangazi University, Eskisehir, TURKEY.

Although their therapeutic utility, nonsteroidal anti-inflammatory drugs have significant side effects, such as gastrointestinal and renal toxicities. Indomethacin -induced reactive oxygen species (ROS) play important role in renal toxicity (1). It has been demonstrated that *Hypericum perforatum* have an antidepressant, antiseptic, antioxidant and wound-healing effect, and may have also indicated a renal protective effect (2). We also aimed to investigate the protective and antioxidant effects of *Hypericum perforatum* oil on the indomethacin-induced renal failure.

Hypericum perforatum oil (2 ml/kg, ig) was given 1 hour before administration of indomethacin. After 6 hours of indomethacin application, rats were dissected. In the collected kidney samples, malondialdehyde (MDA) which is an indicator of oxidative stress, and antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) levels were measured in kidney homogenates. Kidney tissues were examined histologically via H&E staining method.

MDA and CAT levels of indomethacin-induced kidney damage group were high, whereas decreased levels were found in treated group. The histological examination observed that, necrosis of renal tubules, tubular damage and intravenous congestion were seen in acute kidney injury group. *Hypericum perforatum* oil treatment alleviated these effects.

Our results showed that, *Hypericum perforatum* oil have antioxidant properties and a protective effect on indomethacin-induced acute kidney injury.

Acute kidney injury, antioxidant, *Hypericum perforatum* oil, oxidative stress, rat.

References

1. Basivireddy J, Jacob M, Pulimood AB, Balasubramanian KA. Indomethacin-induced renal damage: role of oxygen free radicals. Biochem Pharmacol. 2004 Feb 1;67(3):587-99.
2. Zdunić G, Godevac D, Milenković M, Vucićević D, Savikin K, Menković N, Petrović S.: Evaluation of Hypericum perforatum Oil Extracts for an Antiinflammatory and Gastroprotective Activity in Rats. Phytother Res. 2009 Nov;23(11):1559-64.

Poster No. 113

Investigation kinetics curves of chemiluminescence in the Oncology process.

Iryna Oliynyk

Department of Electronic Devices, Lviv Polytechnic National University, Lviv, Ukraine. Centre of scientific and technical creativity of youth, Lviv, UKRAINE.

To investigate the free-radical oxidation (FRO) under the monitoring of cancer diseases, the methods of chemiluminescence (CL) are generally used. The CL of the blood serum, initiated by hydrogen peroxide, is most informative in this situation. Under investigations, as the main parameters one usually considers the maximal CL intensity and the integrated plot area.

These parameters characterise the number of short-living radicals, and the general number of radicals, which appear as a result of reaction, correspondingly (only the radicals, which recombinate with light elimination, are taken into

account). However, the kinetics of this process is still not clarified.

We consider the simulation and analysis of the kinetics under the cancer pathologies as very important.

In our study, we performed the simulation of kinetics. Assuming the non-branching of oxidation chain, we have shown the straightening of initiated CL curve in determined coordinates.

The straightening of CL curves appear both in all samples of blood serum taken from healthy people and the main part of samples under the pathologies. The bending angle of these curves can determine the intensity of oncological process. Under the medical treatment, this angle is modified and approaches the normal value.

The strongly reactive onco-processes are characterised by absence of straightening. These curves are also characterized by two maximum values, which signalize the degeneracy of oxidation process in several cases of oncopatologies. The oncomarker, unfortunately, cannot present any differences between these two types of onco-processes.

Poster No. 114

Oxidative stress induces mitochondrial alterations under diabetic neurodegeneration in cell culture models

Egor V. Dzyubenko, Stanislav I. Shram

Laboratory of Neuropharmacology, Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov Sq. 2, Moscow 123182, RUSSIA.

Diabetic neurodegeneration is being extensively researched over the last decades, but still, the mechanisms are not fully described yet. This is probably due to the lack of experiments performed using cell culture models. Only few such approaches were proposed by (Vincent, 2005) to study new drugs to treat diabetic neuropathy. In this work we have developed two different cell culture systems for modeling hyperglycemic injury in neurons of central and peripheral nerve systems. Primary cultures of neonatal rat hippocampus and chicken embryonic dorsal root ganglion (DRG) were exposed to 45 mM glucose thus modeling diabetic encephalopathy and neuropathy respectively. Both models exhibited well-defined response to hyperglycemia: mitochondrial membrane potential, shape and transport were altered significantly, while hippocampus culture was more resistant. These results are consistent with other data obtained using neuronal cultures (Schmeichel, 2003; Vincent, 2011; Chowdhury, 2012). The similar alterations were also seen under oxidative stress induced by 50 uM tert-butil-hydroperoxide (THBP). Comparing the effects of hyperglycemia and THBP in two nerve cell cultures we conclude that oxidative stress plays the key role in the development of neural disorders in both central and peripheral nerve system under hyperglycemia. These two cell culture systems may serve the specialists who need to watch neurodegenerative processes in dynamics and become a useful platform for testing new pharmaceuticals in vitro.

This work was partially supported by the Russian Foundation for Basic Research (project No 12-04-90444).

Poster No. 115

Association between TRPM5 gene polymorphisms and colorectal cancer

Serdar Öztuzcu[1], Esma Özkara[1], Celaletdin Camcı[2], Abdullah T. Demiryürek[3]

Departments of [1]Medical Biology, [2]Medical Oncology, and [3]Medical Pharmacology, Faculty of Medicine, University of Gaziantep, Gaziantep, TURKEY.

TRPM5 are expressed in the stomach, small intestine, and colon of humans. However, the contribution of the TRPM5 in colorectal cancer (CRC) is not known. The aim of this study was to investigate a possible association between TRPM5 gene polymorphisms and CRC development. A total of 85 patients operated due to CRC and 173 healthy controls with similar age and sex were included to this study. Polymorphisms were analyzed in genomic DNA using a BioMark 96.96 dynamic array system. There were significant associations between TRPM5 gene rs4929982 (Arg578Gln), rs34551253 (Ala456Thr), rs3986599 (Val254Ala) and rs886277 (Asn235Ser) polymorphisms with CRC development. CT genotype frequency in rs4929982 polymorphism was markedly high in patients. TT genotype and T allele in rs34551253 polymorphism were significantly high in the CRC group. CC genotype and C allele frequencies in rs3986599 polymorphism were low in the patients group, but CT genotype and T allele frequencies were high in the patients group. Although TT genotype and T allele frequencies were high in the patients group for rs886277 polymorphism, TC and CC genotypes and C allele frequencies were low in the CRC group. These results are the first to demonstrate the contribution of TRPM5 channel in CRC development. Our data showed that the

TRPM5 channel gene might be a risk factor for CRC, and suggested that genetic polymorphisms in the these genes modify individual susceptibility to CRC in the Turkish population.

