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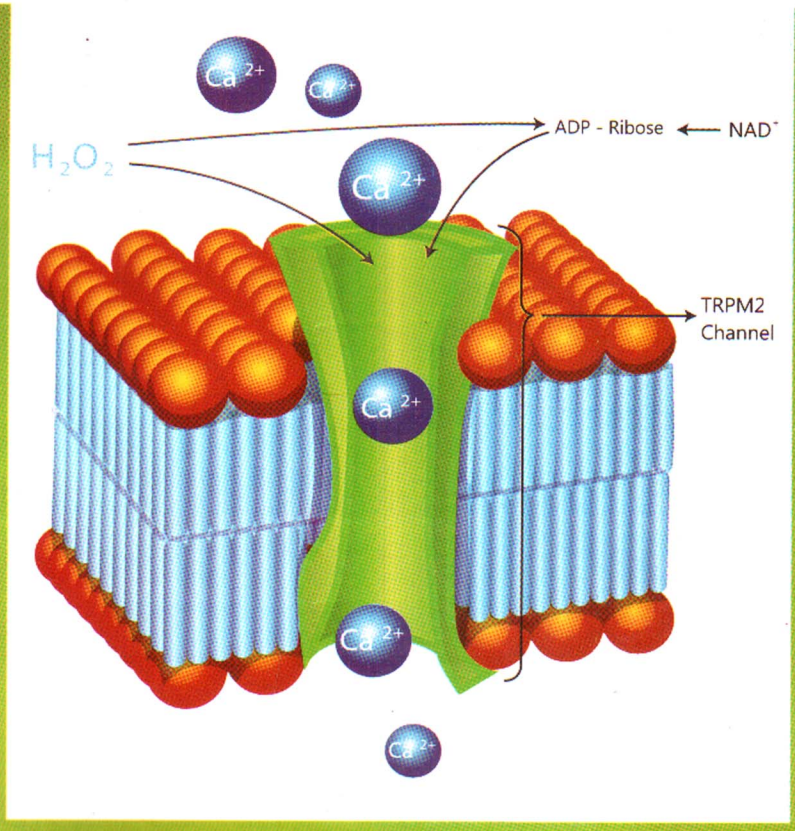

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Cell Membranes and Free Radical Research

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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, the biophysics of the radicals which springed up from oxygen),

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.

CONFERENCES

Conference 1

Calcium channels in non-excitable cells.

James W. Putney, Jr.

National Institute of Environmental Health Sciences-NIH, Research Triangle Park, NC 27709 USA

In non-excitable cell types, agonist regulation of calcium signaling often involves a mechanism initiated by release of intracellular calcium and a subsequent entry of calcium across the plasma membrane. This entry of calcium is believed to be signaled by the depletion of calcium stores, and has been termed *capacitative* or *store-operated calcium entry*. The best characterized electrophysiological current associated with capacitative calcium entry is the calcium-release-activated calcium current, or I_{crac}. The mechanism of activation of SOC or CRAC channels involves an endoplasmic reticulum Ca^{2+} sensor, STIM1, and this in turns signals to and activates CRAC channels composed of Orai pore-forming subunits.

We have investigated the mechanism of action of STIM1 and Orai proteins and their regulation by phosphorylation in a kidney epithelial cell line, HEK293. STIM1 activates Orai channels by a mechanism that depends upon its co-localization with Orai at endoplasmic reticulum – plasma membrane junctions. STIM1 is organized within the endoplasmic reticulum by mechanisms dependent on the microtubular cytoskeleton and collects upon store depletion at specific sites where it draws Orai to collect as well. During mitosis, store-operated entry is down-regulated by complex mechanisms involving changes in protein expression as well as co-valent modification of signaling proteins. The dynamic nature of regulation of STIM1 and Orai function and expression may play an important role in the plasticity of cellular responses during development and under conditions of environmental stress.

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Conference 2

TRPC1 is regulated in response to store depletion and contributes to SOCE

Indu Ambudkar

Molecular Physiology and Therapeutics Branch, NIDCR, NIH, Bethesda MD.

Store-operated Ca^{2+} entry (SOCE) is activated in response to depletion of the ER-Ca^{2+} stores by the ER Ca^{2+} sensor protein, STIM1 which oligomerizes and moves to ER/PM junctional domains where it interacts with and activates channels involved in SOCE. Two types of channel activities have been described. I_{CRAC}, via Ca^{2+} release-activated Ca^{2+} (CRAC) channel, which displays high Ca^{2+} selectivity and accounts for the SOCE and cell function in T lymphocytes and mast cells. Orai1 has been established as the pore-forming component of CRAC channels and that interaction of Orai1 and STIM1 is sufficient for generation of the CRAC channel. Orai1 is gated by a C-terminal region of STIM1 referred to as SOAR or CAD. Store depletion also leads to activation of relatively non-selective cation currents (referred to as I_{SOC}) that contribute to SOCE in several other cell types; including salivary gland, pancreatic, as well as some types of smooth muscle and endothelial cells. TRPC channels, including TRPC1, TRPC3, TRPC4, have been proposed as possible candidate channels. Our studies have primarily focused on the role of TRPC1 in SOCE. TRPC1-mediated I_{SOC} and SOCE in cells stimulated by agonist or thapsigargin display similar pharmacological properties as I_{CRAC} (e.g. inhibition by low [Gd^{3+}] and 10-20 μM 2APB). Further, knockdown of TRPC1 expression results in a reduction in SOCE and I_{SOC} in several different cell types. In $TRPC1^{-/-}$ mice, there was severe loss of salivary gland function as well as SOCE in acinar cells. Importantly, STIM1 also associates with and activates TRPC channels via electrostatic interaction between STIM1 and TRPC1 C-terminal residues. Further, store depletion induces dynamic recruitment of a TRPC1/STIM1/Orai1 complex and knockdown of Orai1 completely abrogates TRPC1 function. The functional interaction between Orai1 and TRPC1 that is required for SOCE and generation of I_{SOC} has not yet been

resolved. Nevertheless, there has been much debate regarding the activation of TRPC1 by store depletion as well as the role of Orai1 and STIM1 in SOC channel function.

Our recent studies have been directed towards understanding the mechanism of activation of TRPC1 in response to store depletion. We have reported that TRPC1 activation requires STIM1 and Orai1. TRPC1 is scaffolded by caveolin1, which holds inactive TRPC1 within the ER/PM domains where STIM1 puncta are formed. Further, STIM1 associates with TRPC1 and gates the channel which also results in dissociation of TRPC1 from caveolin1. We have now assessed the role of Orai1 in regulation of TRPC1-function. In brief, our studies reveal that I_{SOC} that is activated in HSG cells in response to store depletion (as previously described by us) is composed of Orai1/STIM1-mediated I_{CRAC} and TRPC1/STIM1-mediated non-selective cation current. Orai1-mediated I_{CRAC} was unmasked by expression of a STIM1 mutant that cannot activate TRPC1. We will discuss our new findings which elucidate the functional interaction between Orai1 and TRPC1 that is required for TRPC1-SOC channel regulation within ER/PM junctional domains. We suggest that TRPC1-STIM1 channels contribute to local Ca^{2+} entry and Ca^{2+} signaling triggered by Ca^{2+}-store depletion.

Conference 3

Reciprocal control of Orai and CaV1.2 Channels by STIM Proteins

Donald L. Gill

Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA 19140

Ca^{2+} entry channels, crucial in providing cellular Ca^{2+} signals regulating a spectrum of cellular functions, are controlled by diverse sensing mechanisms including membrane voltage, surface receptors, and Ca^{2+}-sensing STIM proteins in the ER. The operation and role of these transduction processes in excitable vs. nonexcitable cells were considered very different, but new information shows intriguing overlap in the coordinated function. Voltage-operated L-type channels ($Ca_V1.2$) well known in excitable cells, also exist at high densities in most immune cells, including T cells, B cells, dendritic cells, and mast cells, yet their channel activity appears suppressed. Conversely, the dynamic ER-resident Ca^{2+}-sensing STIM proteins thought to control Ca^{2+} signaling mostly in nonexcitable cells, are present in excitable cells in large quantities, yet their role is unclear. We reveal that $Ca_V1.2$ channels are under the direct control of STIM proteins. STIM proteins sense ER Ca^{2+} stores and rapidly translocate into ER-PM junctions where they interact with and activate the highly Ca^{2+}-selective Orai family of PM channels. The widely expressed TRPC group of receptor-induced nonselective cation channels, have also been implicated as STIM-regulated channels; however, while STIM proteins play a decisive role in receptor-induced Ca^{2+} signaling through Orai channels, we reveal no STIM-mediated control of TRPC channels. In contrast, we determined that STIM proteins mediate profound inhibitory control over the function of voltage-activated $Ca_V1.2$ channels. This action is independent of Orai channel function or changes in cytosolic Ca^{2+}, and mediated by a direct action of STIM1 on the $Ca_V1.2$ α_{1C}-subunit. STIM1 activation by store-depletion or mutational modification, strongly suppresses voltage-operated $Ca_V1.2$ channels while activating store-operated Orai channels, both actions being mediated by the short STIM-Orai activating region (SOAR) of STIM1. STIM1 interacts with $Ca_V1.2$ channels and when activated, localizes in discrete endoplasmic reticulum/plasma membrane junctions precisely co-localized with clusters containing both CaV1.2 and Orai1 channels. Thus, STIM1 reciprocally controls Orai and $Ca_V1.2$ channels, indicating a hitherto unknown and potentially crucial regulatory link between receptor-induced Ca^{2+} store-depletion and the control of voltage-activated Ca^{2+} signals. Such coordinated control of the widely expressed $Ca_V1.2$ and Orai channels has major implications for Ca^{2+} signal generation in excitable and nonexcitable cells. STIM proteins may play a crucial role in suppressing $Ca_V1.2$ function in cells such as immune cells and STIM-mediated reciprocal control of $Ca_V1.2$ and Orai channels is likely a decisive mechanism controlling Ca^{2+} signal generation.

Conference 4

Vascular smooth muscle STIM/Orai proteins in vascular occlusive disease

Mohamed Trebak

Center for Cardiovascular Sciences, Albany Medical College, New York 12208, USA

Vascular smooth muscle cell (VSMC) proliferation and migration is a hallmark of vascular occlusive disease such as atherosclerosis and restenosis. We showed that proliferative migratory VSMCs (called "synthetic") display up-regulated STIM1/Orai1 proteins and store-operated calcium entry (SOCE) compared with quiescent contractile freshly isolated VSMC. Synthetic VSMCs isolated from rat aorta display SOCE with classic features, namely inhibition by 2-aminoethoxydiphenyl borate, ML-9, and low concentrations of lanthanides. On store depletion, synthetic VSMCs and A7r5 cells display currents with characteristics of I(CRAC). Protein knockdown of either STIM1 or Orai1 in synthetic VSMCs greatly reduced SOCE, whereas Orai2, Orai3, TRPC1, TRPC4, and TRPC6 knockdown had no effect. Orai1 knockdown reduced I(CRAC) in synthetic VSMCs and A7r5 cells. Knockdown of STIM1 and Orai1 inhibited synthetic VSMC proliferation and migration in response to serum and PDGF, whereas STIM2, Orai2, and Orai3 knockdown had no effect. STIM1, Orai1, and PDGFRbeta mRNA levels were upregulated *in vivo* in VSMC from balloon-injured rat carotid arteries compared with sham non-injured control vessels. Protein levels of STIM1 and Orai1 were also upregulated in medial and neointimal VSMC from injured carotid arteries compared with non-injured vessels, as assessed by immunofluorescence microscopy. *In vivo* silencing using lentiviral shRNA vectors of either STIM1 or Orai1 after balloon injury in rats largely prevented neointima formation. These results establish STIM1 and Orai1 as important components for SOCE, proliferation and migration in VSMC. The upregulation of VSMC STIM1/Orai1 is required in vivo during vascular injury for neointima formation.

Conference 5

Is ER Ca^{2+} signaling the portal to mitochondrial dysfunction and vascular stress?

Madesh Muniswamy

Department of Biochemistry, 627 Kresge Building, 3440 N. Broad St, Philadelphia, PA 19140

Reactive oxygen species (ROS) play a divergent role in both cell survival and cell death during ischemia-reperfusion (I/R) injury and associated inflammation. In physiologic conditions, mitochondrial respiratory chain proteins continually produce superoxide ($O_2^{\cdot-}$), which can be dismutated into hydrogen peroxide (H_2O_2) or react with nitric oxide (NO) to produce peroxynitrite (ONOO). During inflammation $O_2^{\cdot-}$ production is substantially increased and is accompanied by vascular injury. However, the molecular mechanism(s) by which ROS lead to mitochondrial dysfunction and cell death is poorly understood. In this study, ROS generation by activated macrophages evoked an intracellular Ca^{2+} ($[Ca^{2+}]i$) transient in endothelial cells that was ablated by a combination of superoxide dismutase and an anion channel blocker. The $O_2^{\cdot-}$-induced $[Ca^{2+}]i$ transient initiated mitochondrial depolarization and downstream apoptotic cascades that were independent of reactive nitrogen species. The transmembrane paracrine $O_2^{\cdot-}$ flux occurs through ClC-3 channels and results in $\Delta\Psi_m$ alterations and mitochondrial $O_2^{\cdot-}$ production. This novel finding elucidates a potential mechanism by which extracellular $O_2^{\cdot-}$ is propagated to the intracellular milieu to trigger endothelial cell signaling or dysfunction associated with oxidative stress. Interestingly, the autocrine mitochondrial ROS production promotes proinflammatory signaling and leukocyte/EC firm adhesion. In addition to cell-cell adhesion, intracellular oxidative stress perturbs receptor-mediated Ca^{2+} oscillation and promotes cytoplasmic Ca^{2+} overload and cell death. These findings demonstrate a unique relationship between ROS and Ca^{2+} signaling in cell death and inflammation.

Conference 6

STIM1-mediated store-operated calcium entry uses a protein unfolding/oligomerization-coupled mechanism

P. B.Stathopulos, L. Zheng, G.-Y. Li, M. J. Plevin and Mitsu Ikura

Division of Signaling Biology, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada M5G 1L7

Stromal interaction molecule-1 (STIM1) activates store operated Ca^{2+} entry (SOCE) in response to diminished luminal Ca^{2+} levels. We have recently determined the solution structure of the Ca^{2+}-sensing region of STIM1 consisting of the EF-hand and sterile α motif (SAM) domains (EF-SAM) (Stathopulos et al. Cell 135, 110-122, 2008). The canonical EF-hand is paired with a previously unidentified EF-hand. Together, the EF-hand pair mediates mutually indispensable hydrophobic interactions between the EF-hand and SAM domains. Structurally critical mutations in the canonical EF-hand, 'hidden' EF-hand or SAM domain disrupt Ca^{2+} sensitivity in oligomerization *via* destabilization of the entire EF-SAM entity. In mammalian cells, EF-SAM destabilization mutations within full-length STIM1 induce punctae formation and activate SOCE independent of luminal Ca^{2+}. More recently we have investigated STIM2, a homologoue of human STIM1, and identified key structural features which contribute to the properties of the Ca^{2+} sensory function of STIM proteins. We provide atomic resolution insight into the molecular basis for STIM-mediated SOCE initiation and show that the folded/unfolded state of the Ca^{2+} sensing region of STIM is crucial to SOCE regulation. (Supported by CIHR and CFI).

Conference 7

The role of ion channels in platelet function

Martyn Mahaut-Smith, C. McCloskey, G. Tolhurst, S. Amisten, R. Carter, E. Fung & S. Jones

Department of Cell Physiology & Pharmacology, University of Leicester, UK

Platelets play a central role in the development of arterial thrombosis and cardiovascular disease. Ion channels contribute to cellular responses in virtually all cell types, however the importance of this major family of membrane proteins during platelet signalling has proven difficult to investigate. This is mainly due to the difficulty of conducting direct electrophysiological recordings in the small and fragile platelet. We have demonstrated that its giant precursor cell, the megakaryocyte, represents a *bona fide* surrogate for studies of platelet activation. For example the synergy between Gαq and Gαi receptors in generating inside-out activation of the $\alpha_{IIb}\beta_3$ integrin is fully conserved in the megakaryocyte (Tolhurst *et al.* 2005, *Blood* 106:1644-51). Expression profiling in the platelet and megakaryocyte, together with patch clamp recordings of megakaryocytes, have now provided evidence for several Ca^{2+} entry pathways evoked by major agonists and has also recently identified the main K^+ channel of this cell type. Five different TRP channel isoforms were demonstrated: TRPC1, TRPC6, TRPM1, TRPM2 and TRPM7 (Carter et al. 2006, J.Physiol. 576, 151-162). The physiological P2Y agonist ADP stimulated inward cation currents whose properties are consistent with a major contribution by both TRPC6 and Orai1 store-operated Ca^{2+} channels. A further major pathway for Ca^{2+} entry has proven to be the P2X1 ATP-gated ionotropic receptor, which contributes to the Ca^{2+} response stimulated by all major platelet agonists, including thrombin, thromboxaneA_2 and collagen (Fung et al. 2007 *J.Thromb Hameost* **5,** 910-917). Strikingly, up to ≈80% of the peak Ca^{2+} increase generated by the collagen is due to secondary activation of P2X1 by released ATP. P2X1 is also unique as a pathway for Ca^{2+} entry in the platelet as it is resistant to elevations of cytosolic cyclic AMP and cyclic GMP. Thus, P2X1-evoked signalling can continue even in the presence of the major

endogenous anti-platelet agents prostacyclin and nitric oxide. Kv1.3 is exclusively responsible for the voltage-gated K^+ conductance of the platelet and megakaryocyte (McCloskey et al. 2010 *J.Physiol.* **588,** 1683-93), which is the highest density ion channel recorded to date in these cells. Kv1.3 sets the resting membrane potential and promotes Ca^{2+} influx, although other K^+ channels can contribute to the driving force for Ca^{2+} entry following block of Kv1.3. Kv1.3$^{-/-}$-deficient mice also have higher platelet counts, which may result from the role of this channel in apoptosis since platelet lifespan is controlled by an intrinsic programme of apoptosis. Thus, with the aid of megakaryocyte electrophysiological recordings and transgenic models, a picture is emerging of the functional role of different ion channels in the platelet.