Poster No. 116

Association between TRPM4 gene polymorphisms and colorectal cancer

Serdar Öztuzcu[1], Esma Özkara[1], Celaletdin Camcı[2], Abdullah T. Demiryürek[3]

Departments of [1]Medical Biology, [2]Medical Oncology, and [3]Medical Pharmacology, Faculty of Medicine, University of Gaziantep, Gaziantep, TURKEY.

Colorectal cancer (CRC) is an important global health problem. CRC arises as a consequence of the progressive accumulation of genetic and epigenetic alterations in colonic epithelial cells. The contribution of the TRPM4 in CRC is not known. The aim of this study was to investigate a possible association between TRPM3 gene polymorphisms and CRC development. A total of 85 patients operated due to CRC and 169 healthy controls with similar age and sex were included to this study. Polymorphisms were analyzed in genomic DNA using a BioMark 96.96 dynamic array system. There were significant associations between TRPM4 gene rs71352737 (Trp525Ter) (stop-gain mutation), rs56355369 (Asp561Ala) and rs75615609 polymorphisms with CRC development. GG genotype frequency in rs71352737 polymorphism was markedly low in patients. AG and AA genotypes and A allele were absent in the control group, but present in CRC group. Similarly, AA genotype frequency in rs56355369 polymorphism was low in the patients group. AC and CC genotypes and C allele were absent in the control group, but present in CRC group. In the rs75615609 polymorphism, CC genotype and C allele were significantly low in the patients group. However, TT genotype and T allele frequencies were significantly high in the patients group. These results are the first to demonstrate the contribution of TRPM4 channel in CRC development. Our data showed that the TRPM4 channel gene might be a risk factor for CRC, and suggested that genetic polymorphisms in the these genes modify individual susceptibility to CRC in the Turkish population. The presence of A allele in rs71352737 polymorphism and C allele in rs56355369 polymorphism may provide diagnostic markers for the CRC patients.

Poster No. 117

The ameliorating role of Caffeic Acid Phenethyl Ethyl Ester on human Choline Esterase inhibition induced Diazinon

Erkan Kıbrıslı[1], Adnan Tüfek[2], Orhan Tokgöz[2], Yaşar Yıldırım[3], Osman Evliyaoğlu[4], Osman Gökalp[3]

[1] Dicle University, Medical Faculty, Department of Family Medicine , 21280, Diyarbakır/TURKEY.
[2] Dicle University, Medical Faculty, Department of Anesthesiology and Reanimation, 21280, Diyarbakır, TURKEY.
[3] Dicle University, Medical Faculty, Department of Pharmacology, 21280, Diyarbakır, TURKEY.
[4] Dicle University, Medical Faculty, Department of Biochemistry, 21280, Diyarbakır, TURKEY.

Organophosphate (OP) poisoning is still a major cause of death in countries engaged in agriculture. Especially, when high-dose OPs pass into the bloodstream, even if oximes used in treatment, mortality rate is very high. Today, the new drugs are needed to reduce mortality in OP intoxications (OPI). In a study in rats, it is reported that Caffeic acid phenethyl ester (CAPE) prevents plasma cholinesterase (ChE) inhibition by caused Malathion. It is not investigated that the effects of CAPE on human tissues against the OPI. For this purpose, the effect of CAPE is investigated on human sera ChE inhibition caused by Diazinon which is a commonly used OP.

Effects of various concentrations of Diazinon on the activity of ChE in human sera were studied. A 10-ml venous blood sample was obtained from each of ten volunteers (five male, five female). The activities of serum enzymes were determined in each sample and these served as 0 h. Each sample was divided into 12 portions and each one served as experimental groups, as follows: control, only CAPE, Diazinon and Diazinon+CAPE groups. CAPE was only added to human sera at 0.33 M concentration. Also Diazinon was added to human sera at concentrations ranging from 0.0033, 0.033, 0.33, 3.3, and 33 M. After now, all samples was incubated with previously prepared human sera at +4°C for 24 h. The activities of serum ChE was measured in each sample at 24 h.

CAPE/diazinon ratio 1/1, 1/10 and 1/100 at concentrations, percentage of protection of the CAPE's to ChE inhibition was respectively, 35%, 63% and 66%.

CAPE can be used in OPI according to our study. But we believe that CAPE should be used in patients with ChE inhibition decreased 40% or more. Also, the concentrations of CAPE must be set not more than concentration of OPs in environment. We believe that this protocol is an important therapeutic

option in OPI because of fatal OPI occurs in patients with high blood concentrations OPs. In addition, further studies need to be put out the availability of combination of pralidoxim and CAPE in serious OPI.

Poster No. 118

The protective effects of Caffeic Acid Phenyl Ethyl Ester on human plasma cholinesterase against variety organophosphate agents

Adnan Tüfek[1], Orhan Tokgöz[1], Yaşar Yıldırım[3], M.Uğur Yüksel[1], Erkan Kıbrıslı[2], Osman Evliyaoğlu[4], Osman Gökalp[3]

[1] Dicle University, Medical Faculty, Department of Anesthesiology and Reanimation, 21280, Diyarbakır/TURKEY.
[2] Dicle University, Medical Faculty, Department of Family Medicine , 21280, Diyarbakır/TURKEY.
[3] Dicle University, Medical Faculty, Department of Pharmacology, 21280, Diyarbakır/TURKEY.
[4] Dicle University, Medical Faculty, Department of Biochemistry, 21280, Diyarbakır/TURKEY.

It is investigated that the efficacy of various antioxidant agents (AO) such as Vit-E, Vit-C, Melatonin, Caffeic Acid Phenethyl Ethyl Ester (CAPE) on organophosphate intoxications (OPIs) and expressed that its positive effects on antioxidant system. However, besides the AO activity of these agents, are found to protect from cholinesterase (ChE) inhibition induced organophosphate (OPs).

Aim: In our study, we aimed to investigate the protective effect of CAPE on inhibition of ChE with OP (3 different agents and 5 different concentrations) induced.

In our study, we investigated that the effect of 33 M concentrations of CAPE on 3 different OPs (Malathion, Methidathion and Clorpirifos etil) at 5 different concentrations (0.0033, 0.033, 0.33, 3.3 M) as in vitro on human serum which obtained 10 healthy volunteers.

In our study, we found that CAPE prevents the inhibition of plasma ChE by induced of high concentration of OPs in human blood. It is found that 0.33 M concentration of CAPE has significantly protective effects on ChE inhibition when it's concentrations is equal with the concentration of OPs (0.33) or OPs concentrations higher 10 (3.3 M) and 100 (33 M) times than CAPE concentrations.

Conclusion: CAPE can have ameliorating effects that preventing the inhibition of ChE besides AO efficiency on OPI occurred high concentrations OPs.

Poster No. 119

Effects of melatonin on antioxidant enzymes in ligature-induced periodontitis in rats lung

Muhsin Özdem[1], F. Yeşim Kırzıoğlu[2], H. Ramazan Yılmaz[3], Ayşe Yiğit[4]

[1]Abant İzzet Baysal University, Faculty of Dentistry, Department of Periodontology. Bolu, TURKEY.
[2]Suleyman Demirel University, Faculty of Dentistry, Department of Periodontology. Isparta, TURKEY.
[3]Mevlana University, Faculty of Medicine, Department of Medical Biology. Konya, TURKEY.
[4]Suleyman Demirel University, Faculty of Medicine, Department of Medical Biology and Genetics. Isparta, TURKEY.

Introduction

Melatonin is synthesized and secreted by the pineal gland and other organs (1). Melatonin hormone (N-acetly-5-methoxytryptamine) has potential action in the regulation of many physiological functions such as endocrine rhythm, protecting the nervous system, stimulation of immun system, and the protection of free radicals (2). Also melatonin stimulates the gene expression of several important endogenous antioxidant enzymes, including GSH-Px and SOD. The organs and tissues exposed to oxygen radical formation such as liver, lung, heart, brain, and skin produced melatonin lower levels (3,4). The aim of this study was to evaluate the effects of melatonin on the activities of antioxidant enzymes in the lung tissue of ligature-induced periodontitis rats.

Twenty eight male rats were divided into four groups as follows: Healty(H) saline solution(s), Hmelatonin(m), Periodontitis(P)s and Pm. Following 2-week, all rats were anaesthetized and then were sacrificed. Lung samples were collected in order to determine levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px).

MDA levels were higher in P groups when compared with H groups. GSH-Px levels lower in Ps group compared to Pm group as well as MDA level lower in Pm group. While SOD and GSH-Px levels in P groups were lower than H groups.

Melatonin might caused a decrease in MDA levels and an increase in SOD and GSH-Px levels and might regulate the activities of antioxidant enzymes of ligatured-induced periodontitis in rat lung.

References

1. Susko I, Mornjakovic Z, Alicelebic S, Cosovic E, Beganovic A. Retinal and pineal melatonin-from acircadian signal to therapeutic use. Med Arh 2004; 58: 61-64.

2. Stefulj J, Hortner M, Ghosh M, et. Al. Gene expression of the key enzymes of melatonin synthesis in extrapineal tissues of the rat. J Pineal Res 2001; 30: 243-247.
3. Lee PP, Pang SF. Melatonin and its receptors in the gastrointestinal tract. Biol Signals 1993; 2: 181-193.
4. Stefulj J, Hortner M, Ghosh M, et. Al. Gene expression of the key enzymes of melatonin synthesis in extrapineal tissues of the rat. J Pineal Res 2001; 30: 243-247.

Poster No. 120

Selenium-mediated cardioprotection against adriamycin-induced mitochondrial damage

Nurcan Dursun, Eylem Taşkın, Kalender Özdoğan

Department of Physiology, University of Erciyes, Kayseri, TURKEY.

Adriamycin (ADR) causes morphological and functional alterations in mitochondrial structure in the heart. The study's aim was to determine whether there is a protective effect of selenium (Se) on ADR-induced cardiac damage. Rats were divided into four groups: The first group was injected saline intraperitoneally (i.p.) for 21 days; the second group received 4 mg/kg i.p. ADR every alternate day for 8 days; the third group received 50 μg/kg i.p. Se for 21 days; and the fourth received the Se (for 21 days) and ADR (for 8 days) coadministration i.p. Left ventricular functions, electrocardiography parameters, and blood pressures were assessed. Mitochondrial membrane potential (MMP), adenosine triphosphate (ATP) level, and thioredoxin reductase (TrxR) activity were determined. Total antioxidant (TAS) and oxidant status (TOS) in cytosol, mitochondria of myocytes, and plasma were measured. Left ventricular data demonstrated left ventricular systolic pressure (LVSP) decreased, left ventricular developed pressure (LVDP) decreased, and left ventricular end-diastolic pressure (LVEDP) increased in ADR-treated animals, compared to the control and Se groups. ADR decreased the membrane potential and ATP level in myocyte mitochondria. TrxR activity decreased in the ADR group, compared to the Se group. Cytosolic and mitochondrial TAS decreased and mitochondrial and plasma TOS increased in the ADR group, compared to the control. The coadministration of Se with ADR attenuated left ventricular dysfunction, improved MMP and ATP levels, and prevented oxidative stress by increasing antioxidants (especially TrxR) and decreasing oxidants. We concluded that Se is effective against ADR-induced cardiac damage via the restoration of TAS and TOS, which prevented mitochondrial damage.

Poster No. 121

Beneficial Effect of Levetiracetam on Electrophysiological, Histological and Motor Strength Test of Streptozotocin-Induced Diabetic Rats

Oytun Erbaş[1], Mustafa Yılmaz[2], Fatih Oltulu[3], Altuğ Yavaşoğlu[3], Dilek Taşkıran[1]

[1] Ege University School of Medicine, Department of Physiology, Izmir, TURKEY.
[2] Mugla University School of Medicine, Department of Neurology, Mugla, TURKEY.
[3] Ege University School of Medicine, Department of Histology and Embryology, Izmir, TURKEY.

Oxidative stress and inflammation causing brain damage to the peripheral nervous system.