Conference 8

Calcium signatures of prostate tumours

Natalia Prevarskaya

INSERM U1003, Laboratory of Cell Physiology, University of Lille1, France

Prostate cancer is the most common non-cutaneous human malignancy and the second most lethal tumour among men, with the highest incidence in industrialized countries. Understanding the processes leading to prostate cancers and developing new therapeutic targets are necessary to improve both the survival and the everyday life of patients. Until now, the molecular nature and the regulation of ion channels involved in the prostate carcinogenesis and cancer evolution towards androgen independence remain poorly understood. This is why our project was centred on a novel aspect of human prostate cancer research: the study of the role of intracellular calcium and membrane ion channels in the pathophysiology of the human prostate.
Our work has allowed us to identify various ion channels expressed in normal or cancerous prostate cells which take a significant part in the prostate's pathophysiology (apoptosis, proliferation and cellular differentiation). According to these results, ion channels determining the calcium signature (channels from Transient Receptor Potential (TRP) family: TRPV6, TRPV2, TRPM8; Store Operated Channels (SOC) and voltage dependent CaV3.2 calcium channels) would appear to us to be the best potential candidates as tumour markers and pharmacological targets. Indeed, the aberration of channel's regulation could lead to the development of the physiopathological processes associated with prostate cancer.

Conference 9

TRPM8 modulation during prostate cancer

Loïc Lemonnier, D. Gkika, G. Bidaux, N. Prevarskaya

INSERM U1003, Laboratoire de Physiologie Cellulaire, 59655-CEDEX, Villeneuve d'Ascq, FRANCE

Recent studies indicate that alterations in calcium homeostasis and in ion channels could play a central role in the regulation of processes such as proliferation, differentiation and oncogenesis. Every cell phenotype is characterized by a specific "calcium signature" dependent on kinetics, magnitude and sub-cellular localization of calcium signals. Therefore, quantitative and functional variations of ion channels disturb the physiological status of the cell and may lead to the incidence of a pathology called channelopathy. Recent progresses in modern medicine have shown that numerous pathologies are actually channelopathies (cystic fibrosis, myotonias, hypertension etc). Accumulating evidence tends to demonstrate that the development of some cancers could also involve such ion channel aberrations and, therefore, could be classified as channelopathies.
Over the last few years, we have identified TRPM8 channel as being critical in prostate cancer development, whose hallmarks are 1) self-sufficiency in growth signals and aberrant cell proliferation, 2) insensitivity to antigrowth signals, 3) evasion of programmed cell death (apoptosis), and 4) tissue invasion and metastasis. Furthermore, we made efforts for establishing TRPM8 involvement in "calcium and ion channels signatures" of androgen-dependent and androgen-independent stages of prostate cancer in order to develop reliable prognostic markers and define new therapeutic treatment strategies

Conference 10

The genetic arthitecture of calcium channels in complex disorders

Hilmi Ozcelik

Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto Ontario Canada

The factors underlying the genetic susceptibility to complex disorders continue to remain largely unknown. The emergence of high-density genome-wide association (GWA) studies has generated great hopes to unravel their genetic etiology but also skepticism because only limited genetic information have been obtained, which unfortunately did not improve the risk detection or clinical management of the complex diseases. Here, we applied gene- and pathway based bioinformatics analyses to the publicly available genomic data from several complex disorders including cancer, neurodegenerative and mental disorders. Our model has identified statistically significant associations of “ion channel activity” and the “protein kinase activity” with all of the disorders studied. Further analyses of gene-sets representing the identified function have shown that a set of large genes from each activity was found to commonly impact the common disorders to variable degree. In this study, we have taken advantage of GWA data and demonstrated the role of the biological processes and functions that may be critical for complex disorders. The innovative application proposed here is promising since it allows the study of the collective impact of the genetic variation on the functionally similar gene families, compared to traditional SNP- or gene-based genetic analysis of the GWA data. Therefore, taken together the critical role of the identified gene networks in human disorders, the approach used here represents an effective biological discovery tool that can be applied to all types of GWA data for the discovery of biological networks associated with human conditions.

Conference 11

Hypoxia-induced production of reactive oxygen species and TRPC6-activation in pulmonary arterial smooth muscle and endothelial cells

Alexander Dietrich[1], H. Kalwa[1], A. Sydykov[2], U. Storch[3], B. Fuchs[2], T. Gudermann[3], N. Weissmann[2]

[1]Institute for Pharmacology and Toxicology, School of Medicine, University of Marburg, Karl-von-Frisch-Str. 1, 35033 Marburg, Germany

[2]University of Giessen Lung Center (UGLC), Department of Internal Medicine II/V, Klinikstrasse 36, 35392 Giessen, Germany

[3]Institute for Pharmacology and Toxicology, Medical Faculty, University of Munich, Goethestr. 33, 80336 Munich, Germany

Transient receptor potential (TRP) channels are regulators of $[Ca^{2+}]_i$ in many different tissues including vascular smooth muscle and endothelial cells. Among the classical TRP (TRPC) channels, TRPC3, 6, and 7 are gated by pathways involving C-type phospholipases (PLCs) as well as by direct exposure to diacylglycerols (DAG). Because TRPC6 is highly expressed in the lung, we analyzed its function in the hypoxic pulmonary vasoconstriction (HPV) of pulmonary arterial smooth muscle cells (PASMC) and lung ischemia reperfusion injury (LIRI) using a TRPC6-deficient mouse model (1).
HPV adapts the regional blood flow in the lung to the local alveolar ventilation and can be divided in an acute, prolonged and chronic phase. Most interestingly, TRPC6 activation is essential for the acute but not for the prolonged and chronic HPV, demonstrating that the acute phase is using different signal transduction components. We isolated precapillary PASMC from mouse and identified TRPC1 and 6 as the predominantely expressed TRPC channels. After priming with endothelin (4nM), application of hypoxia induced DAG accumulation and direct activation of TRPC6 resulting in Ca^{2+} influx and contraction which was completely absent in

TRPC6 deficient PASMC (2). The fact that H_2O_2, is able to initiate cation influx in wild-type (WT) but not TRPC6-deficient (TRPC6-/-) PASMC suggest a mechanistically important role of superoxide anions in the signal transduction process.
LIRI is a life-threatening condition that is accompanied by pulmonary edema caused by endothelial dysfunction. We show that lungs from mice lacking NADPH oxidase (NOX2-/-) or TRPC6 are protected from LIRI. Generation of chimeric mice by bone marrow cell transplantation suggested that endothelial, but not leukocytic NOX2 or TRPC6 are responsible for LIRI. Accordingly, lung endothelial cells isolated from NOX2- or TRPC6-deficient mice showed attenuated ischemia-induced Ca^{2+} influx, actin stress-fiber formation, cellular shape changes, and impaired barrier function. Production of reactive oxygen species (ROS) was completely abolished in NOX2-/- cells. A mechanistic model comprising NOX2-derived production of ROS, activation of C-type phospholipase γ, inhibition of diacylglycerol (DAG) kinase, DAG-mediated activation of TRPC6 and ensuing LIRI is conclusively supported by pharmacological and molecular evidence.

Conference 12

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. Redox modulation of SERCA structure and function has been observed under various biological conditions associated with increased levels of oxidative stress, such as atherosclerosis and aging, and has been mimicked *in vitro* to some extent through the exposure of SERCA to peroxyl radicals, peroxynitrite, and small amino acid-derived peroxides. Free radical pathways initiated through the exposure of SERCA to these reactive oxygen species lead to the formation of primary protein radicals, which, depending on their nature, have the opportunity to trigger secondary radical reactions. For example, SERCA thiyl radicals, either generated through direct reaction of SERCA thiols with reactive oxygen species, or through secondary reaction with potential SERCA tyrosyl radicals, have the opportunity to abstract hydrogen atoms from nearby C-H bonds, generating carbon-centered radicals, which could serve as origins for further protein oxidation, aggregation and/or fragmentation. We have monitored such reactions in SERCA through covalent H/D-exchange coupled to mass spectrometry, and identified very specific regions of SERCA subject to such hydrogen abstraction reactions. These processes add another level of complexity to SERCA redox modulation, important to understand for a full rationalization of oxidative stress effects on SERCA structure and function. For example, SERCA recovered from the aorta of diabetic animals contains a significant extent of oxidized and fragmented SERCA isoforms, which may form via free radical pathways as outlined above.
We will also present data on the interaction of SERCA with the anti-apoptotic protein Bcl-2. The incubation of SR membranes with Bcl-2 leads to a loss of SERCA activity, measured through ATP hydrolysis and Ca^{2+}-uptake. Through photoaffinity-labeling experiments a tentative SERCA domain interacting with Bcl-2 was identified, and several Bcl-2 loss-of-function and gain-of-function mutants were designed for mechanistic studies. Bcl-2 inactivation of SERCA was reduced in the presence of Hsp70. A potential biological role of the Bcl-2/SERCA interaction will be discussed.

Conference 13
Giorgio Aicardi

Conference 14

Isoform-Specific modulation of Inositol (1, 4, 5)-triphospate receptors by cytosolic ATP

David I. Yule

Department of Pharmacology & Physiology, University of Rochester, Rochester, NY, USA

Inositol (1, 4, 5)-trisphosphate receptors ($InsP_3R$) are the predominant route of calcium release in non-excitable cells and they play a major role in regulating calcium signaling in numerous physiological systems. There are three known isoforms ($InsP_3R$-1, InsP3R-2 and $InsP_3R$-3) and multiple splice variants of $InsP_3R$ expressed in mammalian cells. This sequence diversity along with varied tissue distributions hints at important isoform-specific regulatory mechanisms. One such regulatory mechanism is the modulation of calcium release from $InsP_3R$ by cytosolic free ATP. This form of regulation likely links the metabolic status of the cell to Ca^{2+} release and as such is important for normal physiology and in pathological situations. All three isoforms contain putative ATP binding domains, with $InsP_3R$-1 expressing two such domains (ATPA, and ATPB) in the S^{2+} isoform and three sites (ATPA, ATPB and ATPC) in the S2- splice variant while $InsP_3R$-2 and $InsP_3R$-3 each express a single ATPB site. Functionally, ATP has been shown to positively regulate $InsP_3R$-1 and $InsP_3R3$ while InsP3R-2 is thought to be insensitive to ATP modulation. The purpose of this study was to examine the contributions of ATP binding sites to the calcium release properties of the individual isoforms both in isolation and when expressed in native exocrine acinar cells. TNP-ATP binding assays using GST-fusion proteins containing the ATP binding domains were used to confirm ATP binding. Calcium release assays from permeabilized cells were used as a means of measuring the effects of ATP on endogenous InsP3R in native exocrine acinar cells and on individual wild type or mutant isoforms expressed in DT40-3ko cells. The results presented here demonstrate that, contrary to prior studies, $InsP_3R$-2 can indeed be modulated by ATP. In addition, even though $InsP_3R$-2 and $InsP_3R$-3 contain identical ATP biding sites, they exhibit dramatically different sensitivities to ATP. The impact of this differential modulation can therefore depend on the metabolic state of the cell and on the relative abundance and localization of the three $InsP_3R$ isoforms.

Conference 15

Modulation of TRP channels: impact on pain sensation

Peter A. McNaughton

Department of Pharmacology, University of Cambridge, UK

One of the distinguishing characteristics of the sensation of pain is that it is increased by inflammatory mediators such as prostaglandin E2, bradykinin and nerve growth factor (NGF). At least three different signalling mechanisms mediate the actions of inflammatory mediators on the heat-sensitive ion channel, TRPV1. Bradykinin and PGE2 enhance the probability that TRPV1 channels will be activated by a heat stimulus, and they act by promoting phosphorylation of TRPV1 by protein kinases C and A, respectively. NGF by contrast increases the expression of TRPV1 channels in the neuronal cell membrane by promoting trafficking from a subcellular vesicle store. Phosphorylation of TRPV1 by PKC and PKA depends critically on a scaffolding protein, AKAP79, which binds PKA and PKC into a signalling complex together with TRPV1. AKAP79 is therefore a final common element in heat hyperalgesia, on which the effects of multiple proinflammatory mediators converge. In more recent work we have examined the effect of inflammatory mediators on other members of the TRP family. TRPV4 is activated by pleasant warm temperatures, and it is potentiated by phosphorylation by PKA and PKC, in a similar manner to TRPV1. TRPM8, which is activated by cool temperatures and menthol, is by contrast inhibited by inflammatory mediators, apparently by a quite different mechanism. Because of their central roles in pain sensation, the modulation of TRP channels may prove an attractive target for the development of novel analgesics.

Conference 16

Novel aspects of TRPC (Patho) physiology

Klaus Groschner

Institute of Pharmaceutical Sciences – Pharmacology and Toxicology, Graz, Austria

TRPC proteins are highly versatile components of the cellular Ca^{2+} signalling network serving as multifunctional sensors for a wide array of stress factors. Ca^{2+} entry channels formed by TRC subunits are considered to enable the selective conversion of distinct input stimuli, including chemical, oxidative and mechanical stress, into highly specific Ca^{2+} and/or electrical signalling pattern. This function of TRPC channels allows for crucial adaptation processes in various tissues and organs. Versatility of TRPC signalling is based on promiscuity and complexity in gating as well as highly versatile coupling of TRPC proteins to different signalling partners. We have recently explored the role of TRPC-mediated cation (monovalent and Ca^{2+}) transport as a determinant of remodelling processes in the cardiovascular system, with particular focus on endothelial cells and cardiac myoctes. By over expression of mutant channels with distinct changes in ion permeation and/or gating properties in native cardiovascular cells, we identified TRPC3 as potential key player in electrical remodelling of the heart and TRPC4 as a determinant phenotype transitions in the endothelium. Our results demonstrate that the role of TRPC4 as part of an endothelial Ca^{2+} entry channel is restricted to a surprisingly narrow phenotype window. Moreover, our recent results suggest an additional, ion transport independent function of TRPC4, based on participation in the Wnt/β-cateinin signalling pathway. Dynamic targeting, assembly and disintegration of TRPC channel complexes appear essential for the control of function and fate of cardiovascular cells. Emerging concepts for therapeutic intervention based on TRPC targets will be discussed.

Conference 17

Redox regulation of protein kinases A and G: From detection to functional consequences

Joseph Burgoyne

Department of Cardiology, Cardiovascular Division, King's College London, The Rayne Institute, St Thomas' Hospital, London, UK

The regulatory RI subunits of protein kinase A (PKA) were identified using a proteomic screen of cardiac proteins sensitive to interprotein disulphide formation (i.e. oxidation). By trapping the protein in its in vivo oxidised state using alkylating agents during lysis we were able to demonstrate that PKA disulphide bond formation between its two regulatory RI subunits occurs in response to cellular hydrogen peroxide. This modification may increase the affinity of PKA for its binding partners the AKAPs leading to increased substrate phosphorylation and enhanced ventricular myocyte contractility by altering myocardial Ca^{2+} flux. Protein kinase G (PKG), which is a homodimer like PKA, was also found to be sensitive to oxidant-induced interprotein disulphide formation. The disulphide forms in the N-terminal region linking its two monomers; this increases the enzymes affinity for substrate and so induces activity. This novel form of activation underlies in part vasodilation in isolated vessels treated with hydrogen peroxide independent of cyclic nucleotide formation. PKG mediates vessel relaxation by decreasing intracellular Ca^{2+} sensitivity and Ca^{2+} concentration in vascular smooth muscle cells. This novel redox mechanism for PKA and PKG activation highlights an important mechanism for oxidant mediated regulation of intracellular Ca^{2+} with simultaneous modulation of cardiac contractility and vascular smooth muscle relaxation.

Conference 18

Calcium, mitochondria and the control of apoptosis and autophagy

Cristina Mammucari[1], D. De Stefani[1], V. Granatiero[1], M. Murgia[1], G.

Pallafacchina[1], M. Patron[1], A. Picard[1], A. Raffaello[1], A. Romagnoli[1], R. Siviero[1], E. Zecchini[1], Rosario Rizzuto[1]

[1]Department of Biomedical Sciences, University of Padova, Italy

Mitochondria rapidly accumulate Ca^{2+} through a low-affinity uptake system (the mitochondrial Ca^{2+} uniporter, MCU) because they are exposed to high [Ca^{2+}] microdomains generated by the opening of ER Ca^{2+} channels. These rapid [Ca^{2+}] changes stimulate Ca^{2+}-sensitive dehydrogenases of the mitochondrial matrix and hence rapidly upregulate ATP production in stimulatd cells. Ca^{2+} also sensitizes to cell death mediators, e.g. ceramide. Accordigly, we demonstrated that Bcl2-reduces the state of filling of ER Ca^{2+} stores, and this alteration is effective in reducing the sensitivity to apoptotic challenges. I will here review our latest data focusing on: (1) the role of p66shc, an isoform of a growth factor adapter, in the response to oxidative stress. Based on our previous in vitro results, linking hyperglycemia to ROS production and adipocyte differentiation of muscle precursors, we aimed on disclosing the physiological relevance of these observations. We have studied the role of p66 during adipogenic differentiation in a context of muscle damage and regeneration. Oxidative stress causes cell damage which can be counteracted by a number of defense responses, including autophagy. I will discuss the role of p66 in mediating the autophagic response to oxidative stress; (2) the molecular elements of the mitochondrial-ER Ca^{2+} connection. I will discuss the role of VDAC in rapidly challenging Ca^{2+} through the outer mitochondrial membrane and the specific funcions of VDAC isoforms in autophagy and apoptosis.

Conference 19

Myeloperoxidase, oxidants and damage to ion pumps

H. M. Viola[1], N. Cook[2], V. S. Sharov[3], C. Schoneich[3], L. C. Hool[1], Michael J. Davies[2]

[1] School of Biomedical Biomolecular and Chemical Sciences, University of Western Australia, Crawley, Australia

[2] The Heart Research Institute, 7 Eliza Street, Newtown, Sydney, Australia

[3] Dept. of Pharmaceutical Chemistry, University of Kansas, Lawrence, USA

The heme enzyme myeloperoxidase (MPO), released by activated white cells, kills invading pathogens by generating powerful oxidants. Thus H_2O_2 is used to convert Cl^- and SCN^- to hypochlorous (HOCl), and hypothiocyanous (HOSCN) acids. However oxidant formation at the wrong time, place, or concentration can result in host tissue damage – a process linked with multiple human inflammatory pathologies including heart disease, asthma, rheumatoid arthritis, cystic fibrosis, kidney disease and some cancers.

Kinetic data indicate that proteins and particularly Cys residues are major targets for oxidation. As many ion pumps and channels contain critical Cys residues, we postulated that oxidant generation by MPO may alter intracellular calcium (and other ion) levels.

Treatment of human coronary artery endothelial cells with HOCl increased intracellular calcium, with the magnitude of increase dependent on the oxidant concentration. The absence of extracellular Ca^{2+}, nisoldipine (inhibitor of L-type channels Ca^{2+} influx), or Ru360 (which blocks mitochondrial Ca^{2+} uptake) did not modulate this increase. In contrast, thapsigargin, which blocks Ca^{2+} re-uptake into internal stores (including via SERCA, the sarco / endoplasmic reticulum Ca^{2+}-ATPase) abolished this increase. HOSCN induced a greater increase than HOCl at identical concentrations, but SCN^- alone, or decomposed oxidant had no effect.

These data indicate that MPO-derived oxidants can modulate intracellular Ca^{2+} in a thapsigargin-sensitive manner. We propose that this arises from oxidation of critical Cys residues on a Ca^{2+}-regulatory protein, such as SERCA. Experiments with semi-purified SERCA indicate that this pump is rapidly inactivated by pathophysiological levels of HOCl and HOSCN.

Conference 20

Chloride Current During Beta-Amyloid Oxidative Stress Generation in Microglia

S. Averaimo[1], K. Kyritsi[1], R. Abeti[2], M. Duchen[2] and Michele Mazzanti[1].

[1]Università di Milano, via Celoria 26, 20133 Milano, Italy.

[2]University College of London, WC1E 6BT, London, UK.

One of the hallmarks of Alzheimer's Disease (AD) is the presence of amyloid plaques in the brain surrounded by astrocytes and reactive microglia. Activated microglia, stimulated by beta-amyloid (Aβ), produces reactive oxygen species (ROS) through the enzyme NADPH oxidase. We previously showed that ROS production causes translocation of CLIC1 (Chloride Intracellular Ion Channel 1) from the cytosol to the plasma membrane in microglia cell lines, microglia primary culture, and in a triple mutant AD mouse model. Once in the membrane, CLIC1 is able to promote a chloride conductance. CLIC1conductance is specifically blocked with 50 μM of IAA94 and down regulated by CLIC1 specific siRNA transfection.
Some authors believe that NOX 2 activation is the key event converting resting microglia into activated, proliferating, cytotoxic microglia. In particular, during bacterial invasion of the central nervous system (CNS) microglia cells are activated to destroy the invaders. Thus, physiologically, microglia cells have to efficiently produce toxic agents to prevent the infection spread. Increasing concentration of aberrant proteins, like amyloid compounds, are recognized by the brain immune system as a threat. Although amyloid aggregates are self produced, microglia actively works to remove them. Since the presence of amyloids is not localized, microglia toxic molecules release is wide, damaging a large number of neurons. Therefore, the idea is that blocking oxidase activation may block inflammatory neurodegeneration. As inhibition of the NADPH oxidase will cause systemic immunosuppression, it is imperative to identify more selective targets that will permit specific inhibition of CNS inflammatory responses. The decrease of CLIC1 functional expression impairs ROS production via a negative feed-back on the membrane oxidase NOX2. Thus, CLIC1 is an essential element in the ROS production during the progression of a neurodegenerative cellular process. Our experiments suggest that the CLIC1 chloride current works to balance the excessive charges extruded by the oxidase. The activation of the CLIC1 chloride current allows charge compensation, maintaining the membrane potential hyperpolarized where NOX2 is supposed to be more efficient. These results suggest that NOX2 in microglia cells has significant voltage dependence with more efficient ROS production at more hyperpolarized potentials.
In conclusion we found that CLIC1 is involved in ROS production by the NADPH oxidase in Aβ stimulated microglia cells. The compensating current mediated by CLIC1, indeed, sets the membrane potential and avoids an excessive depolarization that could in turn impair the optimal functioning of the oxidase.

Conference 21

Oxidized protein degradation and repair in aging and oxidative stress.

Bertrand Friguet

Laboratoire de Biologie Cellulaire du Vieillissement, UR4, IFR83, Université Pierre et Marie Curie, Paris, France.

Oxidatively modified protein buildup with age results, at least in part, from the increase of reactive oxygen species and other toxic compounds coming from both cellular metabolism and external factors, but experimental evidence has also indicated that failure of protein maintenance (degradation and repair) is a major contributor to the age-associated accumulation of damaged proteins. Oxidized protein degradation is mainly achieved by the proteasomal system in the cytosol and the nucleus while the Lon protease has been implicated in oxidized protein degradation within the mitochondrial matrix. Oxidized protein repair is limited to few protein oxidative modifications, such as methionine oxidation, that can be reversed by the methionine sulfoxide reductase (Msr) enzymes, MsrA and MsrB. Importantly, the Msr system has been implicated in increased longevity and resistance to oxidative stress in different cell types and model organisms. Interestingly, it has also been involved in the functional regulation of calcium binding

proteins such as calmodulin and calcineurin that are important players in calcium signaling. To analyze the relationship between oxidative stress, protein oxidative damage and Msr, MsrA full-length cDNA has been overexpressed in SV40 T antigen-immortalized WI-38 human fibroblasts while MsrA and MsrB2 full-length cDNA have been overexpressed in Molt-4 lymphoblastoid cells. Overexpression of either MsrA or MsrB2 was found to protect the cells against oxidative stress-induced cell death. After hydrogen peroxide-induced oxidative stress, both MsrA- and MsrB2-overexpressing cells exhibited lower protein oxidative damage than control cells. Moreover, we demonstrated that the mechanisms by which MsrB2 protects against oxidative stress include: maintenance of a lower level of intracellular ROS, preservation of mitochondrial integrity, prevention of oxidized protein accumulation and protection of the proteasome against oxidative stress-induced inactivation. Using replicative senescence of human WI-38 fibroblasts as a model for studying cellular aging, we have recently provided evidence that oxidized proteins and also proteins modified by the lipid peroxidation product (4-hydroxy-2-nonenal: HNE) and glycoxidation (AGE) adducts are increased in senescent fibroblasts compared to young ones. Identification of proteins selectively targeted by oxidation and by modification with HNE and AGE, have shown that a large number of the identified proteins are mitochondrial proteins. Investigation of the protein repair system Msr has revealed that the decrease in Msr activity in senescent cells is more important in the mitochondria than in the cytosol. Taken together, these results indicate that the Msr system may play an important role in cellular defenses against oxidative stress by protecting proteins against oxidation and limiting the accumulation of oxidized proteins.

Conference 22

Drug delivery proteins and hypoxia in brain

Ertuğrul Kılıç

Deparment of Physiology, Faculty of Medicine, Yeditepe University, Istanbul

Neuroprotection therapies have made limited progress in recent years. Several compounds shown to be efficacious in animals were tested in humans in cost-expensive trials. Unfortunately none of these studies were able to demonstrate efficacy under clinical conditions in patients. In order to establish treatments that are of benefit not only in animals but also humans, new strategies are clearly needed, comprising (i) new factors mimicking intrinsic mechanisms that the brain itself makes use of, (ii) novel delivery techniques allowing drugs to pass the blood-brain barrier more efficaciously than before, (iii) better, functionally relevant readouts of brain recovery and (iv) strategies that are of usefulness not only in the acute, but also post-acute stroke phase. In this presentation, our recent studies will be reviewed.

Conference 23

Relationship between calcium signalling, reactive oxygen species and apoptosis

José A. Pariente

Department of Physiology, Neuroimmunophysiology Research Group, Faculty of Science, University of Extremadura, 06006 Badajoz, Spain

The calcium ion is one of the cellular signaling 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. Previous studies have indicated that the reactive oxygen species (ROS) release calcium from both mitochondrial and non-mitochondrial stores. Additionally, ROS can inhibit the extrusion or exit of calcium to the exterior, thus inhibiting the activity of the plasma membrane calcium pump (PMCA), and in some cases even, suppress inhibiting the activity of the sarcoendoplasmic reticulum calcium pump (SERCA), with the subsequent

inhibition the refilling of intracellular stores of calcium. The stimulation of capacitative calcium entry (CCE) induced by ROS also contribute to increases in the cytosolic calcium signal. Therefore, ROS can be regarded as an excellent tool for increasing $[Ca^{2+}]_c$ and producing intracellular calcium overload.

On the other hand, of all the intracellular organelles, the mitochondrion is considered to be one of the most important cellular sources of oxidizing agents due to its high consumption of oxygen during the process of ATP synthesis in the respiratory chain. Mitochondria are currently considered to be one of the cellular compartments involved in calcium homeostasis. Moreover, mitochondria participate very significantly in the processes of apoptosis or programmed cell death, which is a physiological process of vital importance in the development of multicellular organisms, being essential in some physiological processes.

This is the context in which we have evaluated the participation of the calcium signal in apoptosis, and its relationship with ROS. Our results suggest that stimulation with physiological agonists that increase calcium release from intracellular stores produces a depletion of intracellular calcium, and a stimulation of caspases 3 and 9 and their association with the actin cytoskeleton in human platelets, a process which requires activation of protein kinase C (PKC) and is dependent on the reorganization of the actin filaments, but is independent of intracellular calcium increases while requiring the depletion of calcium stores. We have been able to show that, in human platelets, stimulation with thrombin causes early activation of caspase 3 activity, which is not related to the apoptotic process and seems to be involved in certain cell processes, at least in human platelets and mouse pancreatic acinar cells.

We also found that hydrogen peroxide (H_2O_2) produced a dose- and time-dependent increase in the activity of caspases 9 and 3, and it also produced a dose-dependent increase in phosphatidylserine externalization. Thrombin induced a dose-dependent mitochondrial depolarization, which was dependent on the presence of both extra- and intra-cellular calcium. We were also able to observe that thrombin produced endogenous H_2O_2, and that the treatment with catalase (an enzyme that breaks H_2O_2 down into H_2O plus O_2) significantly reduced the phosphatidylserine externalization and caspase 3 and 9 activation induced by thrombin. This implies that the endogenous production of H_2O_2 induced by thrombin is required for the development of apoptosis in human platelets.

Our results also showed that thrombin stimulates the pro-apoptotic proteins Bax and Bid, the effects being dose- and time-dependent. Additionally, thrombin increased the mitochondrial association of active Bax and Bid.

Finally, we can conclude that we have developed a model of apoptosis induced by ROS and calcium overload that is characterized by: i) a modification of mitochondrial activity, ii) cytochrome c release from mitochondria to cytosol, iii) externalization of phosphatidylserine., iv) activation of caspase 3 and 9 (this includes an early activation of caspase 3 which is not related to the apoptotic process but is required for certain cell functions, in particular in the capacitative calcium entry and the aggregation of human platelets, and in the secretion of amylases of mouse pancreatic acini), v) translocation of caspases from cytosol to cytoskeleton; and finally, vi) Bax and Bid activation and translocation to mitochondria.

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Conference 24

Melatonin: Helping cells cope with oxidative disaster.

Sergio D. Paredes

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

Melatonin possesses the capability of donating electrons, consequently reducing the reactivity of molecules with an unimpaired electron in their valence orbital, i.e. free radicals. A plethora of studies support the role of this indoleamine in diminishing molecular damage associated with massive free radical generation both *in vitro* and *in vivo*. Melatonin protects against ischemia/reperfusion injury, acts as a radioprotector, and counteracts free radical toxicity due to its essential role in antioxidant protection. At the intracellular level, it reduces electron leakage from the mitochondrial respiratory chain complexes as well as scavenging radicals generated in the cytosol (exclusive of its actions in mitochondria) and nucleus. Comparative

studies with other well-known naturally occurring antioxidants show that melatonin's efficacy is equal to or better in neutralizing highly toxic oxygen and nitrogen-reactants. However, not only melatonin but a series of its metabolites are also capable of detoxifying free radicals and related species in what is referred to as the antioxidative cascade. Thus, melatonin may be actually seen as a prodrug for a family of other molecules that also have the capability of reducing oxidative / nitrosative stress. Taken together, the results reviewed here point to consider melatonin as a key element in mainstream antioxidative medicine in the context of the antioxidative defense system.
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Conference 25

Melatonin in the treatment of breast cancer

Ahmet KORKMAZ

Department of Physiology, Gulhane Military Medical Academy, Ankara, Turkey.

Recent studies have suggested that the pineal product melatonin, as nature's most versatile and pleiotropic molecule, may protect/treat breast cancer, and the mechanisms underlying its actions are becoming clearer. Experimental studies suggest that the antineoplastic action of melatonin arises through many different mechanisms, including melatonin's antioxidant, antimitotic, and antiangiogenic activity, as well as its ability to modulate the immune system and alter fat metabolism. Melatonin interacts with membrane and nuclear receptors, and may be linked to the regulation of tumor growth. An intriguing mechanism has recently been suggested; melatonin may regulate a variety of epigenetic mechanisms and affects the tumor biology. Of particular relevance to breast cancer risk, melatonin may also block the estrogen receptor ERα and impact the enzyme aromatase, which produces estradiol. A growing number of epidemiologic studies have evaluated the relationship between night shift works as well as how varying duration of sleep affects peak melatonin secretion at night. In addition, independently of receptors melatonin can modulate estrogen dependent pathways and reduce free-radical formation, thus preventing mutation and cellular toxicity. The fact that melatonin works through a myriad of signaling cascades that are protective to cells makes this molecule a good candidate for use in the clinic for the prevention and/or treatment of cancer.

Conference 26

Hyperbaric oxygenation and oxidative stress: using reactive molecules for its action.

Sukru Oter

Department of Physiology, Gulhane Military Medical Academy, Ankara, Turkey.

The therapeutic mechanisms of action for hyperbaric oxygen (HBO_2) are classically explained via two main ways: (i) the physical effect which depends directly to the elevated ambient pressure and causes a reduction in the volume of gas-filled spaces according to Boyle's law; (ii) the chemical effect acting through increased partial oxygen pressure (PaO_2). Nevertheless, these general explanations fail to elucidate the molecular details which are responsible for the beneficial effects of HBO_2 therapy.
It is definitely known that exposure to HBO_2 results in increased production of reactive oxygen species (ROS) in living organisms and, consequently, can lead to oxidation of bio-molecules such as membrane lipids and/or proteins. Recently, a series of experimental studies conducted in our institution clearly presented increased levels of oxidation products in the blood, lung and brain tissues of rats after HBO_2 exposure within its approved therapeutic limits (max. 3 atm for 2 h); the increase of oxidation products were found to be directly related to the exposure-time and –pressure of HBO_2. On the other hand, activities of endogenously produced antioxidant enzymes, namely superoxide dismutase, glutathione peroxidase and catalase, also presented enhanced levels in the abovementioned studies. Therefore, it seems that HBO_2 treatment causes a moderate level of oxidative stress which physiologically lasts under the control of endogenous antioxidant mechanisms.

It is obvious that free radicals also present physiological functions and that they have the ability to play role as signaling molecules in pathophysiological processes. An interesting 'counteracting bidirectional' action, i.e. causing oxidative stress itself but limiting the level of oxidative stress which is present due to other stressing agents or conditions, was described for HBO_2 exposure. The hypothesis that HBO_2 exerts at least some of its beneficial effects via ROS is now widely accepted; particularly for its use in wound healing, post-ischemia and inflammatory pathologies.
Taken together, this presentation will discuss the potential oxidative action of HBO_2 exposure and the importance of the accompanying enhanced levels of antioxidant molecules during treatment.