Diabetes Mellitus (DM) is also associated with oxidative stress. Levetiracetam (LEV) is a used antiepileptic drug and activated N-type calcium channels. Recent evidence suggests that LEV exhibits both anti-inflammatory and anti-oxidative effects. Therefore, we aimed of this study was to evaluate the effects of the animal model of diabetes induced by streptozotocin on electrophysiological, histological and engine power test.

In this study 21 Sprague-Dawley adult male rats is used. First of all streptozotocin 60 mg/kg was injected to rats. The blood glucose levels was measured from the venous sample by a glucometer at the end of the 2rd day. The rats with blood glucose levels 250 mg/dl and higher were included in the study. 20 days after , sciatic nerve was stimulated and recordings of distal latency, amplitude and duration of the compound muscle action potential (CMAP) were recorded from the 2-3. interdigital muscle. Rats were divided into

3 groups (n=7). Group 1; normal group 1 mL/kg saline, Group 2; LEV 300 mg/kg,

Group 3; LEV 600 mg/kg. Drugs were given intraperitoneally (i.p.) to rats for thirty consecutive days. 30 days after, EMG and motor tests were repeated. Rats were sacrificed and sciatic nerves were examined histologically (epineural thickness, bax-bcl-caspase immunohistohemistry) and drug effects have been revealed.

LEV groups CMAP duration and distal latency measurements were shorter and CMAP amplitude was longer compared with group1($p < 0.001$) and consisted of an increase in motor strength. In addition LEV decreased in thickness of epineural fibrosis and expression of bax-caspase (apoptotic markers)

This study demonstrated the effectiveness of LEV in the prevention of neuropathy. LEV may be considered in the prophylactic therapy of diabetic patients.

Poster No. 122

DNA-PK IS Regulated BY PKC-δ In Non-Tumorigenic And Androgen-Independent Tumorigenic Prostate Epithelial Cells

Nilgun Gurbuz[1,3], Hakan Darici[2], Aslı Baykal[1]

[1] Akdeniz University, School of Medicine, Department of Biochemistry, Antalya, TURKEY.
[2] Suleyman Demirel University, School of Medicine, Department of Histology and Embryology, Isparta, TURKEY.
[3] Suleyman Demirel University, School of Health Sciences, Department of Nursing, Isparta, TURKEY.

PKC-δ, a proapoptotic protein, has the inhibitory effect on cell growth. This effect is resulted from the loss of mitochondrial membrane potential and then the increase of cytochrome-c release from mitochondria, which are the markers of irreversible apoptotic cell death. The molecular basis of prostate cancer is strongly related with inactivation of pro-apoptotic, tumor-suppressive isozyme PKC-δ. PKC-δ not only induces apoptosis, but also regulates other cellular pathways related to tumorigenesis including DNA repair via critical mediators. DNA dependent protein kinase (DNA-PK), a nuclear serin/threonine protein kinase, plays a critical role in double-strand DNA break repair, as it has the ability to recognize any DNA damages and to initiate the binding of other repair proteins to the damaged region in double-strand DNA. We aimed to investigate whether or not DNA-PK was regulated by PKC-δ in non-tumorigenic, RWPE-1, and androgen-independent tumorigenic, PC-3, prostate epithelial cells. For this purpose, mRNA levels of DNA-PK were quantitatively determined by real-time PCR followed by the transfection of both prostate cells with PKC-δ targeted siRNA to inhibit PKC-δ gen expression. We first identified that DNA-PK was regulated by PKC-δ in both prostate cells. To take into account of many cellular carcinogenic mechanisms involved in PKC-δ and DNA-PK, the demonstration of PKC-δ/DNA-PK signaling axis in prostate cells will guide us for future investigations. We believe that the clarification of such molecular mechanisms in prostate cell will help to create new approaches in prostate cancer treatment.

Poster No. 123

Use of two biomarkers (malondialdehyde and glutathione) in liver tissue of *Capoeta umbla* (heckel, 1843) for the biomonitoring of Uzuncayir dam lake (Tunceli-TURKEY)

Cemil Ergin[1], Nuran Cikcikoğlu Yildirim[1]

[1] Tunceli University, Faculty of Engineering, Department of Environmental Engineering, TR62000, Tunceli, TURKEY.

Household wastes of Tunceli being directly discharged to Munzur and Pülümür Rivers without any pre-purification process. As a result of this, over time, the physico-chemical properties of this water system will change and creating some ecotoxicological impacts of ecosystem on the living elements are inevitable.

Fish samples will be taken from the certain places before and after discharges of household liquid wastes to the Munzur and Pülümür Rivers, from the different points and depths of the dam lake and from just after dam wall exit and from the point where the water poured to the Keban Dam Lake. *Capoeta umbla* (Heckel, 1843) was used as the indicator organism. The malondialdehide (MDA) and glutathione (GSH) were determined in samples of the liver tissue.

The results obtained from this study show that the changes of GSH and MDA levels of *Capoeta umbla* (Heckel, 1843) liver tissue can used as an indicator for water pollution monitoring. In polluted sites, the fish exposed to xenobiotics, causing an interaction between these chemicals and biological systems has led to negative changes in biochemical parameters.

Poster No. 124

Effect of maternally exposed colouring food additives on renal oxidant and anti oxidant systems in rats

Firdevs Aylak[1], Duygu Kumbul Doğuç[1], Betül Mermi Ceyhan[2], Esin Kulaç[3], Fatih Gültekin[1]

[1] Medical Biochemistry Department, Medical Faculty of Suleyman Demirel University.
[2] Central Hospital, Biochemistry Laboratory.
[3] Medical Education and Informatics of Suleyman Demirel University.

Consequences of maternally exposure to artificial food colourings (AFCs) which have been used all over the world for many years are not clear. We aimed to investigate the effect of maternally exposed AFCs on renal oxidant and anti oxidant biomarkers in when the rats became adult. Thirty female rats were included to the study which were divided into two groups as control (n=15) and experiment (n=15) groups. A mixture of nine food colours (erythrosine, ponceau 4R, allura red, sunset yellow, tartrazine, brilliant blue, azorubine, indigotin) were given daily to experiment group during period of four weeks

from the preconception to birth. The control group was given tap water during the same period. Three months after the birth, 24 offspring from each group were selected randomly as control and experiment groups. At the end of experiment the offspring were sacrified and oxidant and antioxidant biomarkers were determined from their kidney homogenate. Lipid peroxidation product MDA was measured by Draper and Hadley's double heating method. SOD activity was measured by the method of Woolliams et al., GSH-Px activity was measured by Paglia and Valentina's method and Catalase activity was measured by Aebi's method. Mann-Whitney U test was used for statistical analyses. While GSH-Px activity was found to be significantly lower, MDA level was found to be significantly higher in experiment group when compared with control group ($p< 0.05$). Comparison of SOD and Catalaz activity of the groups showed no statistically significant difference from each other. Maternally exposure to AFCs caused decrease in GSH-Px activity which is an antioxidant enzyme. Decrease in GSH-Px level may be related to consumption of enzyme in response to increase in MDA levels, and also may be resulted from the effect of AFCs on enzyme synthesis of kidney in intrauterin period.