Conference 27

Melatonin receptors in human cancer: State of the art and future perspectives

Cem Ekmekcioglu

Department of Physiology, Medical University Vienna, Austria

In addition to its antioxidative effects melatonin acts through specific membrane and nuclear receptors. To date, two G-protein coupled melatonin membrane receptors, MT_1 and MT_2, have been cloned in mammals, while the newly purified MT_3 protein belongs to the family of quinone reductases. Screening studies have shown that various tissues of rodents and humans express MT_1 and/or MT_2 melatonin receptors. The role of melatonin in human physiology is still under investigation. In addition to sleep inducing effects there is growing evidence that melatonin is involved in the regulation of circadian rhythms and seasonal changes.
Regarding cancer, melatonin exerts oncostatic actions on a variety of cancer cells from different organs, such as those from breast, prostate, skin, liver and colon. In extensively investigated breast cancer cells the anticancerogenic effects of melatonin are mainly mediated through the MT1 receptor. But also antioxidant and immunostimulating effects seem to be important factors in the oncostatic actions of melatonin. Furthermore clinical investigations showed that melatonin secretion is impaired in patients suffering from breast, endometrial, or colorectal cancer. However, due to a lack in large scaled intervention studies, the effect of melatonin administration on human cancer development and progression is still unclear. Future experimental studies could focus on the connection between melatonin and clock genes. Both are not only essential components of the circadian system but also are implicated in human breast cancer and cancer cell proliferation.

Conference 28
Hüseyin Bağcı

Conference 29
Ozcan Erel

Novel, Easy and Applicable Tests to Measure Oxidant and Antioxidant System Homeostasis and Open Research Areas

Özcan EREL

Department of Biochemistry, Ataturk Training and Research Hospital, Ankara,Turkey

Reactive oxygen species (ROS) are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms which remove them via enzymatic and nonenzymatic antioxidative mechanisms. Under certain conditions, the increase in oxidants and decrease in antioxidants cannot be prevented, and the oxidative / antioxidative balance shifts towards the oxidativestatus.
Consequently, oxidativestress, which has been implicated in over 100 disorders, develops. Serum (or plasma) concentrations of different oxidant and antioxidant species can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive and costly and require complicated techniques. Since the measurement of different oxidant and antioxidant molecules separately is not practical and their oxidative and antioxidative effects are additive, the total oxidant status (TOS) and the total antioxidant status (TAS) of a sample is measured. TAS levels of all biological fluids and tissues can be measured by using our third generation TAS assay kit, which has high analytical performance characteristics. TOS levels of all biological fluids and tissues can be measured by using our TOS assay kit,

which has high analytical performance characteristics.
A specific antioxidant enzyme paraoxonase (PON1) hydrolyses lipid hydroperoxides to native lipids and it protects against atherosclerosis. This enzyme test may take a place in routine clinical biochemistry tests in near future. It has been suggested that PON1 test can be used to evaluate the functional status of liver.Manual methods to measure PON1 activity are present but they are not used in routine biochemistry laboratories because the used substrates hydrolyze spontaneously and they are very toxic. We developed novel, stable, practice and fully automated PON1 activity measurement methods and assay kits which can be used in routine biochemistry laboratories.

The developed tests which are easy, stable, reliable, sensitive, inexpensive, manual and fully automated methods can be used to measure total oxidant status, total antioxidant status, oxidative stress index, PON1-paraoxonase and PON1-arylesterase levels of samples.

Oral Presentations

Oral Presentation 1

Angiogenic and antiangiogenic factors in South African Black Preeclamptic women and in a rat model of preeclampsia

Prem Gathiram[1], L. Govender [1], S. Ramesar [1], I. Mackraj [1] and J. Moodley[2]

[1]Department of Human Physiology and [2]Department of Obstetrics and Gynecology and Women's Health and HIV Research Unit, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa.

Preeclampsia, a pregnancy specific syndrome, is characterised by the onset of hypertension and significant proteinuria after 20 weeks of gestation. The etiology and pathogenesis of preeclampsia remains elusive. It has been suggested that ischemia in the placental bed may cause the release of excess antiangiogenic factors over angiogenic factors that lead to multi organ endothelial damage resulting in clinical features of vasoconstriction and platelet activation presenting as high blood pressure, proteinuria and platelet consumption. In this study we compared the blood levels of antiangiogenic and angiogenic factors in South African black preeclamptic women with a rat model (n=24) in which preeclampsia-like syndrome was produced by L-Name (0.3 g/l drinking water) administration. Fifty eight patients were recruited from a South African Hospital. ELISA technique was used for serum sFlt-1 and VEGF assays. Compared to the normotensive control group, the preeclamptic group had significantly elevated serum sFlt-1 levels, whilst the levels of VEGF were undetectable. Compared to the control rats, the preeclamptic group had a significantly raised systolic blood pressure and a concomitant and significant rise in serum sFlt-1 concentrations on gestation day 21. The levels of serum VEGF were low in both groups with no significant difference between the groups. Microalbuminurea levels were raised in preeclamptic rats compared to the controls. Sildenafil citrate (10 mg/kg, b.w., s.c.) administration to preeclamptic rats significantly reduced the high blood pressure, serum sFlt-1 concentrations and microalbuminurea but not the VEGF concentrations. We conclude that the changes in rat model are similar to that found in preeclampsia.

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Oral Presentation 2

Medical application of photosensitization

Mehmet Dinçer Bilgin

Department of Biophysics, School of Medicine, Adnan Menderes University, Aydin, Turkey

Photosensitization is a light-activated process in which the presence of a chromophoric agent (referred to as a photosensitizer) leads to changes in a substrate that otherwise would not take place. Biological pigments (e.g. chlorophyll, carotenoids) and synthetic dyes (e.g. porphyrins, chlorines, etc.) are typical photosensitizers. The pathway of photosensitized reactions is divided into two categories. In Type I photosensitization, the optically-excited photosensitizer reacts first with a major substrate under the anoxic condition. The substrate may then react with molecular oxygen (e.g. Photosythesis, PUVA therapy). In Type II photosensitization, the optically-excited photosensitizer reacts first with molecular oxygen, leading to "active" oxygen intermediates that initiate further reactions (e.g. Photodynamic Therapy). There are three main medical application of photosensitization: PUVA photochemotherapy of skin disorders, "blue light" phototherapy of jaundice in newborns and photodynamic therapy of cancer and non-cancer disorders such as aged-related macular degeneration, actinic keratosis and atherosclerotic vascular disease. Also, extracorporeal photopheresis, an implementation of PUVA, is used for the treatment of cutaneous T-cell lymphomas. The photosensitization of viral and bacterial contamination in blood products and photosensitization of dental bacteria are the other applications. Improvements in light delivery and dosimetry, standardization of study protocols, development of new photosensitizing agents and new light sources are required to generalize the medical applications of photosensitizers.

Oral Presentation 3

Dehydroepiandrosterone ameliorates hepatocellular damage in obstructive jaundice

Metehan Gümüş[1], F. Çelebi[2], A. Böyük[1], N. Gürsan[3], F. Akçay[4]

[1]Department of General Surgery, Medical Faculty, Dicle University, Diyarbakir, Turkey

[2]Department of General Surgery, Medical Faculty, Atatürk University, Erzurum, Turkey

[3]Department of Pathology, Medical Faculty, Atatürk University, Erzurum, Turkey

[4]Department of Biochemistry, Medical Faculty, Atatürk University, Erzurum, Turkey

We aimed to investigate the ameliorating effect of dehydroepiandrosterone (DHEA) on the potential hepatocellular damage in experimental obstructive jaundice. Twenty-four male rabbits in the study were randomly allocated into three groups. In the sham group, the choledochal canal was identified and explored. In the obstructive jaundice and treatment groups, the choledochal canal was ligated. Placebo and DHEA were administered to the obstructive jaundice and treatment groups, respectively. Blood samples were obtained at baseline, and both blood samples and liver tissue samples were obtained by re-laparotomy performed on day 8. Biochemical parameters were measured in blood samples, and liver samples were histopathologically evaluated. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and bilirubin levels were lower in the treatment group than in obstructive jaundice. Mononuclear inflammation in the portal region and hepatocyte degeneration were milder in the treatment group compared to obstructive jaundice group. These findings suggested that DHEA may reduce the obstructive jaundice-induced hepatocellular damage.

Oral Presentation 4

Energetics of ion transfer through potassium channels.

Özlem Mercan[1], M. Çavuş[2], T. Baştuğ[3], S. Kuyucak[4]

[1]Faculty of Science and Art , Department of Physics, Süleyman Demirel Üniversity, Isparta, Turkey.

[2]Faculty of Science and Art , Department of Physics, Gazi University, Ankara, Turkey.

[3]TOBB Economy and Technology University, Faculty of Science and Art Ankara, Turkey.

[4]School of Physics, University of Sydney, NSW, Sydney, Australia.

Ion channels are formed by specific proteins embedded in the cell membrane and provide pathways for fast and controlled flow of ions down the electrochemical gradient. This activity generates action potentials in nerves, muscles and other excitable cells, and forms the basis of all movement, sensation and thought processes in living beings. While the functional properties of ion channels are well known from physiological studies, lack of structural knowledge has hindered development of theoretical models necessary for understanding and interpretation of these properties. Recent determination of the molecular structures of potassium and mechanosensitive channels from x-ray crystallography has finally broken this impasse, starting a new age in the field of ion channels where study of structure-function relationships will take a central stage.

There are by now several potassium channel structures available both in the open and closed states. Using these structures in free energy molecular dynamics simulations, it is possible to study the structure-function relations in potassium channels in a rigorous fashion. Here we use free energy simulations in a comparative study of selectivity and energetics of ion permeation in the KcsA and Shaker potassium channels.

Oral Presentation 5

Effects of selenium on TRPM2 cation channels activated by hydrogen peroxide and ADP-ribose

Mustafa Nazıroğlu[1], M. Küçükayaz[1], Ö. Çelik[1], E. Sözbir[1], C. Özgül[1]

[1]Department of Biophysics, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

The TRPM2 cation channels can be gated either by ADRR in concert with Ca^{2+} or by H_2O_2, an experimental model for oxidative stress, binding to the channel's enzymatic Nudix domain. Selenium is an antioxidant trace element and it is also cofactor of antioxidant enzyme glutathione peroxidase (1). Recently we observed protective effects of selenium on cytosolic Ca^{2+} release in HL-60 cell line (2). To our knowledge, direct TRPM2 channel blocker has not discovered till today. Since the mechanisms that lead to TRPM2 inhibiting in response to ADP- ribose and H_2O_2 are not understood, we tested the effects of selenium on TRPM2 currents in Chinese hamster ovary (CHO) cell lines. The cells were incubated by 10 μM selenium and 15 minutes before patch-clamp and Ca^{2+} release analysis (2). The control and selenium incubated cells studied with the conventional whole-cell patch clamp technique. H_2O_2 (10 mM) was added extracellularly although the ADP- ribose (0.3 mM) was applied intracellularly (i.e. through the pipette). Non-selective cation currents in whole cell experiments of control and selenium incubated cells were consistently induced by ADP- ribose, NAD^+ and H_2O_2. The time cause of ADP- ribose and NAD^+ in the cells was characterized by a delay of 0.6-3.0 min and a slow current induction to a plateau although the time cause of H_2O_2 was characterized by a delay of 2-5 min and it didn't reach a slow current induction to a plateau. There were statically differences on the mean values of TRPM2 channel opening time and capacitance between control and selenium incubated cells. In conclusion, these results demonstrated that all three agonists, H_2O_2, ADP- ribose and NAD^+, are capable of activating TRPM2 although selenium did not inhibiting effect on CHO cell line TRPM2 cation channels activated by hydrogen peroxide and ADP-ribose.

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Oral Presentation 6

Characterization of conserved amino acid mutations in *cbb*$_3$-type oxidase enzyme of *Rhodobacter capsulatus.*

M. Öztürk[1], Gülgez Gökçe Yıldız[1], D. Erdoğan[2]

[1]Abant İzzet Baysal University, Faculty of Science and Literature, Department of Biology, 14280, Bolu, TURKEY

[2] Gebze Institude of Biotechnology, Faculty of Science, Department of Biology, 41400, Kocaeli, TURKEY.

Terminal oxidases of membrane-bound electron transfer chains from mitochondria, many aerobic bacteria and archea that catalyze the reduction of molecular oxygen to water to the translocation of protons across the plasma membrane. The *cbb*$_3$-type oxidases are distinct class of proton-pumping respiratory heme-copper oxidases and represent identical channels in all eukaryotic and in several but not all bacterial *cbb*$_3$ enzymes. Two such channels have been characterized by site-specific mutations in bacteria and are called D- and K-channels after their central, conserved amino acids which serve the same function of coupling electron transfer and proton transport. Since the C-type oxygen reeducates have been shown to pump protons, it is certain that there must be proton-conducting channels in this family that play analogous roles to the D- and/or K-channels. In this study, conserved Asn346, Thr272, Tyr280 and Tyr37 in *Cco*N subunit of *cbb*$_3$-type oxidase in *R. capsulatus* were substituted to Val346, Ala272, Phe280 and Phe374 by site-directed mutagenesis technique in order to investigate their roles on the activity of *cbb*$_3$–type oxidase. The effects of these mutations on the catalytic activity were determined by using NADI staining. The results indicate that Asn346 and Tyr374 mutations led to a complete loss of enzyme activity while Thr272 and Tyr280 mutations cause a partial loss of catalytic activity of the enzyme. These results suggested that these conserved amino

acid residues may play important role in the enzyme activity or assembly.

Oral Presentation 7

Effect of antioxidants on microscopic semen parameters, lipid peroxidation and antioxidant activities in Angora goat semen following cryopreservation

Purhan Barbaros Tuncer [1], M. N. Bucak [1], S. Sarıözkan [4], P. A. Ulutaş [2], O. Büyükleblebici [3], H. İ. Akçadağ [1]

[1] Ministry of Agriculture and Rural Affairs, Lalahan Livestock Central Research Institute, Lalahan, Ankara, Turkey

[2] Department of Biochemistry, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, Turkey

[3] Department of Biochemistry, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

[4]Erciyes University, Faculty of Medicine, Hakan Çetinsaya Experimental and Clinical Research Center, Kayseri

The aim of this study was to determine the effects of the antioxidants glutamine and hyaluronan and the inclusion of different levels on microscopic semen parameters, lipid peroxidation and the antioxidant activities following the freeze–thawing of Angora goat semen. Ejaculates collected from three Angora goat bucks, were evaluated and pooled at 37 °C. The semen samples which were diluted with a Tris-based extender containing additives including glutamine (2.5; 5mM) and hyaluronan (500; 1000 µl/ml), and an extender containing no antioxidants (control) were cooled to 5 °C and frozen in 0.25 ml French straws and stored in liquid nitrogen. Frozen straws were thawed individually (37 °C) for 20 s in a water bath for microscopic evaluation. Freezing extenders supplemented with 2.5 and 5mM glutamine led to higher sperm motility and hypo-osmotic swelling test (HOST) values compared to the control (p<0.05) following the freeze–thawing process. The addition of 500 µl l/ml hyaluronan resulted in a higher HOST percentage, compared to the addition of 1000 µl/ml hyaluronan and the control (p< 0.001). No significant difference was recorded in the percentage acrosome and total sperm abnormalities, following supplementation with antioxidants. The addition of antioxidants did not prevent malondialdehyde (MDA) formation. Antioxidant treatment however decreased (p< 0.01) the superoxide dismutase activity. The maintenance of catalase activity was demonstrated to be insignificant following addition of antioxidants. Further studies are required to obtain more repeatable results regarding the characterization of the enzymatic and non-enzymatic antioxidant systems in cryopreserved goat sperm.

Oral Presentation 8

Comparison of the effects of glutamine and an amino acid solution on post-thawed ram sperm parameters, lipid peroxidation and anti-oxidant activities

Mustafa Numan Bucak [1], P. B. Tuncer [1], S. Sarıözkan [1], P. A.Ulutaş [2], O. Büyükleblebici [3]

[1]Ministry of Agriculture and Rural Affairs, Lalahan Livestock Central Research Institute, Lalahan, Ankara, Turkey

[2]Adnan Menderes University, Faculty of Veterinary Medicine, Department of Biochemistry, Aydın, Turkey

[3]Ankara University, Faculty of Veterinary Medicine, Department of Biochemistry, Ankara, Turkey

Ram semen contains sufficient quantities of superoxide dismutase (SOD) and much lower concentrations of glutathione peroxidase (GSH-Px) and catalase (CAT) to prevent oxidative damage. The anti-oxidant capacity of the sperm cell is limited, due to a small cytoplasmic component, which contains these anti-oxidants to scavenge the oxidants. However, the concentration of these anti-oxidants may decrease considerably by the dilution of the semen. The aim of the present work was to study the effect of two anti-oxidants, namely, glutamine and an amino acid solution (BME) in a Tris-based extender on ram sperm parameters, lipid peroxidation and

anti-oxidant capacity after the cryopreservation/thawing process. Ejaculates collected from 4 Akkaraman rams were evaluated and pooled at 37°C. Semen samples which were diluted with the tris-based extender containing glutamine (2.5 or 5 mM), BME (13 or 26%), and no anti-oxidants (control) were cooled to 5°C and frozen in 0.25-ml French straws and stored in liquid nitrogen. Frozen straws were thawed individually at 37°C for 20 s in a water bath for evaluation. The freezing extender supplemented with 5mM glutamine led to higher motility rate (68.0±4.4%) and hypo-osmotic swelling test (HOST) (64.1±5.5%), when compared to glutamine (2.5 mM) and BME (13 and 26%) ($p< 0.05$). No significant differences were observed regarding sperm motility and HOST, following the supplementation of the freezing extender with glutamine 2.5 mM and BME (13 and 26%) after thawing. CAT activity remained significantly higher following the addition of glutamine 5mM (6.4±0.9 kU/g protein), compared to the other treatments ($p< 0.01$). The anti-oxidants at different levels were not effective in the elimination of malondialdehyde (MDA) formation and maintenance of SOD activities, when compared to the control ($p< 0.05$). Findings showed that glutamine (5 mM) supplementation in semen extenders, was of greater benefit to frozen–thawed ram sperm. Future efforts are needed to find the appropriate anti-oxidants and their effective concentrations to improve post-thaw sperm parameters (e.g. motility, membrane integrity, fertility) and anti-oxidant activities when frozen–thawed ram sperm is used.

Oral Presentation 9

The influence of cysteine and taurine on microscopic–oxidative stress parameters and fertilizing ability of bull semen following cryopreservation

Serpil Sarıözkan[1], M.N. Bucak[2], P.B. Tuncer[2], P.A. Ulutaş[3], A. Bilgen[2]

[1]Hakan Çetinsaya Experimental and Clinical Research Center, Faculty of Medicine, University of Erciyes, Kayseri, Turkey

[2]Ministry of Agriculture and Rural Affairs, Lalahan Livestock Central Research Institute, Ankara, Turkey

[3]Department of Biochemistry, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydın, Turkey

Oxidative stress significantly damages sperm functions such as motility, functional integrity, endogenous antioxidant enzyme activities and fertility due to lipid peroxidation induced by reactive oxygen species (ROS). The aim of this study was to determine the effects of antioxidants such as taurine and cysteine in Bioxcell extender on standard semen parameters, fertilizing ability, lipid peroxidation (LPO) and antioxidant activities comprising reduced glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) after the cryopreservation/thawing of bull semen. Nine ejaculates for each bull were included in the study. Three groups, namely taurine (2 mM), cysteine (2 mM), and control, were designed to analyze the antioxidants in Bioxcell. The addition of cysteine led to higher motility, compared to the other groups ($p< 0.001$). Cysteine showed a greater protective effect on the percentages of acrosome and total abnormalities in comparison to the other groups ($p< 0.001$). No significant differences were observed in hypo-osmotic swelling test (HOST), following supplementation with antioxidants during the freeze–thawing process. No significant difference was observed in non-return rates among groups. In biochemical assays, the additives did not show effectiveness on the elimination of malondialdehyde (MDA) formation and maintenance of GSH and GSH-Px activities, when compared to controls. CAT activity was demonstrated to be significantly higher upon the addition of 2 mM taurine ($p< 0.001$), while the level of MDA increased, indicating oxidative stress in this group. SOD activity was significantly elevated in the group with cysteine, compared to the other groups ($p< 0.001$).

Oral Presentation 10

The Effect of antioxidants on Sperm Parameters, Lipid peroxidation (LPO), Total glutathione (Total GSH) and Antioxidant Potential (AOP) Activities of Post-Thawed bovine Semen

Pınar Peker Akalın[1], M.N. Bucak[2], P. B. Tuncer[2], S. Sarıözkan[3], N.Başpınar[4], K. Çoyan[5], A. D. Ömür [5]

[1] Ministry of Agriculture and Rural Affairs, Central Veterinary Control and Research Institude, Ankara, Turkey

[2] Ministry of Agriculture and Rural Affairs, Lalahan Livestock Central Research Institute, Lalahan, Ankara, Turkey

[3] Erciyes University, Safiye Cikrikcioglu Vocational College, Kayseri, Turkey

[4] Selcuk University, Faculty of Veterinary Medicine, Department of Biochemistry, Konya, Turkey

[5] Selcuk University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Konya, Turkey

This study was conducted to determine the effects of methionine, inositol and carnitine on standard sperm parameters, LPO (lipid peroxidation), total glutathione (total GSH) and antioxidant potential (AOP) activities of bovine semen after the freeze–thawing process. Nine ejaculates collected with the aid of an artificial vagina twice a week from each Simmental bovine were included in the study. Each ejaculate, splitted into seven equal groups and diluted in Tris-based extender containing methionine (2.5 and 7.5 mM), carnitine (2.5 and 7.5 mM), inositol (2.5 and 7.5 mM) and no additive (control), was cooled to 5^{o}C and then frozen in 0.25 ml French straws. Frozen straws were thawed individually at 37^{o}C for 20 sec in a water bath for the evaluation.

The extender supplemented with 7.5 mM doses of carnitine and inositol led to higher subjective motility percentages (61.9±1.3% and 51.3±1.6%) compared to the other groups. The addition of methionine and carnitine at doses of 2.5 and 7.5 mM and inositol at doses of 7.5 mM provided a greater protective effect in the percentages of total abnormality in comparison to the control and inositol 2.5 mM ($p<0.001$). In biochemical parameters, supplementation with antioxidants did not significantly affect LPO and total GSH levels in comparison to the control group ($p>0.05$). The maintenance of AOP activity in methionine 2.5 mM was demonstrated to be higher (5.06±0.38 mM) than that of control (0.96±0.29 mM) following the freeze-thawing ($p<0.001$).

Poster Presentations

Poster N^{o} 1

How the cortisol level and brain antioxidant activity change in chronic REM-sleep deprivation.

A. Seda Artış[1], L. Şahin[1], A. Çetin[2], N. Dolu[1], C. Süer[1]

Departments of Physiology[1] and Biochemistry[2], Faculty of Medicine, University of Erciyes, Kayseri 38039, Turkey.

In this study, it was aimed to measure the oxidative damage and any changes that might occur in antioxidant defense system in the brain together with plasma cortisol levels following 21 days of REM-sleep deprivation.
Rats were randomly assigned to one of three groups (n=10 for each): cage control (Group1), tank control (Group2) or sleep-deprived (Group3). Rats were subjected to sleep deprivation by multiple platform method. Group2 rats were kept in a similar environment as Group3 rats, but they were able to sleep. At the end of the 21st day the rats were sacrificed. Plasma cortisol changes were determined together with levels of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) from the whole brain homogenates.
No significant difference between the groups was found for cortisol levels. There was a significant group effect for MDA, SOD and GSH-Px activities in the whole brain homogenates. In the Group3 MDA levels were elevated, and the activities of SOD and GSH-Px were significantly lower in comparison to the Group1 or 2. In the Group 2, MDA levels were also elevated and the activities of SOD and GSH-Px were also decreased in comparison to the Group 1.
We speculate that an adaptation in the HPA axis occurs in chronic REM-sleep deprivation. Chronic REM-sleep deprivation caused a decrease in the antioxidant defenses in brain in the present study. So, we think it is also noteworthy to investigate to what extend these changes occur in different brain regions.

Poster N^{o} 2

The protective effect of ethyl pyruvate on liver following experimental partial hepatectomy.

T. Artış[1], A. Abdülrezzak[1], F. Mutlu[1], A.Seda Artış[2], K. Deniz[3], Z. Yılmaz[1]

Departments of General Surgery[1], Physiology[2] and Pathology[3], Erciyes University Medical Faculty, Kayseri 38039, Turkey.

Following liver resection improving the hepatic blood flow and controlling a series of inflammatory process are nowadays gaining more importance. Ethyl pyruvate is secreted from cells and found in the extracellular fluid as its anionic conjugate, pyruvate. It is a stabile form of pyruvic acid which is the last product in glycolysis, and at the same time an effective free radical scavenger. In this study we aimed to investigate its antioxidant, anti-inflammatory, and protective effects on tissue against the free oxygen radicals and cytokines.
Rats were equally divided into two as the control and the experimental groups. Each group was then subdivided into two (n=8 for each). The experimental rats were given 40 mg/kg i.p. ethyl pyruvate after liver resection of 70%. Physiologic saline was administered to control groups as 40 mg/kg i.p. Rats were sacrificed at the 48th and 72th hours to measure AST, ALT, PT, IL–6, IL–10, and TNF-α from plasma and MDA from liver samples.
After 48th and 72th hours AST, ALT, PT, IL–6, TNF-α, and MDA levels were found to be significantly lower, whereas IL-10 levels was significantly higher in the experimental groups.
Our findings demonstrated that ethyl pyruvate, which inhibits secretion of IL-6 and TNF-α cytokines and has anti-inflammatory and antioxidative effects, shows a protective effect on liver following partial hepatectomy.

Poster N^{o} 3

Influence of vanadium supplementation on oxidative stress factors in the muscle of STZ-diabetic rats

Ö. Kurt[1], T. Yılmaz[1], N. Özsoy[1], S. Tunalı[2], Ayşe Can[1], R. Yanardağ[2]

[1]Department of Biochemistry, Faculty of Pharmacy, Istanbul University, 34116, Istanbul, TURKEY.

[2]Department of Chemistry, Faculty of Engineering, Istanbul University, 34320, Istanbul, TURKEY.

In recent years the role of free radical damage consequent to oxidative stress is widely discussed in diabetic complications. In this aspect, the protection of cell integrity by trace elements is a topic to be investigated. Vanadium is a trace element believed to be important for normal cell function and development. The aim of the present study was to investigate the effect of vanadyl sulfate supplementation on the antioxidant system in the muscle tissue of diabetic rats. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ, 65 mg/kg body weight) to male Swiss albino rats. The rats were randomly divided into 4 groups: Group I, control; Group II, vanadyl sulfate control; Group III, STZ-diabetic untreated; Group IV, STZ-diabetic treated with vanadyl sulfate. Vanadyl sulfate (100 mg/kg) was given daily by gavage for 60 days. At the last day of the experiment, rats which were fasted overnight were sacrificed, muscle tissue was taken, homogenized in cold saline to make a 10% (w/v) homogenate. Antioxidant enzymes, catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) as well as myeloperoxidase activities and protein carbonyl content were determined in muscle tissue. The results were evaluated using an unpaired *t*-test and ANOVA variance analysis with the NCSS statistical computer package. It was shown that vanadium supplementation significantly decrease antioxidant enzyme levels which were elevated in the muscle tissue of untreated diabetic group, showing that this trace element could be used as preventive for diabetic complications.

Poster Nº 4

Effect of extremely low frequency electromagnetic fields on biofilm formation of *S.epidermidis*; correlation to surface charge and hydrophobicity

B. Aksu[1], M. E. Cüce[2], G.Ayşe İnhan Garip[2]

[1]Marmara Univ. School of Medicine, Microbiology Dept.

[2]Marmara Univ. School of Medicine, Biophysics Dept.

Extremely low frequency electromagnetic fields (ELF-EMF) have been shown to change physicochemical properties of cell membranes. Cell surface hydrophobicity and cell surface charge have been implicated to biofilm formation. *Staphylococcus epidermidis*, are the predominant cause of implanted medical-device related infections. The formation of adherent multilayered biofilms on implanted devices is believed to be essential for the pathogenesis of *S. epidermidis* infections. *Staphylococcus epidermidis* was subjected to 50Hz, 1mT ELF-EMF and the change in hydrophubicity, membrane charge and biofilm formation was evaluated. Quantitative determination of biofilm production was performed by using a microtiter assay.
Bacterial cell-surface hydrophobicity was determined by measuring the bacterial adhesion to hydrocarbon in a hydrocarbon-water system. Change in surface charge was determined by electrophoretic mobility assay. Biofilm formation and hydrophobicity was seen to increase under the effect of ELF-EMF while there was a decrease in zeta potential. This work showed that ELF-EMF affected biofilm formation by changing the physicochemical properties of the cell surface.

Poster Nº 5

Effects of desflurane and vitamin C and E combination on element and oxidative stress levels in blood of operative patients

Berit G. Ceylan[1], M.Nazıroğlu[2,3], A. C. Uğuz[2], C. Barak[4], B. Erdem[1], L. Yavuz[1]

[1]Department of Anesthesiology and Reanimation, Medical Faculty,

Suleyman Demirel University, Isparta, Turkey.

[2]Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, Turkey.

[3]Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey.

[4]Department of Chemistry, Science Institute, Balıkesir University, Balıkesir, Turkey.

Volatile agents are frequently used in general anesthesia practice without complications. Clinical trials of desflurane on oxidative stress and antioxidant systems are still limited (1). In a recent study the agent has been found to produce lipid peroxidation (LP) by decreasing vitamin E levels (2). In this study we aimed to investigate the effect of the new volatile anesthetic's oxidant property on element, LP and antioxidative response (as measured by GSH-Px, GSH, β-carotene, vitamins A, C and E) of patients who undergone elective surgery.

Forty adult ASA I or II Physical Status patients scheduled for elective surgery was randomly divided into two groups. First group has been designed as control group and single dose of intravenous VCE were preoperatively given to the patients consisting second group. Baseline values in venous blood samples were preoperatively taken from control and treatment group before VCE administration. The blood samples from the control and VCE groups were also taken post operatively at the 1st, the 24th and 72th hours of desflurane exposure.

Erythrocytes and plasma lipid peroxidation (LP) levels in the postoperative period at the 1st, 24th and 72th hours of control group were higher than baseline values while vitamin E levels at the 1st and 24th hours, erythrocyte glutathione peroxidase (GSH-Px) activity at the 1st, 24th and 72th hours in postoperative period of control group were lower than preoperative period of control group. Erythrocyte GSH-Px activity, plasma vitamin A, C and E levels at the 1st, 24th and 72th hours were higher in the treatment group than control group whereas erythrocyte and plasma LP levels were lower in treatment group than the control group. Erythrocyte and plasma reduced glutathione, plasma β-carotene and serum copper, zinc, selenium, aluminum, iron, magnesium and calcium levels were not different between pre- and postoperative periods.VCE combination prevents the desflurane-induced vitamin E and glutathione peroxidase consumption under general anesthesia; however further clinical trials planned in wider series are required to determine the exact role of vitamin pre-supplementation before desflurane anesthesia.

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Poster N° 6

The oxidative stress effect of thermal water on natural distribution asphoedolus aestivus Brot. plant in Kestanbol Tuzla (Çanakkale-Turkey) geothermal field and the effect of water on the regional public

Berrak Damla Yağan[1], A. Gönüz[1]

[1]Department of Biology, Çanakkale 18 Mart University, Çanakkale, Turkey

Kestanbol-Tuzla is one of the most important geothermal fields of our country within the boundaries of the town of Canakkale Ayvacik. Area is quite low considering the diversity of flora is observed. Asphoedolus aestivus Brot. species are distributed in an area close to the source. The observations show that; the plant has lost its ability to produce its flowers, so vegetative reproduction occurs to depends on stress effect. The plant that has resistance against osmotic and salt stress, so that situation suggested us to oxidative stress related cellular damage cause the inhibit flowering period. In this study; plants growing in the geothermal area were compared to a pilot plant that grows healthy plants in the region was chosen as an example in terms of biochemical properties. Analysis carried out in this study, the plants showing the geothermal distribution accumulate Mn and Fe relatively

high amounts then pilot plant, thus these elements cause oxidative stress and also that results supported by multiple literature. This plant has been used as fodder crops and folk remedies in the region; and that cause the transfer of stress agent from plant thought the other organisms including human by food chain. Although soil and water is transported to agricultural land for agricultural activities; depends on geographical position and seasonal fluctuations the area is contaminated. As a general opinion; the increasing cancer cases in this region has close relation with the current situation.

Poster N° 7

Adaptation of rat gastric tissue against indomethacin toxicity

Beyzagul Polat[1], H. Suleyman[1], H.H. Alp[2]

[1]Ataturk University, Faculty of Medicine, Department of Pharmacology, 25240 Erzurum, Turkey.

[2]Ataturk University, Faculty of Medicine, Department of Biochemistry, 25240 Erzurum, Turkey.

This study investigated whether adaptation occurred after various dosages of repeated (chronic) indomethacin in rats to the gastro toxic effects of indomethacin. It also examined whether the adaptation was related to oxidative DNA damage in gastric tissue. To illuminate the adaptation mechanism in the gastric tissue of rats given various dosages of chronic indomethacin, the levels of oxidative DNA damage (8-OHd Gua/ 10^5 Gua) were measured. Results were compared to the control group, which received a 25-mg/kg single dose of indomethacin, and the role of oxidative DNA damage in the adaptation mechanism was evaluated. The average ulcer areas of gastric tissue of the 1-, 2-, 3-, 4-, and 5-mg/kg dosages of chronic indomethacin given to rats were 19.5±3.7, 12.5±3.3, 10±5.2, 4.5±3.6, 8.6±2.4, and 9.5±2.1 mm^2, respectively. This rate was measured as 21.3±2.6 mm^2 in the control group. Consequently, after various dosages of repeated (chronic) indomethacin administration in rats, it was observed that a clear adaptation developed against gastric damage and that gastric damage was reduced. The best adaptation was observed in the gastric tissue of the 3-mg/kg chronic indomethacin group. In parallel with the damage reduction oxidative DNA damage (8-OHd Gua/ 10^5 Gua) were reduced. This circumstance shows that oxidation play a partial role in the adaptation mechanism.