Poster No. 125

Determinations of Mefv Gene Mutations Prevalence in the Familial Mediterranean Fever (FMF) at the Region of East Anatoli

Numan Taşpınar[1], Hasan Doğan[2], G. Zişan Şahin[2], Eda Diyarbakır[2], Atilla Topçu[1], İbrahim Pirim[3]

[1] Department of Medical Pharmacology, Medical Faculty, Ataturk University, Erzurum, TURKEY.
[2] Department of Medical Biology, Medical Faculty, Ataturk University, Erzurum, TURKEY.
[3] Department of Medical Biology, Medical Faculty, Katip Çelebi University, İzmir, TURKEY.

Familial Mediterranean fever (FMF) is an autosomal inflammatory recessive disease characterized by recurrent episodes of fever accompanied by peritonitis, pleuritis, or arthritis. The FMF gene, MEFV, is located on chromosome 16p13.3. It has been identified by positional cloning and comprises 10 exons. Until now with 200 mutations and polimorfisms having been identified in the world. Exon 2 and 10 carry most mutations. The four mutations; M694V, V726A, M680I and R761H are the most common mutations in East Mediterrian populations.

The study involved in 2530 cases who were clinically pre-diagnosed as FMF and studied exon 2 and exon 10 of MEFV gene by DNA sequencing techniques. Peripheral blood taken and DNA was isolated. The PCR was performed with exon 2 and exon 10 primers and exons analysis were made by the gene alignment sequence.

As the result of the analysis, we established 58.58% wild genotype and 41.42% mutant genotype. In FMF pre-diagnosed 1048 patient with muations MEFV gene mutations frequency were M694V 57,16% (n=599), V726A 27.19% (n=285), M680I 20.80% (n=218), R761H 8,87% (n=93) on exon 10 and 2 E148Q 6,68% (n=70) on exon 2 respectively. The rare mutations were detected as A744S (n=15), M694I (n=3), E148V (n=3), T267I (n=3), E167D (n=2) and L110P (n=1) in the East Anatolian region.

In conclusion, in our patients M694V mutation was found higher than the other mutations that has been detected commonly in patients with FMF.

Poster No. 126

Determination Of Cisplatin Induced Neurotoxicity In Newborn Rat Neuron Culture

Ahmet Hacımüftüoğlu, Damla Çetin, Adem Alemdar, Abdulgani Tatar, Fatih Saruhan.

Department of Pharmacology, Faculty of Medicine, Ataürk University, Erzurum, TURKEY

Anti-cancer drug induced neurotoxicity is a crucial side effect and this restricts the usage and doses in treatment. In this study it was investigated whether neurotoxicity caused by cisplatin. In the conducted experiment newborn *Spraque Dawley* rats were decapitated in sterile atmosphere and neuron culture was made from their cerebral cortex. On the sixteenth day 50μ ve 100μ cisplatin were applied. MTT analysis was conducted for the test of cell vitality. The fact that group averages were 70,50±2,07 – 63,83±8,51 in the groups to which only cisplatin was applied while it was 84,83±3,43 in the control group on the other plate showed that neurotoxicity appeared. Therefore, the neurotoxicity resulting from administration of the anti-cancer drug cisplatin is become clear in newborn rat neuron culture. Second part of this study is going to focus on many different agents to reveal more accurate neuroprotective agents to minimize cisplatin induced neurotoxicity.

Poster No. 127

Investigation of Possible Oxidative Stress of 99m-Tc Dimercaptosuccinic Acid (DMSA) Application in Rats

Serdal Öğüt[a], Gökhan Cesur[b], Duygu Kumbul Doğuç[c], Mümin Polat[d], Mustafa Yıldız[e]

[a] Suleyman Demirel University, Research and Application Hospital, Blood Center, Isparta, TURKEY.
[b] Department of Physiology, Adnan Menderes University, Faculty of Medicine, Aydin, TURKEY.
[c] Department of Physiology, Suleyman Demirel University, Faculty of Medicine, Isparta, TURKEY.
[d] Suleyman Demirel University, Sarkikaraagac Vocational school, Isparta, TURKEY.
[e] Department of Nuclear Medicine, Suleyman Demirel University, Faculty of Medicine, Isparta, TURKEY.

In renal scintigraphy one of the most widely used pharmaceuticals is technetium-99m (99mTc) dimercaptosuccinic acid (DMSA). 99m Tc DMSA the best radiopharmaceutical substance that displays renal cortex. In this study, oxidative stress was investigated pose of 99mTc DMSA in rats.

For this purpose, total antioxidant capacity (TAC), total oxidant capacity (TOC) and oxidative stress index (OSI) was investigated DMSA injected 8 Sprague Dawley rats' in blood. In addition, the kidney tissues of rats with SOD, CAT, GSH-Px and MDA levels are also calculated. 8 rats were evaluated as a control group without application of 99mTc DMSA.

End of the study, compared with the control group, rats with 99mTc DMSA applied to the OSI, and high levels of MDA, SOD, CAT and GSH-Px levels were found to be low. Conclusion: These results indicate a negative impact on the activities of antioxidant enzymes of rats with 99mTc DMSA application.

Poster No. 128

Paclitaxel Induced Neurotoxicity and its Relationship with Antioxidant Parameters

Damla Çetin, Ahmet Hacımüftüoğlu, Abdulgani Tatar, Fatih Saruhan

Department of Pharmacology, Faculty of Medicine, Ataürk University, Erzurum, TURKEY.

Peripheral neurotoxicity is a side effect that restricts the usages and doses of anti-cancer drugs like paclitaxel. In this study it was investigated whether neurotoxicity caused by anticancer drugs, reason for which is unknown can be treated with antioxidants. In the conducted experiment, newborn *Sprague Dawley* rats were decapitated in sterile atmosphere and neuron culture was made from their cerebral cortex. On the tenth day salicylic acid, a 10^{-4}M and 10^{-5}M antioxidant agent, was added to culture flasks. On the sixteenth day 10^{-7}M and 10^{-8}M paclitaxel was applied. MTT analysis was conducted for the test of cell vitality. The fact that group averages were consecutively 47±7 - 53±14 in the groups to which only paclitaxel was applied while it was 74±33 in the control group, showed that neurotoxicity appeared. In the total antioxidant capacity tests the control groups which were obtained as 1,23±0,32 and 1,12±0,27 - 1,11±0,19 in the groups to which only paclitaxel was applied showed that antioxidant capacity decreased. In the total oxidant capacity tests the control groups which were obtained as 1,44±0,51 and 1,68±0,48 - 1,71±0,62 in the groups to which only paclitaxel was applied showed that the capacity increased. As a result in this study it was seen that salicylic acid, an antioxidant for the formation of free radicals, can prevent the neurotoxicity caused by anticancer drugs.

Poster No. 129

PKC-Epsilon Downregulates DNA-Pk Gene Expression in Tumorigenic and Non-Tumorigenic Prostate Cell Lines

Nilgun Gurbuz[1,3], Hakan Darici[2], Aslı Baykal[1]

[1] Akdeniz University, School of Medicine, Department of Biochemistry, Antalya, TURKEY.
[2] Suleyman Demirel University, School of Medicine, Department of Histology and Embryology, Isparta, TURKEY.
[3] Suleyman Demirel University, School of Health Sciences, Department of Nursing, Isparta, TURKEY.

Protein kinase C-epsilon (PKC-ε) is a member of new PKC family which are activated by phosphatidyl serine (PS) and diacilglicerol (DAG) but not Ca^{2+}. Because of its direct interaction with Raf-1 kinase, Akt, Bax and Retinoblastoma (Rb) proteins, PKC-ε plays an oncogenic role via modulating cell growth and apoptosis and involved in prognosis of some cancers including prostate cancer,.

Disregulation of DNA repair mechanisms and resulting DNA damage is one of the causes of cancer. DNA depended protein kinase (DNA-PK) is a nuclear serin/threonin protein kinase which is activated by DNA damage and modulates gene activation, DNA repair and apoptosis.

In order to explain oncogenic effects of PKC-ε, we hypothesized that PKC-ε might suppress DNA-PK therefore causing increased DNA damage. To proof our hypothesis, PKC-ε gene expression was silenced via specific siRNA within androgen-independent prostate cancer cell line (PC-3) and non-tumorigenic prostate cell line (RWPE-1). mRNA levels of DNA-PK were quantitatively determined by real-time PCR.

Our results showed that DNA-PK gene expression was three times higher in cancer cells than normal prostate cells without any transfection. PKC-ε siRNA inhibited DNA-PK gene expression in both cell lines thereby proving our hypothesis. Interestingly, the inhibition was two times more effective in cancer cells than normal cell lines. Therefore we showed for the first time that DNA-PK was regulated by PKC-ε in normal and tumorigenic prostate cells. Identifying other molecules, mediating PKC-ε/DNA-PK pathway in prostate cells will be helpful to create new treatment models for prostate cancer.

Poster No. 130

The Neuroprotective Effects of Pregabaline On Ischemia and Reperfusion in Carotid Artery Occlusion Rat Model

Sanem Aşci[1], Serpil Demirci[1], Duygu Kumbul Doğuç[2], Halil Aşci[3], İbrahim Onaran[4]

[1] Suleyman Demirel University, Faculty of Medicine, Neurology Department / Isparta, TURKEY.
[2] Suleyman Demirel University, Faculty of Medicine, Biochemistry Department / Isparta, TURKEY.
[3] Suleyman Demirel University, Faculty of Medicine, Pharmacology Department / Isparta, TURKEY.
[4] Suleyman Demirel University, Faculty of Medicine, Medical Biology Department / Isparta, TURKEY.

In acute ischemic stroke, core of the brain tissue where the blood perfusion is minimum, damages irreversibly in minutes. Reperfusion of ischemic area may results in free radicals production. Pregabaline has been shown to promote neuroprotection in spinal ischemia.

In this study, 36 Wistar Albino type male rats was used. Rats were divided into six groups:control, pregabaline, ischemia, pregabaline+ischemia, ischemia+reperfusion and pregabaline+ischemia+re perfusion. Pregabaline was administered with oral gavage through two days in 50 mg/kg/day dose. Ischemia was performed by occluding the right internal carotid artery with buldog clamp for 30 minutes, then reperfusion was performed through 20 minutes. TBARS, NO, SOD, GSH-Px, CAT levels and NMDA-NR2A and NR2B receptor concentrations were evaluated.

TBARS and NO levels in ischemia and ischemia+reperfusion groups were significantly more increased in comparison to control group and were significantly more decreased in prega baline+ischemia+reperfusion group. NO level in ischemia+pregabaline group was significantly more decreased than was in ischemia group. GSH-Px level was decreased in both damage group showing the usage of enzyme. GSH-Px levels in pregabaline+is chemia+reperfusion group were significantly more increased in comparison to ischemia+reperfusion group. NR2B receptors concentrations were significantly reduced in Pregabaline+ischemia and pregabaline+ischemia+reperfusion groups.

Pregabaline led to decreased NMDA-NR2B receptor levels and NO levels in ischemia and ischemia+reperfusion groups, supporting a significant role of pregabaline in decreasing oxidative stres along with neuroprotection in ischemia-reperfusion damage and suggests that pregabaline may be used in acute ischemic brain injury for both to decrease the oxidative stres and also to provide neuroprotection.

Poster No. 131

RAS/RAF/MAP Kinase pathway genes in relation to bladder cancer

*Pınar Aslan Koşar, **Erdem Çapar, **Alim Koşar, *Gülçin Yavuz

*Department of Medical Biology, Faculty of Medicine,Suleyman Demirel University, Isparta, TURKEY.
**Department of Urology, Faculty of Medicine, Suleyman Demirel University, Isparta, TURKEY.

After initial TURB, 70% of patients with non-muscle-invasive bladder cancer have develop one or more recurrences and one third of them will progress to invasive tumor.