Poster N° 8

Glutathione peroxidase activities in Jurkat cell line: The antioxidant effects of melatonin

Canan Aslan[1], M.İnanır[2], G.Ş. Karatoprak[3], M.B. Yerer-Aycan[2]
University of Erciyes, Faculty of Pharmacy, Department of Biochemistry[1], Pharmacology[2], Pharmacognosy[3]

As an endogenous indolamin melatonin is known to be potent antioxidant in addition to its regulatory roles in many functions via biological rhythm. In this study, melatonin and its effects on glutathione peroxidase activity in Jurkat cell line has been investigated. Jurkat cell line (E-Clone E6) is grown in RPMI1640 medium and the cell number is adjusted to $1x10^6$ cells in each well of the plate. The cells were incubated with different doses of melatonin: 1mM, 2mM, 5mM and 10mM at 37°C in 5%CO_2 incubator for 24 hours. Then the GSH-Px activity was measured by microplate reader spectrophotometrically. GSH-Px activities were diminished in a dose-dependent manner compared with the control group, however the dose of 5mM were more effective than all the other groups to lower the activity. As a result, 5mM melatonin is found to be the most effective dose to decrease the oxidative damage in Jurkat cell lines. And this study is a pre-trial to investigate further effects of melatonin on oxidatively-stressed Jurkat cell lines.

Poster N° 9

Store-operated calcium entry increases in shTRPC1-expressing vector-transfected hepatocellular carcinoma cell line

Cigdem Selli[1], Y. Erac [1], B. Kosova[2], E. Erdal[3],M.Tosun[1]

[1]Departments of Pharmacology, Faculty of Pharmacy and [2]Medical Biology, Faculty of Medicine, Ege University, Izmir

[3]Medical Biology, Faculty of Medicine, Dokuz Eylul University, Izmir

We previously observed that TRPC1 and TRPC6 expression levels were reciprocally altered along with potentiated adrenergic receptor responses in aging rat thoracic aorta[1]. Furthermore, TRPC6 and store-operated Ca^{2+} were upregulated in TRPC1-silenced A7r5 embryonic rat aortic smooth muscle cell line[2]. Therefore, the purpose of this study was to investigate any regulatory role of transient receptor potential canonical 1 (TRPC1) in store-operated Ca^{2+} entry in hepatocellular carcinoma cell (HCC) lines. For this purpose, HCCs were cultured in RPMI-1640 and transiently transfected with shRNA-expressing vector (pSUPERIOR) targeting TRPC1 mRNA for *in vitro* post-transcriptional gene silencing. After 72 h incubation period, total RNA and protein samples were isolated. To determine the changes at transcriptional and protein level, quantitative real-time PCR (LightCycler 2.0, Roche Diagnostic) and immunoblot analyses were performed, respectively. Changes in intracellular Ca^{2+} levels were monitored via front surface spectrofluorimetry (PTI QM8/2005) in fura-2-loaded cells grown on coverslips[2]. In shTRPC1-coding vector-transfected HCCs, TRPC1 protein expression significantly decreased (p<0.05) without any change in mRNA levels. Furthermore, Ca^{2+} released from endoplasmic reticulum (p<0.05) and entered via store-operated Ca^{2+} both induced by cyclopiazonic acid, a selective sarco-endoplasmic Ca^{2+} ATPase blocker, were significantly increased (p<0.01). These results suggest that TRPC1 may play a regulatory role in store-operated Ca^{2+} entry in HCCs. Despite the decreased TRPC1 protein levels, no apparent change in mRNA levels may be due to the presence of alternative mRNA spliced isoforms of TRPC1 in cancer cells.

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Poster Nº 10

TNF-α-induced apoptosis in human myeloid HL-60 cells is dependent of ROS generation

David González-Flores, C. Barriga, A. B. Rodríguez, J. A. Pariente

Department of Physiology, Neuroimmunophysiology and Crononutrition Research Group, University of Extremadura, Badajoz, 06006, Spain.

In the present work the authors investigated whether oxygen reactive species (ROS) play a role in the activation of extrinsic pathway apoptosis induced by TNFα in human leukemia cell line HL-60.
We have analysed ROS generation and caspase -3, and -8 activity by fluorimetric methods using the fluorescent dye dihydrorhodamine 123 and specific fluorigenic substrate, respectively. We also determined cell survival using the MTT-method.
Our results show that the treatment of HL-60 cells with TNFα (100 ηg/ml) produces a time-dependent increase of intracellular ROS production followed as well as caspase -3 and -8 activation and finally cell death. Pretreatment of cells with 1 mM trolox, an α-tocopherol analogue which works as an antioxidant, for 30 minutes, reduced significantly both caspase activation. Similar results were obtained when the cells were pretreated with 1 mM N-acetyl-cysteine for 30 min, a derivative of cysteine which is well-known for playing a role as an antioxidant.
Our results suggest that TNFα-induced apoptosis in human myeloid cell line HL-60 is dependent of intracellular ROS production.

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Poster Nº 11

Nutritional and functional characterization of Crimson globe Japanese plums: Antioxidant effect of their consumption.

David González-Flores[1], B. Velardo[2], M. Garrido[1], D. González-Gómez[2], M. Lozano[2], M.C. Ayuso[3], C. Barriga[1], S.D. Paredes[1], A.B. Rodríguez[1]

[1]Department of Physiology (Neuroimmunophysiology and Chrononutrition Research Group), Faculty of Science, University of Extremadura, 06006, Badajoz, Spain

[2]Technological Institute of Food and Agriculture of Extremadura, 06071, Badajoz, Spain

[3]Agricultural Engineering School, University of Extremadura, 06007, Badajoz, Spain

Plums are considered a source of phytochemical compounds with beneficial health effects. The study was aimed at characterizing nutritionally and functionally Japanese plums (*Prunus salicina* Lindl. cv. Crimson globe) and evaluating the antioxidant effect of a diet enriched in these fruits in young (20±10 yr-old), middle-aged (45±10 yr-old) and elderly (75±10 yr-old) individuals. Participants consumed 200 g of plums twice a day (as the lunch and dinner desserts) for 5 days. First-void morning urines were collected before treatment (basal values), the immediate day after the last ingestion of plums (assay), and one day afterwards (post-assay). Urinary 6-sulfatoxymelatonin and total antioxidant capacity were measured by means of commercial ELISA and colorimetric assay kits, respectively. Nutritionally, Crimson globe plums were shown to contain lower carbohydrate values than the reported levels for other cultivars. From a functional point of view, detectable levels of serotonin, phenolic compounds and anthocyanins were found. Melatonin was detected but not quantifiable. The consumption of plums induced a significant increase in the participants' urinary 6-sulfatoxymelatonin and total antioxidant capacity levels in relation to their corresponding basal and post-assay values. Japanese plums of the cultivar Crimson globe may be seen as source of antioxidants with potential properties for counteracting oxidation.

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Poster N° 12

Effects of water pipe smoking on oxidative stress

Ebru Derici Eker[1], M. Berköz[2], N.Ö. Şahin[1], E. Jaroch[2]

Mersin University, Faculty of Pharmacy, [1]Department of Pharmaceutical Biotechnology, [2]Department of Pharmaceutical Technology, Yenisehir Campus, Mersin, Turkey

Water-pipe smoking (WPS) has been practiced extensively for almost five hundred years. It is a common habit in Turkey, Middle Eastern countries and Asia. In recent years, there has been a revival of WPS, notably among adolescents. The aerosol of water pipe smoke has been reported to have a composition of carbon monoxide, nicotine, tar, and heavy metals. Therefore, it can not be stated that water pipe smoking sets a safe alternative to cigarette smoking. Cigarette smoking induces lipid peroxidation resulting in suppression of antioxidant system. Although there is intensive research conducted on the effect of cigarette smoking causing oxidative damage, no significant study was found in literature investigating the relation of water pipe smoking with oxidative stress. In this study, it was aimed to determine the effect of water-pipe consumption on the activities of lipid peroxidation and antioxidant enzyme activities. 24 water pipe users (experimental group) and 25 nonsmokers (control group) were included to the study (n_T = 49). All subjects were selected among the people without a habit of smoking or any other use of tobacco. Catalase and superoxide dismutase (SOD) activities were investigated to determine the antioxidant statue and MDA level for lipid peroxidation. All blood samples were collected from both groups prior to taking any food in the morning and processed to separate serum. Catalase activity was determined utilizing the method of Aebi. SOD activity was found by the method of Sun et al. Finally, MDA levels were investigated using Ohkawa's method based on thiobarbituric acid reaction. The data obtained for two groups were compared statistically using SPSS v16.0 and Independent T-Test. In conclusion, catalase and SOD activities were found to be lower and MDA level was determined to be higher in water-pipe smokers than the control group ($p<0.05$). Therefore, it can be suggested that water pipe consumption reduces antioxidant enzyme activities and

increases lipid peroxidation. In turn, it induces oxidative stress and triggers many diseases as with cigarette smoking.

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Poster N° 13

Calcium impression on proliferation and phenotype changes of differentiated cardiomyocytes *in vitro*

Esin Akbay[1], G. Ayaz[1], H. Sevim[1], E. M. Albayrak[1], Ö. A. Gürpınar[1], M. A. Onur[1]

[1]Hacettepe University, Faculty of Science, Department of Biology, 06800 Beytepe, Ankara, Turkey

Intracellular calcium (Ca^{2+}) signaling plays a role in cardiac physiology and modulates cardiac gene expression. Changes in intracellular Ca^{2+} levels play an important role in the cardiac myocyte physiology. Although the activities of intracellular Ca^{2+} on the physiology and metabolic functions of heart cells have been studied extensively, the extracellular Ca^{+2} activities has been analyzed scarcely. The aim of this study was to investigate the effects of various Ca^{2+} concentrations on the proliferation and phenotype regulation of cultured cardiomyocytes. Differentiated cardiomyocytes were cultured in media with different concentrations of Ca^{2+} supplements in 24-well. The cultures were maintained at 37˚C in a humidified atmosphere of 5% CO_2 and 95% O_2. The cell proliferative activity was evaluated after 24, 48, and 72 h, counted with hemositometer by using trypan blue dye exclusion method. Cellular morphology of live cultures was observed by inverted microscope. Results of analysis indicated that, at a calcium concentration of 1, 8 mmol L^{-1} enhance cell proliferation rate as from second day. But the other doses of calcium showed toxic effects on cell morphology and proliferation. Our consequence means that the differentiated cardiomyocytes proliferative activity may regulate by intracellular calcium, whose concentration depends on the inflow of extracellular calcium through various ionic channels without involvement of intracellular calcium stores.

Poster N° 14

Protective effect of selenium on adriamycin-induced mitochondrial damage of rat renal tissue

N. Dursun[1], Eylem Taşkın[1], M. B. Yerer Aycan[2]

Department of Physiology[1] and Pharmacology[2], Faculty of Medicine and Pharmacy, University of Erciyes, Kayseri, Turkey

It is well known that oxidative stress is related to the pathogenesis of adriamycin (ADR) nephropathy[1, 2]. The aim of the study was to show the protective effect of selenium (Se) on ADR-induced mitochondrial–mediated apoptosis in nephropathic rats.

ADR were injected intraperitoneally for 8 days (ADR and Se+ADR groups, 4 mg/kg, every alternate day); Se were injected ip for 21 days (Se and Se+ADR groups, 0.15 mg/kg, every day) and Saline were injected ip for 21 days (Control groups, everyday). Mitochondrial membrane potential (MMP), ATP levels were determined with Cambrex and Cayman kits, respectively. Cytosolic, mitochondrial total antioxidant (TAS) and oxidant status (TOS) were measured with Rel assay kit. The oxidative stress index (OSI) was defined as TOS to TAS ratio for both cytosol and mitochondria.

ADR administration caused oxidative stress, showing that increased total oxidants and decreased total antioxidants in cytosol and mitochondria. Selenium normalized oxidative stress induced by ADR treatment. ADR decreased the ATP level in myocyte mitochondria. Coadministration Se with ADR improved ATP levels. ADR decreased the mitochondrial membrane potential and, coadministration Se with ADR improved the mitochondrial membrane potential.

The results provide evidence for the role of selenium in renoprotection against ADR nephropathy in rats possibly by changing oxidant/antioxidant balance and modulating ATP production and apoptosis.

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Poster N^{o} 15

Oxidative stress in the blood of farm workers following intensive pesticide exposure

Serdal Ö.[1], Fatih Gültekin[2], A.N. Kişioğlu [3], E. Küçüköner [4]

[1]Süleyman Demirel University, Faculty of Medicine, Blood Bank, 32260, Turkey

[2]Süleyman Demirel University, Faculty of Medicine, Department of Biochemistry, 32260, Turkey

[3]Süleyman Demirel University, Faculty of Medicine, Department of Public Health, 32260, Turkey

[4]Süleyman Demirel University, Faculty of Engineering and Architecture, Department of Food Engineering, 32260, Turkey

The aim of this study was to evaluate oxidative stress in workers using organophosphate, synthetic pyrethroid and carbamate pesticides in their farm. In this survey, 94 farm workers (exposed to pesticides at least 5-year during apple and cherry production) and 45 control subjects (living in the same region with no exposure to pesticides) were included to the study. Lipid peroxidation level, catalase, superoxide dismutase and glutathione peroxidase activities in erythrocytes were analysed as biomarkers of oxidative stress. In addition, the acetylcholinesterase activity was measured as a biomarker of toxicity. As a result of the analysis, lipid peroxidation level and activities of antioxidant enzymes, catalase and superoxide dismutase significantly increased ($p<0.05$). Acetylcholinesterase activity did not show any significant changes between the two groups ($p>0.05$). Consequently, chronic exposure to pesticides may negatively affect antioxidant system.

Poster N^{o} 16

The antiallodynic effect of Penicillin G in paclitaxel induced mechanoallodynia

A. Hacimuftuoglu[1], Fatih Saruhan[2], D. Cetin[1], H. Suleyman[1]

[1]Department of Pharmacology, Faculty of Medicine, Ataturk University, Erzurum, Turkey

[2]Department of Pharmacology, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey

Insufficient selectivity of anticancer agents for tumor cells makes them toxic for both tumor and healthy body cells. If nerves are affected by anticancer agents neuropathy may occur and patients may feel pain for even a light touch stimuli which are perceived as innocuous normally. This condition is named as “allodynia” and often resistant to standard analgesics. We know that expression of glutamate transporters (EAAT’s), responsible for transporting excitotoxic major neurotransmitter glutamate thought to be responsible for a variety of neurological disorders from synaptic cleft to astroglias to avoid increasing its concentrations to excitotoxic levels in synaptic cleft, down regulate after administration of anticancer agent “paclitaxel” to rats. Also we know that β-lactam antibiotics increase EAAT’s expression via gene activation. This prompted us to try to heal paclitaxel induced allodynia by increasing EAAT’s expression via β-lactam antibiotics. Two different groups of rats were injected with paclitaxel to induce allodynia and another one was injected with distilled water intraperitoneally. The pilot studies we did show that pain thresholds markedly reduce and the allodynia becomes most severe on 30th day after the first paclitaxel injection we started Penicillin G treatment to first group on 30th day and continued for 10 days. 30 days after the first Penicillin G injection we observed that Penicillin G increase the decreased pain

thresholds (from 75g to16, 5g±5,8to 35g±14, 4) significantly by comparison paclitaxel injected group treated with distilled water. This study shows that glutamate transporter or receptors may be potential targets to treat neurological disorders like allodynia, hepatic encephalopathy.

Poster N^{o} 17

Baseline and salt-stimulated paraoxonase, arylesterase and platelet activating factor acetylhydrolase activities and their relationship with oxidized LDL in polycystic ovary syndrome

S. Uysal[1], Ferah Armutcu[1], A. Çarlıoğlu[2], F. Karakurt[2]

[1]Department of Biochemistry and [2]Department of Endocrinology, Fatih University Medical Faculty, TURKEY

Polycystic ovary syndrome (PCOS) is characterized by hyperinsulinemia and hyperandrogenemia. These metabolic abnormalities in women with PCOS cause the increase in risk of some diseases with high mortality and morbidity, including cardiovascular disease and obesity. We studied a total of 31 patients with polycystic ovary syndrome and 30 healthy comparison subjects. Baseline and salt-stimulated Paraoxonase 1 (PON1), aryl esterase ARE, and platelet activating factor, acetylhydrolase (PAF-AH) activities were determined in control and patiens. PON1192Q/R phenotypes also were determined by using results of ARE activity. PAF-AH activity and oxidized LDL levels were significantly higher in patients with PCOS than in control ($p<0.001$, and $p<0.003$ respectively) although PON1 activity was significantly ($p<0.002$) lower than in the control group. ARE activity was also significantly higher ($p<0.007$) in patients with PCOS, whereas there was no significant difference in salt-stimulated PON1 activities. In PCOS group the phenotypic distributions of paraoxonase compared with the controls were QQ 45.2% - 46.7%, QR 48.4% - 53.3%, and RR 6.4% - 0%, respectively. These phenotypes (QQ, QR, RR) and plasma PON1 levels were statistically ($p<0.05$) significant lower in patients with PCOS than in the control group. Oxidized LDL levels and ARE activities were elevated; on the contrary baseline and stimulated PON1 were reduced in patients with polycystic ovary syndrome. These results suggest that these parameters are closely related, and could be suitable biomarkers for the evaluation of the risk of atherosclerosis in patients having polycystic ovary syndrome.

Poster N^{o} 18

The effect of calcium on the proliferation and viability of rat mesenchymal stem cells

Gamze Ayaz[1], E. Akbay[1], H. Sevim[1], M.E. Albayrak[1], Ö.A. Gürpınar[1], M.A. Onur[1]

[1]Hacettepe University, Faculty of Science, Department of Biology, 06800 Beytepe, Ankara

Several in vitro studies have shown that calcium is involved with a range of cell functions including attachment, tissue morphology, signal transduction and proliferation. Different concentrations of calcium in the culture medium may change the permeability of plasma membrane and intracellular signaling systems significantly (Osipenko et al., 2007). The aim of this study was to determine the effect of increasing extracellular calcium ion concentrations on growth and viability of mesenchymal stem cells (MSC) in vitro. MSCs were isolated from 5-6 week old rat's tibias and femurs. The cells were primarily cultured in DMEM/Ham's F-12 media supplemented with 15% fetal bovine serum (FBS) and 0.5% penicillin /streptomycin in a humidified atmosphere of 5% CO_2 , 95% O_2 at 37 $^{\circ}$C. The media was changed every 3 or 4 days and the cells were subcultured two times before employing them in the study. To evaluate the effect of the calcium on the growth of MSCs, 10^4 cells/ml were seeded into the 96-well culture plates. Different concentration of calcium (1.8 mM, 3.6 mM and 7.2 mM) were used in the experiments. After 24, 48 and 72 hours, the cell proliferative activity and viability of MSCs were determined by MTT assay. Our study showed that the 1.8 mM calcium concentration enhanced cell proliferation in MSCs and it was time dependent. However, higher concentration of calcium (7.2 mM) would decrease the viability of MSCs.

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Poster N° 19

Sport climbing causes oxidative stress in sedentary individuals.

Gökhan Cesur[1], E. Atay[2], S. Öğüt[3], M. Polat[3], H. Oruç[2].

[1]Department of Physiology, [2]Department of Sport Sciences, [3]Blood Bank, University of Süleyman Demirel, Isparta, Turkey

Sport climbing is a worldwide sport with particular physiological and physical demands. The topic of exercise-induced oxidative stress has received considerable attention in recent years. This study was aimed to investigate the effects of sport climbing on oxidative stress, antioxidant levels and hematologic parameters. Seven voluntary male students whose average age is 20,57 ± 1,27 years old, participated in this study. None of the subjects had performed regular exercise. These subjects were climbed 28±3.13 times during eight weeks. Blood samples were collected at rest, 24h before, and 24 h after climbing protocols to analyze total oxidant status (TOS), total antioxidant status (TAS) and hematologic parameters (Hemoglobin (Hb), hematocrit (Hct), platelet (Plt) and leukocyte (Wbc)). Resting heart rates were measured at the same time. According to our findings, climbing increased TOS (1,49 ± 0,04, 1,52 ± 0,03), decreased TAS (1,54 ± 0,06, 1,51 ± 0,06) and significantly increased oxidative stress index (TOS/TAS) (0,97 ± 0,01, 0,99 ± 0,02) ($p<0.05$). Only Hct levels (45,01 ± 1,42, 44,80 ± 1,39) of hematologic parameters increased after climbing protocols ($p<0.05$). There has been no statistically relation among the other parameters. These data demonstrate that sport climbing leads to increased plasma oxidative stress in sedentary individuals and negatively affect the athletes' performance.

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Poster N° 20

The effect of chronic swimming exercise on several target tissues of the stressed female rats with or without functioning ovarian hormones: the role of the HPA axis

Gülsün Memi[1], S. Özgür[2], A. Yarat[2], B.Ç. Yeğen[1]

[1]Marmara University School of Medicine, Department of Physiology, Haydarpaşa, Istanbul
[2]Marmara University School of Dentistry, Department of Biochemistry, Nişantaşı, Istanbul

When a threat to homeostasis occurs, hypothalamus-pituitary-adrenal (HPA) axis is activated as a regulatory mechanism of the stress response, where oxytocin plays an important role. The present study was aimed to investigate the role of oxytocin receptors in stress-induced inflammation in intact or overiectomized rats that were sedentary or exercised prior to chronic stress exposure. Female Sprague-Dawley rats underwent ovariectomy (n=30) or sham operation (n= 30). Then, subgroups were divided as sedentary or swimming groups (9 weeks). Each group was further divided as "non-stress" group or "stress" group exposed to psychological electric shock stress for 3 days. Rats were injected with either saline or oxytocin antagonist (atosiban, 1mg/kg) intraperitoneally for 3 days following stress. On the 3rd day, following decapitation colonic and gastric tissues were examined to measure myeloperoxidase (MPO) activity and superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdeyde (MDA) levels. The data were analyzed using ANOVA. Stress-induced increased colonic MPO activity,

gastric and colonic MDA levels, as well as decreased colonic GSH levels were reversed by exercise ($p<0.05$), while reduced gastric SOD level in the ovariectomized rats was increased by exercise. On the other hand, stress-induced gastric and colonic injury, which was aggravated by ovariectomy, was partially alleviated by atosiban ($p<0.01$). The results demonstrate that stress-induced gastric and colonic injury appears to be ameliorated by exercise in the presence of ovarian hormones, which may be acting though increased antioxidant enzymes. Moreover, the findings show that oxytocin is an important mediator in the regulation of HPA axis.

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Poster N^{o} 21

Zinc deficiency and 8-hydroxy-2-deoxyguanosine levels as a marker of DNA oxidation in children

Halil Ibrahim Atasoy[1], G. Bugdayci[2], A.P. Uyan[1].

Abant Izzet Baysal University, Izzet Baysal School of Medicine, Department of Paediatrics[1], Department of Biochemistry[2], Bolu, Turkey

Zinc deficiency is known to result in retardation of growth, immune and cognitive deficits in children. Zinc has antioxidant properties. Deficiency may cause imbalance in the prooxidant-antioxidant status. We aimed to demonstrate DNA oxidant damage in zinc deficient children by calculating 8-hydroxy-2-deoxyguanosine as a marker of genetic damage.

Serum zinc levels were calculated by colorimetric method in 190 male and 274 female children ages of which were between 5 to 15 years. Out of 466 children sex and age matched 35 children with zinc deficiency (serum zinc levels less than 70 mcg/L) and control plasma 8OH2dG levels were calculated by enzyme immunoassay.

Work-up of serum zinc levels 35 healthy age and sex matched zinc deficient children and control children showed statistically significant increase of 8-OH-2-dG levels in zinc deficient group ($p < 0.05$). 8-OH-2-dG levels in zinc deficient and non-deficient children were 1,94 ± 0,47 and 1,53 ± 0,45 ng/mL (mean ± SD) respectively.

Oxidative stress affects cellular lipids, proteins and nucleic acids. 8OH2dG is valuable marker frequently used for revealing damage to nucleic acids. When nucleic acids are oxidized point mutations and chromosomal breaks could form which might result in carcinogenesis. We used 8-OH-2-dG levels as markers of DNA damage in zinc deficient children and showed a significant increase compared to control children which might pose them to worse clinical consequences.

Zinc deficiency in children should be screened and corrected if consequences are being avoided.

Poster N^{o} 22

Increased occupational coal dust toxicity in blood of central heating system workers

Y. Tuluce [1], Halil Ozkol[1], I. Koyuncu[2], H. Ine[2]

[1]Department of Medical Biology, Medical Faculty, Yuzuncu Yil University, Van, Turkey

[2] Department of Biology, Science & Art Faculty, Harran University, Sanliurfa, Turkey

Coal dust causes lung diseases in occupational exposure. Reactive oxygen species have been implicated in the pathogenesis of its toxicity. In this study serum enzymes, lipid profile and other biochemical values with oxidant/antioxidant status in whole blood and serum of central heating system workers (CHSW) (the persons who are responsible for heating the apartments with coal) were determined to reflect the cell injury. Blood samples were obtained from CHSW (n= 25) and healthy individuals (n= 25). All values were measured in whole blood and serum. ANOVA was used for the estimation of statistical data. In the group of CHSW, creatinine, ferritin, alanin aminotransferase, aspartate aminotransferase, creatine phosphokinase, gamma glutamil transferase, lactate dehydrogenase and glutathione reductase activities as well as triglyceride, very low density lipoprotein, protein carbonyl and malondialdehide were significantly higher, while transferrin, high density lipoprotein and catalase activities were lower than the group of healthy individuals. This result is consistent with hypothesis that respirable coal dust generates lipid and protein oxidation and induces leakage of serum enzymes by cell damage. It also leads to imbalance in antioxidant defense system, lipid profile and other biochemical parameters.

Poster N° 23

Gastroprotective effect of small centaury (*Centaurium erythraea)* on aspirin induced gastric damage in rats

Y. Tuluce [1], Halil Ozkol[1], I. Koyuncu[2], H. Ine[2]

[1]Department of Medical Biology, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey

[2]Department of Biology, Faculty of Science & Art, Harran University, Sanliurfa, Turkey

Small centaury *(Centaurium erythraea)* (family: Gentianaceae), a medicinal plant is used owing to its digestive, stomachic, tonic, depurative, sedative and antipyretic properties in folk medicine. In this study, the gastroprotective effect of small centaury (SC) extract was investigated in the acetylsalicylic acid (ASA)-induced gastric ulcers in rats at a dose of 200 mg/kg body weight. Twenty one Sprague-Dawley albino rats were divided into three groups of 7 rats each as follows: (1) the control group; (2) the acute ASA treated group and (3) ASA plus SC group. At the end of the 4 h drug administration, ulcer index, oxidant and antioxidant levels were measured and compared between the groups. The percentage of lesion area to total gastric surface area (ulcer index) was significantly reduced (77 %) in ASA plus SC group as compared with the acute ASA treated group. The oral administration of ASA decreased catalase (CAT), reduced glutathione (GSH), and increased lipid peroxidation (LPO) levels. Although myeloperoxidase (MPO) activity was increased by ASA, it was found to be lower in the ASA plus SC group. GSH and Vitamin A levels were determined higher in the ASA plus SC group compared with ASA group. These results suggest that SC extract protects against ASA-induced damage due to its anti-oxidizing activity.

Poster N° 24

The effect of exercise and docosahexaenoic acid on the nerve conduction velocity in normobaric hypoxia

H. Ali Erken[1], O. Genç[1], G. Erken[1], R. Çolak[2]

[1]Pamukkale University, Medical Faculty, Department of Physiology.

[2]Pamukkale University, School of Sport Sciences and Technology

Deleterious effects of hypoxia exposure on many nervous system functions are known. There are studies indicating the beneficial effects exercise and docosahexaenoic acid (DHA) on various neurophysiological functions. The aim of this study is to investigate the effects of exercise and DHA supplementation in hypoxic conditions on nerve conduction velocity.

This study was approved by the Pamukkale University Ethics Committee of Animal Care and Usage. A total of 35 Sprague Dawley male rats, were divided into 5 groups: Control (K), hypoxia (H), hypoxia + DHA (HD), hypoxia + exercise (HE), hypoxia + exercise + DHA (HED). All of the rats, with the exception of K group, exposed to hypoxia for 28 days. A treadmill exercise was performed as 1.8 km/h, 0% incline, 20 min/day in exercise groups. DHA were applied by gavage (36 mg /kg/day) to HD and HED groups for every day. At the end of the experimental period, it was removed sciatic nerves of anesthetized rats. Nerve conduction velocity was measured in sciatic nerve. One Way ANOVA and Post Hoc Tukey tests were used for the differences between groups, p values <0.05 accepted as statistically significant.
Nerve conduction velocity of H group was decreased compared to group K. Nerve conduction velocity of HE, HD and HED groups were increased compared to K and H groups.
This study it was shown that exercise and dietary intake of DHA can be used to reduce deleterious effects on nerve conduction velocity of hypoxic conditions.

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Poster N^{o} 25

Antimicrobial and Antioxidant Activities of *Cynanchum acutum*, *Cionura erecta*, *Trachomitum venetum* subsp. *sarmatiense* Grown Wild in Turkey

Hülya Demir[1], E. Ergin[1], L. Açık[1], M. Vural[1]

[1]Atatürk Vocation Training School, Gazi University, 06500-Ankara, Turkey

Wild plants have been reported to have antioxidant properties for centuries. Indigenous plants have been used in herbal medicine for curing various diseases (Sokmen *et al*). Oxidative damage to crucial cellular molecules induced by reactive oxygen species has been implicated as a possible factor in the etiology of several human diseases, including cancer, cardiovascular disease, and aging (Halliwell and Gutteridge, 1989).
DPPH Radical Scavenging Activity:1,1-Diphenyl-2-picrylhydrazyl radical scavenging capacity. DPPH radical scavenging capacity was determined according to the Blois method.
Reducing Power: The reducing powers of extracts were quantified by the method of Oyaizu. Ferric thiocyanate method-total antioxidant activity: The total antioxidant capacities of the extracts were determined according to the ferric thiocyanate method in linoleic acid emulsion.
Determination of the Amount of Total Phenolic Compounds: The phenolic compound content was determined as pyrocatechol equivalents using the following linear equation based on the calibration curve. A is the absorbance, and C is pyrocatechol equivalents (µg). A: 0,006C-0, 0192.
Antimicrobial activities: The antimicrobial effect of ethanol extract of *C.acutum, C. erecta*, *T. venetum* subsp*. sarmatiense* were tested against bacteria strains *Staphylococcus aureus* ATCC 25923*, Pseudomonas aeruginosa* ATCC 27853*, Escherichia coli ATCC* 35218*, Escherichia coli ATCC* 25922, *Bacillus subtilis* ATCC 29213*, Bacillus cereus* NRLL B-3008. *Enterobacter fecalis* ATCC 292112 and two yeast strain *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803.The compound effect was carried out by the agar diffusion method.
The results indicate that the antioxidant activity of ascorbic acid (8.2 µg mL^{-1}) was higher than the ethanol extract of *C. acutum* (196.09 µg mL^{-1})*, C.erecta* (240.14 µg mL^{-1})*, T.venetum* subsp. *sarmatiense* (570.71 µg mL^{-1}). Total antioxidant activities of 54 µg mL^{-1} of *C. acutum*, *C. erecta, T. venetum* subsp. *sarmatiense* extracts, determined according to ferric thiocyanate method. The antimicrobial results obtained using the agar well diffusion method. The highest inhibitory activity was seen against *S.aureus* using the ethanol extract of *C.erecta* concentration of 100 mg/mL.

Poster N^{o} 26

Pro-oxidant effects of melatonin in human myeloid leukaemia HL-60 cells.

Ignacio Bejarano[1], J. Espino[1], C. Barriga[1], J.A. Pariente[1], A.B. Rodríguez[1]

Department of Physiology, Neuroimmunophysiology Research Group, University of Extremadura, 06006-Badajoz, Spain

Melatonin has many effects on a wide range of physiological functions and is involved in a number of pathological events including oncostatic and neoplastic processes. The tissue protective actions of melatonin are attributed to its well-known antioxidant activity though melatonin might also exert pro-oxidant effects, particularly in tumor cells. Here, we evaluated the pro-oxidant effects of melatonin and their role on caspases-3 and -9 activation in a human promyelocytic leukaemia cell line HL-60 cells. Intracellular reactive oxygen species (ROS) cell analysis was quantified by rhodamine-123 fluorescence, MTT assay was performed in order to evaluate the cell viability under melatonin treatments, in addition, DNA cellular was PI stained to discriminate died cells from alive unstained cells. To determine caspase-3 and -9 activities the cleavage of fluorogenic specific substrates, DEVD-AMC and AC-LEHD-AMC respectively, were measured. Melatonin treatment is able to stimulate production of intracellular reactive ROS, as revealed by the increase in rhodamine-123 fluorescence, which was associated with significant cytotoxicity and activation of caspase activities. Furthermore, pretreatment of cells with well-known antioxidants, such as N-acetyl-L-cysteine (NAC), trolox, PEG-catalase and reduced glutathione (GSH), reversed the effects of melatonin on both intracellular ROS production as on the cytotoxicity and caspase activation. This pro-oxidant action may be significant in light of the findings that melatonin has pro-apoptotic actions in tumour cells and it could increase the utility of melatonin as an anticancer agent.