The recurrence risk and risk of progression necessitate a life-long follow-up by cystoscopy.

Otherwise, it is necessary to discover oncogenic mutations to increase effectiviness of cystoscopy for diagnosis of urothelial tumors, and to determine theraphy targets

RAS/RAF/MAP Kinase pathway protooncogene mutations in the human cancers are the most common observed genetic alterations. In urothelial tumors somatic mutations in the RAS/RAF/MAP Kinase pathway genes may be of use for early detection of primary and recurrent tumors, for follow up targeted therapies in tissue-based assays.

We plannned to compare the mutations in the DNA obtained from tumor tissues, healthy tissues and blood of 46 patients who were diagnosed bladder cancer histologically. To determine BRAF and KRAS proto-oncogene polymorphisms we used MassARRAY® (iPLEX®) single nucleotid polymorphism genotyping technic based on the technique of polymerase chain reaction.

We determined BRAF V600E mutation based on T1796A transversion in the tumor tissues of 45

(%97.8) patients. And we determined Gly12Serin mutation at the twelfth codon of KRAS gene based on GGT>AGT transition in only one patient. This patient hasn't got BRAF V600E mutation. There were no mutation in the healthy tissues and blood of 46 patients.

The normal approximation may be inaccurate for small samples in statistical analyses. Fisher's exact test: P-Value = 1,000.

In literature the incidence of BRAF V600E mutations in cancer patients is %90. İn our study the incidence of BRAF V600E mutations in bladder cancer patients is %97.8. Kras mutation doesn't play a role in bladder cancer susceptibility in the Turkish population.

Poster No. 132

Nimodipine modulates Ca^{2+} signaling through TRPM2 channels in dorsal root ganglion of rats following experimental spinal cord injury

Muhammed Borcak[1], Mustafa Nazıroğlu[2], Özgür İsmailoğlu[1], A. Cihangir Uğuz[2], Bilal Çiğ[2], Cemil Özgül[2]

[1] Suleyman Demirel University, Faculty of Medicine, Department of Neurosurgery, Isparta, TURKEY.
[2] Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, TURKEY.

Mechanical injury causes myelin disruption and subsequent axonal conduction failure in the mammalian spinal cord. However, the underlying mechanism is not well understood. Apoptosis and oxidative stress have been occurring through Ca^{2+} influx in dorsal root ganglion (DRG) following the spinal injury although mechanisms of Ca^{2+} influx has not been in DRG neurons. The present study was designed to determine the contribution of TRPM2 cation channels in dorsal root ganglion (DRG) of rats following peripheral nerve injury.

All rats were divided into five groups namely control, sham control, spinal injury, spinal injury+nimodipine injection [0.5 mg/kg for 3 days and intra venous (IV)] and spinal injury+oral nimodiphine (15 mg/kg via gastric gavage for 3 days). Spinal injury except control group was performed by laminoctomy (Th8-Th 12). Cytosolic free Ca^{2+} $[Ca^{2+}]i$ concentrations (by Fura-2), oxidative stress and patch-clamp analyses were performed the DRG neurons.

The concentrations of $[Ca^{2+}]i$ were significantly ($p<0.001$) higher in spinal injury group than in control although their concentrations were decreased ($p<0.001$) by gastric gavage and IV administrations. The TRMP2 currents were also significantly ($p<0.001$) higher in spinal injury group than in control although their concentrations were decreased ($p<0.001$) by IV administration but not gastric gavage. Lipid peroxidation, reduced glutathione and glutathione peroxidase values did not change in the five groups, statistically.

In conclusion, increased influx of Ca^{2+} increased in the DRG neurons following spinal injury indicating TRPM2 cational channel activity. However, the cytosolic $[Ca^{2+}]i$ concentrations were modulated by nimodiphine.

Cell Membranes and Free Radical Research

SUBSCRIPTION ORDER FORM

I would like to subscribe to **Cell Membranes and Free Radical Research**
(ISSN Numbers : 1308-4178 (online) 1308-416X). All prices in Turkish Liras
2012, Vols. (4 issues)

Intitutional Rates:

☐ Print Only: 30 TL per issue + postage 100 TL per year

Credit Card _____ Expiration Date _____
Print Name _____ Signature _____
Receiving Address
Name _____
Department/Title _____
Street _____
_____
City _____ State/Province _____ Post/Zip Code _____ Country _____
Tel _____ Fax _____ E-mail _____

Billing Address ☐ Check here if same as above
Name _____
Department/Title _____
Street _____
Cell Membranes and Free Radical Research

LIBRARY ROUTING CARD

To (Librarian) : _____ Date _____
From: _____ Department _____

I have reviewed **Cell Membranes and Free Radical Research**
(ISSN Numbers : 1308-4178 (online) 1308-416X) and suggest that you subscription with:
2012, Vols. (4 issues) All prices in Turkish Liras.

Intitutional Rates:

☐ Print Only: 30 TL + postage 100 TL per year

Süleyman Demirel Üniversitesi Tıp Fakültesi, Isparta / TURKEY
Tel:+ 90 246 211 33 10 Fax:+ 90 246 237 11 65 EMAIL: mnaziroglu@med.sdu.edu.tr

COPYRIGHT FORM

Cell Membranes and Free Radical Research

Date _____ Contributor Name _____

Contributer Address _____

Manuscripyt Number (if Know) _____

Re: Manuscript entitled _____

''For publication in Cell Membranes and Free Radical Research published by Society of Cell Membranes and Free Oxygen Radicals ''

Submission of a manuscript implies:

- that the work described has not been published before (except in the form of an abstract or as part of a published lecture, review or thesis);
- that it is not under consideration for publication elsewhere;
- that its publication has been approved by all co-authors, if any, as well as by the responsible authorities at the institute where the work has been carried out;
- that, if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to this publisher;
- that the manuscript will not be published elsewhere in any language without the consent of the copyright holders;
- that written permission of the copyright holder is obtained by the authors for material used from other copyrighted sources, and that any costs associated with obtaining this permission are the authors' responsibility. Copyright notice: The contributor and the company/employer agree that all copies the final published version of the contribution or any part thereof distributed or posted by them in print or electronic format as permitted herein will include the notice of copyright as stipulated in the journal and a full citation to the journal as published by Cell Membranes and Free radical Society, Isparta, Turkey.

CHECK ONE BOX

☐ Contributor owned work: Contributor's signature _____ Date _____

Type or print name and title _____

Co-Contributor's signature _____ Date _____

Type or print name and title _____

☐ Company-Institution owned work _____

Company-Institution (Employer for here) _____ Date _____

Authorized signature _____ Date _____

☐ U.S. Government work _____

☐ U.K. Government work _____

☐ Other Government work _____

4th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels

http://www.cmos.org.tr

26-29 June 2012

ISPARTA /TURKEY

Ca^{2+} Signaling

Alexei Tepikin (Liverpool, UK)
From Ca^{2+} signalling to bioenergetics and vice versa

Christian Schöneich (Kansas, USA)
Modulation of SERCA function through redox processes, Bcl-2 and Hsp70

David Criddle (Liverpool, UK)
The roles of calcium and ROS in acute pancreatitis

Emil C. Toescu (Birmingham, UK)
Calcium and mitochondrial interactions in aged neurons

Nicole Mahy (Barcelona, Spain)
Neuroregenerative diseases, calcium and microglia

Saeed Semnanian (Tehran, Iran)
Special electrophysiological characteristics of the nucleus Locus Coeruleus

Antioxidants

Ana Beatriz Rodríguez Moratinos (Badajoz, Spain)
Chrononutrition against oxidative stress

José A. Pariente (Badajoz, Spain)
Calcium signaling and apoptosis: Role of melatonin

Xingen G. Lei (Ithaca, USA)
Do antioxidant nutrients induce diabetes, and how?

TRP Channels

Asrar Malik (Chicago, IL, USA)
TRPM2 homeostatically regulates NADPH oxidase activity in phagocytic cells

Indu S. Ambudkar (Bethesda MD, USA)
TRP channels in cell function and disease

James W. Putney (North Carolina, USA)
Store-operated calcium channels

Leonidas Tsiokas (Oklahoma City, USA)
TRP channels and the primary cilium

Khaled Machaca (Doha, Qatar)
Regulation of SOCE during the cell cycle

Metiner Tosun (İzmir, Turkey)
Functional genomics analysis of TRPC1 gene silencing in liver cancer

Mustafa Nazıroğlu (Isparta, Turkey)
Role of thiol redox system on activation of TRPM2 and TRPV1 channel current in dorsal root ganglion neurons of rats

Mohamed Trebak (New York, USA)
Receptor-activated calcium channels in vascular function

Michael X. Zhu (Houston, USA)
Functional regulation and pharmacology of TRPC channels

Rudi Vennekens (Leuven, Belgium)
TRPM4 as a general regulator of cell functions

Stephan Huber (Tübingen, Germany)
Role of bcl-2 family members on irradiation-stimulated activation of TRPM2 channels in lymphoma cells

Shmuel Muallem (Bethesda MD, USA)
TRPC/Orai1/STIM function and dysfunction

Thomas Voets (Leuven, Belgium)
TRP Channels in noxious temperature sensing

Tomohiro Numata (Kyoto, Japan)
Cysteine-mediated oxidation activates TRP channels

Genetics, Ca^{2+} and Oxidative Stress

Cem Ekmekcioglu (Vienna, Austria)
The expression of clock genes in human cells under various influences

Hilmi Özçelik (Toronto, Canada)
Free radicals and gene networks in human disease and health

Israel Sekler (Beer-Sheva, Israel)
The role of mithochondrial exchanger NCLX in cellular Ca^{2+} signaling

Volker Ullrich (Konstanz, Germany)
Redox regulation of Ca-signaling

Oxidative Stress

Andreas Daiber (Mainz, Germany)
Crosstalk between mitochondrial and NADPH oxidase derived reactive oxygen and nitrogen species - implications for vascular function

Denis Rousseau (Grenoble, France)
ATAD3, a vital inner membrane mitochondrial ATPase involved in fission/fusion processes and interactions with endoplasmic reticulum

Jean-Louis Wayenberg (Brussels, Belgium)
Nitro-oxidative stress in neonatal emergencies: Asphyxia and hypoglycemia

Valerian E. Kagan (Pittsburg, Pennsylvania, USA)
Oxidative lipidomics of cell death signalling-specific involvement of anionic phospholipids

Serge Bottari (Grenoble, France)
Role of protein nitration in insulin-resistance

Tasarım: Serdar DURAN

Prof.Dr. Hasan İBİCİOĞLU
SDÜ Rektörü / Rector

Prof.Dr. Hüseyin YORGANCIGİL
Tıp Fakültesi Dekanı / Dean

Congress Location:

Süleyman Demirel University
Prof. Dr. M. Lütfü Çakmakçı Convention Center
http://www.cmos.org.tr

Congress Language: English

Chairman
Prof. Dr. Mustafa NAZIROGLU
Department of Biophysics, Faculty of Medicine,
Suleyman Demirel University

Vice Chairman
Prof. Dr. James W. PUTNEY Jr.
NIEHS Calcium Regulation Group Leader

Local Vice Chairmans
Assist. Prof. Dr. A. Cihangir UĞUZ and Assist. Prof. Dr. Ömer ÇELİK
Department of Biophysics, Faculty of Medicine
Suleyman Demirel University

Congress Secretariat
I. Suat OVEY & Bilal CIG & Mehmet AYKUR
Department of Biophysics, Faculty of Medicine
Suleyman Demirel University

Accountants
Mustafa KUCUKAYAZ & Cemil OZGUL & Ercan SÖZBİR (webmaster)
Department of Biophysics, Faculty of Medicine
Suleyman Demirel University

Contact:
Suleyman Demirel Universitesi Tip Fakultesi
Biyofizik A.D. Morfoloji Binasi Dogu Kampusu TR 32260
Cunur / Isparta TURKEY
Tel:+90 246 211 36 41 Fax:+90 246 237 11 65
biophysics@med.sdu.edu.tr

TÜBİTAK

HSORD Society of Cell Membranes and Free Oxygen Radicals

ecs european calcium society

Cell Membranes and Free Radical Research

Principal Contact

Prof. Dr. Mustafa NAZIROGLU / Editor in Chief
Suleyman Demirel University Faculty of Medicine (TIP Fakultesi), Department of Biophysics Dogu Kampusu
32260 Cunur -Isparta /TURKEY
Phone: +90 246 2113310
Fax: +90 246 2371165
Email: mnaziroglu@med.sdu.edu.tr
biophysics@med.sdu.edu.tr