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Poster Nº 27

Protective effect of antioxidants on indomethacin-Induced gastric mucosal injury in rats

Ismet Burcu Turkyılmaz, R. Yanardag

Istanbul University, Faculty of Engineering, Department of Chemistry, Istanbul, Turkey

Non-steroidal anti-inflammatory drugs (NSAIDs) are some of the most widely used drugs in the world. It is well-known that gastric damage is the major NSAID-induced side effect. Reactive oxygen species (ROS) also play a vital role in mucosal lesions associated with NSAIDs. Antioxidant defense systems and their components, including antioxidant enzymes, foods and drugs are important in preventing the toxic and disease causing effects of oxygen-derived free radicals. In this study, the effects of antioxidants on indomethacin-induced gastric damage were evaluated in rats. Male Sprague-Dawley rats were randomly distributed into 4 groups. Group I; intact animals. Group II; control animals receiving vit C (100 mg/kg), vit.E (100 mg/kg), β-carotene (15 mg/kg) and sodium selenate (0.2 mg/kg) for 3 days, orally, daily. Group III; rats receiving 25 mg/kg indomethacin. Group IV; animals receiving vit.C, vit.E, β-carotene and sodium selenate for 3 days (in the same dose and time), 2 h prior to the administration of indomethacin. 6 h after the indomethacin administration all the animals were sacrificed. Stomach glutathione peroxidase, catalase acitivities and protein carbonyl and sialic acid levels were increased in indomethacin groups, also, stomach glutathione-S-transferase activity and glutathione and mucus levels were decreased. Administration of antioxidants reversed these effects. In conclusion, the result of present study indicates that antioxidant administration protects gastric tissue against indomethacin-induced damage.

Poster Nº 28

Analysis of the antioxidant activity in human milk, day vs night.

Javier Cubero[1], C.L. Sánchez[1], R. Bravo[1], J. Sánchez[2], A.B. Rodriguez[1], M. Rivero[3], C. Barriga[1]

[1]Laboratory of Chrononutricion Faculty of Sciences, Uex. Badajoz, Spain.

[2]Laboratory of Metalopathies Hospital Perpetuo Socorro. S.E.S. Badajoz, Spain.

[3]Manager Scientific Department of I+D+i. Grupo Ordesa. Barcelona, Spain

It is known all the advantages of the human milk for the development of the breast-fed baby, between the nutritional components they find the vitamins A, E and C, characterized by the defense antioxidant. In the newborn children the stress oxidative is major due to the immaturity of the mechanisms of antioxidant defense and of the digestive system. It is known that it humanizes the nutritional components of the milk, they are changeable throughout the day, being intimately tied to the ingestion of the mother.
The aim of the authors was to analyze the antioxidant capacity of the mother milk along the period of 24 hours, the antioxidant changes between the day and the night.
Samples of milk colostrum of healthy mothers (n=7), quiet (-80 °C) along a period of 24 h, in the Service of Neonatalogy (S.E.S.), Badajoz.
The antioxidant capacity decided for the improved spectroscopic method TEAC (Trolox equivalent antioxidant capacity). By this method there is calculated the percentage of inhibition of the radical cation ABTS - + by means of the Trolox, the analogous soluble one in water of the alfa-tocopherol, which is the antioxidant standard. For the statistical analysis it was used; descriptive statistics (X±DS) and inferential test not parametric Kruskal-Wallis. A value of p <0.00 was considered to be significant.
We found variations in the antioxidant activity between the night and diurnal samples of milk. Being the levels of Trolox Equivalent higher , statistically significant (p <0,00), in the samples collected at 24:00 h opposite to the samples of milk collected at 09:00 h and 21:00 h.
We observed the increase in the antioxidant capacity in the samples of the night period opposite to the rest of samples of the diurnal period, probably as consequence of the immunological mother activity and the ingestion of vitamins and proteins realized during the day.

Poster N° 29

Melatonin blocks intracellular calcium overload- and ROS-dependent caspase activation in human leucocytes.

Javier Espino, I. Bejarano, C. Barriga, J.A. Pariente, A.B. Rodríguez

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

Emerging evidence suggests that melatonin may be involved in the protection of different cell types against damage-induced apoptosis. We aimed to evaluate the effect of melatonin on caspase activation evoked by increases in both intracellular calcium and reactive oxygen species (ROS) levels in human leucocytes.
Human leucocytes were separated from whole blood using Ficoll-Hypaque. ROS generation was quantified with the non-fluorescent, cell-permeable probe dihydrorhodamine-123 (DHR-123). Caspase-3 and -9 activities were determined from the cleavage of their respective specific fluorogenic substrate.
Our results show that the treatment of human neutrophils with the specific inhibitor of calcium reuptake thapsigargin (TG, 1 μM) for 60 minutes induced a rise in intracellular ROS levels, which was forestalled by melatonin (1 mM, 60 minutes) pre-incubation. Likewise, TG-induced ROS production was also abolished by pre-treatments with the well-known antioxidant, N-acetyl-L-cysteine (NAC, 15 mM, 60 minutes), the intracellular calcium chelator, dimethyl BAPTA (10 μM, 30 minutes), and the specific blocker of calcium uptake into mitochondria, Ru360 (10 μM, 30 minutes). Moreover, our results indicated that TG was able to raise the caspase-3 and -9 activities. TG-induced caspase-3 and -9 activation was again inhibited by melatonin pre-incubation, but also by NAC, BAPTA and Ru360 pre-treatments. Similar results were obtained in human lymphocytes.
In sum, our results suggest that melatonin prevents caspase-3 and -9 activation induced by both increases in intracellular calcium and ROS levels in human leucocytes.
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Poster Nº 30

In vitro exposure to melatonin improves sperm motion parameters.

Javier Espino[1], A. Ortiz[2], I. Bejarano[1], F. Monllor[2], G.M. Lozano[2], J.F. García[2], J.A. Pariente[1], A.B. Rodríguez[1].

[1]Department of Physiology, Neuroimmunophysiology and Chrononutrition Research Group, Faculty of Science, University of Extremadura, 06006 Badajoz, Spain, and [2] Extremadura Centre for Human Assisted Reproduction, 06010 Badajoz, Spain

Although human seminal fluid contains melatonin and spermatozoa reportedly possess membrane melatonin receptors, there are no experimental studies that ascertain the relationship between melatonin and sperm motility. To this end, we aimed to evaluate the *in vitro* effects of melatonin on human spermatozoa function by analysing several sperm motion parameters.
Human semen was obtained from fifteen men attending infertility counselling and collected by masturbation after 4-5 days of sexual abstinence. Motility kinematic parameters were assessed by a computer-aided semen analysis (CASA) system in both non-treated and melatonin-treated samples.
Melatonin treatment (1 mM, 30 min) improves motile and progressive motile cells percentage, and decreases static cells count, mainly promoting the proportion of rapid cells, because melatonin enhances sperm velocity, in general, and straightness, in particular.
Briefly, short-term exposure to melatonin ameliorates sperm motion parameters, which is extremely important because melatonin supplementation may be potentially used to obtain a successful assisted reproductive techniques (ART) outcome.

Funded by Merck, S.L. J. Espino and I. Bejarano are supported by Merck, S.L. and Junta de Extremadura (PRE06070), respectively.

Poster Nº 31

Effects of paliperidone on purine catabolizing enzymes and level of nitric oxide in rat brain

Kadir Demirci[1], R. Özcankaya[1], H. R. Yılmaz[2], A. Yiğit[2], K. Karakuş[1]

[1]Department of Psychiatry, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

[2]Department of Medical Biology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

Schizophrenia is a serious mental illness that affects how a person thinks, feels, and behaves. In treatment, antipsychotic drugs are generally used. Paliperidone is a new drug which includes basically the same active metabolite of risperidone which generally used as an antiphysotic drug to treat schizophrenia.
The main objective of the current study is to investigate the effects of paliperidone on enzyme activities of adenosine deaminase (ADA) and xanthine oxidase (XO), and nitric oxide (NO) levels, which has important roles on purine catabolism in rat brain. Twenty Sprague Dawley rats were used in this study. Rats were randomly divided into two equal groups as control (n=10) and paliperidone group (n=10). Physiological saline solution was given to the control group as intraperitoneally (i.p.) once a day during 14 days. 1 mg/kg per day paliperidone was given to paliperidone group once a time in a day during 14 days. 24 hours later after last injection, rats were sacrificed and brain tissues were taken. The enzyme activities of ADA and XO, and NO levels were measured from brain tissues homogenates as spectrofotometrically.
When we compared paliperidone group with control group, ADA and XO enzyme activities were significantly decreased. NO levels were increased but it was not statistically significant.
In conclusion we observed that ADA and XO enzyme activities had significantly decreased in paliperidone group compared to the control group. This may show us the reduction of the purine metabolism. This reduction in the purine metabolism may diminish the activity of dopamine by increasing the amount of adenosine. Paliperidone treatment is thought to be useful to taking under control of the positive effects / symptoms of schizophrenia

through improvement in the reduction of purine metabolism.

Poster Nº 32

Effects of paliperidone on antioxidant system in rat brain

Kadir Demirci[1], H. R. Yılmaz[2], R. Özcankaya[1], A. Yiğit[2], K. Karakuş[1]

[1]Department of Psychiatry, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

[2]Department of Medical Biology, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

Paliperidone, is one of the most commonly used atypical antipsychotic drugs, and it is the active metabolite of risperidone.
The aim of this study is to investigate the effects of paliperidone on the enzyme activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) levels.
Twenty Sprague Dawley male rats were used in this study. Rats were randomly divided into two equal groups as control (n=10) and paliperidone group (n=10). Physiological saline solution was given to the control group as intraperitoneally (i.p.) once a day during 14 days.
1 mg/kg per day paliperidone was given to paliperidone group once a time in a day during 14 days. 24 hours later after last enjection, rats were sacrificed and brain tissues were taken. The enzyme activities of SOD, CAT and, GSH-Px and MDA levels were measured from brain tissues as spectrofotometrically.
When we compared paliperidone group with control group; CAT activity was significantly decreased, SOD activity and MDA levels were increased but not statistically significant. GSH-Px levels were also decreased but also not statistically significant.
As a result, paliperidone could change antioxidant status. According to this result, paliperidone may be a risk factor for oxidative damage.

Poster Nº 33

Comparison of Jerte Valley cherry-based nutraceutical product vs. placebo-controlled trials in terms of 6-sufatoxymelatonin and total antioxidant capacity in urine

Maria Garrido[1], D. González-Gómez[2], M. Lozano[2], T. Hernández[2], C. Barriga[1], S.D. Paredes[1], A.B. Rodríguez[1]

[1]Department of Physiology (Neuroimmunophysiology and Chrononutrition Research Group), Faculty of Science, University of Extremadura, 06006, Badajoz, Spain

[2]Technological Institute of Food and Agriculture of Extremadura, 06071, Badajoz, Spain

The consumption of fruits rich in the antioxidant melatonin increases the circulating and urinary levels of this indole. Jerte Valley cherries (*Prunus avium*) contain melatonin as well as its precursors, the amino acid tryptophan and the neurotransmitter serotonin, which also act as antioxidant bioactive compounds. This study was aimed at comparing the effect of a Jerte Valley cherry-based nutraceutical product (patent nº P200803761) and a placebo product on the urinary 6-sulfatoxymelatonin (aMT6s) and total antioxidant capacity (TAC) in young (20-30 yr-old), middle-aged (45-55 yr-old) and elderly (65-75 yr-old) participants. The nutraceutical product consisted of equal parts of the pulp of 4 cultivars of Jerte Valley cherries from Cáceres, Extremadura, Spain (94%), maltodextrin (5%) and ascorbic acid (1%). The placebo product was a commercial cherry-flavored soft drink. Volunteers ingested either the placebo or the nutraceutical product twice a day for 5 days. aMT6s was analyzed using a commercial ELISA kit in first-void morning urines. TAC was quantified by means of colorimetry in urines collected at 21.00 h. The consumption of the nutraceutical cherry-based product augmented the participants' 6-sulfatoxymelatonin levels and total antioxidant capacity as compared to the values obtained before and after the treatment. The placebo did not modify their urinary antioxidant levels. The Jerte Valley cherry-based nutraceutical product may be used as a source of antioxidants.

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Poster Nº 34

Urinary 6-sulfatoxymelatonin and total antioxidant capacity increase after the intake of a grape juice obtained with high hydrostatic pressure techniques.

D. González[1], E. Gamero[2], María Garrido[1], D. Moreno[2], E. Valdés[2], C. Barriga[1], S.D. Paredes[1], A.B. Rodríguez[1]

[1]Department of Physiology (Neuroimmunophysiology and Chrononutrition Research Group), Faculty of Science, University of Extremadura, 06006, Badajoz, Spain

[2]Technological Institute of Food and Agriculture of Extremadura, 06071, Badajoz, Spain

Red grapes contain elevated concentrations of antioxidant compounds (polyphenols) that may potentially prevent cell aging, cardiovascular disease and oxidation-related disorders. Since functional drinks are presently one of the most dynamic sectors of the market, the aim of the present work was to evaluate the possible antioxidant effect of an experimental grape (*Vitis vinifera* cv. Tempranillo) juice in terms of 6-sulfatoxymelatonin and total antioxidant capacity in the urine of young (20±10 yr-old), middle-aged (45±10 yr-old) and elderly (75±10 yr-old) individuals. The grape juice was obtained by means of high hydrostatic pressure (HHP) techniques. Participants consumed 200 ml of grape juice twice a day (as the lunch and dinner desserts) for 5 days. First-void morning urines were collected before treatment (basal values), the immediate day after the last ingestion of juice (assay), and one day afterwards (post-assay). For the quantification of 6-sulfatoxymelatonin, a commercial ELISA kit was used. Total antioxidant capacity was evaluated using a colorimetric assay kit. The intake of grape juice cv. Tempranillo obtained with HHP induced a significant increase of urinary 6-sulfatoxymelatonin and total antioxidant capacity in the three groups of age analyzed as compared to their corresponding basal and post-assay values. These functional/nutraceutical properties may be of interest for a prospective commercialization of the grape juice.
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Poster Nº 35

The effects of Huperzine A, on traumatic spinal cord injury in rats.

Ş. Yüceli[1], Mediha Eser[2], G. Tanrıverdi[2], O. Akdemir[3], M. Tunçdemir[4], M. Kaya[5], F.Alkan[2]

[1] Taksim Emercency Education and Research Hospital, Department of Neurosurgery İstanbul, Turkey

[2]İstanbul University, Cerrahpaşa School of Medicine, Department of Histology and Embryology, İstanbul, Turkey

[3]Taksim Emercency Education and Research Hospital, Neurosurgery Department, İstanbul, Turkey

[4] İstanbul University, Cerrahpaşa School of M edicine, Department of Medical Biology, İstanbul, Turkey

[5] Kars Kafkas University, Neurosurgery Department, Kars, Turkey

The aim of this study is to investigate the neuroprotective and antiapoptotic effects of Huperzine A on the traumatic rat spinal cord tissue. Thirty six Wistar type albino rats were studied in 3 groups of 12 animals: sham-operated control animals (group 1); trauma-only control animals (group 2); and rats subjected to trauma + Huperzine A treatment (group 3). Spinal cord injury was produced at the thoracic level using the clip compression technique. Responses to injury and the efficacy of Huperzine A were assessed by light microscopy, apoptosis and cellular damage were assessed by staining with TUNEL and

immunostaining for BDNF and TGF-beta. The functional recovery was assessed by field locomotor test at the 3 and 7 days after surgery. Also, the samples were stained with Cresyl Echt Violet and H+E histostaining for investigating the tissue morphology. In the trauma group was observed common hemorrhage, necrosis, severe degeneration of motor neurons and leukocyte infiltration in the gray matter. In the trauma + Huperzine A group was decreased seen cavitations area, hemorrhagic, and edema in the gary matter compared to trauma groups. Apoptotic shows were mostly observed in the glial cells and motor neurons in the trauma group. In the control group decreased number of apoptotic cells per unıt area is observed compared to trauma group ($p<0.05$). In the Huperzine A groups (3 and 7 days) were determined significant decrease compared to trauma 7th day (respectively $p<0.01$ $p<0.01$). Groups were also evaluated semi quantitatively in terms of TGF-β and BDNF immunopositiveness. While it was discovered that TGF-beta positivity was higher in trauma group than in the group applied Huperzine A, it was determined that the number of BDNF positive cells was higher in Huperzine A applied group than in trauma group. In conclusion, in rats local application of Huperzine A after SCI is effective on decreased secondary tissue damage and therefore on protection of the motor function. This effect can be explained by the inhibition of apoptotic death of total cell types in spinal cord.

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Poster N^{o} 36

Funalia Trogii Application on Preventing Oxidative Damage in Brain Produced by Deltamethrin Exposure

Mehmet Berköz[1], S. Yalın[2], Ü. Çömelekoğlu[3], B. Mazmancı[4], M. Ali Mazmancı[5], P. Eroğlu[2], A. Ünyayar[5]

Mersin University, Faculty of Pharmacy, [1]Department of Pharmaceutical Technology, [2]Department of Biochemistry, [3]Faculty of Medicine, Department of Biophysics, [4]Faculty of Art and Science, Department of Biology, [5]Faculty of Engineering, Department of Environmental Engineering, Mersin

Deltamethrin is a dibromo pyrethroid pesticide that has widespread using fields in agriculture industry. For reducing the harmful effects of insecticides on living organisms, using medical plants could be a helpful treatment approach. In this study, we investigated the protective effects of *Funalia trogii* that is a medicinal mushroom species, on oxidative damage in rat brain produced by deltamethrin exposure. Totally 21 Wistar albino rats were used for our study and we separated the animals to 3 groups that contains 7 animals in each groups. Any application wasn't done to the control group during the experiment. Deltamethrin group was taken 1.28 mg/kg deltamethrin during 30 days and deltamethrin+fungus group was taken 1.28 mg/kg deltamethrin and 0.5 mL *Funalia trogii* extract during 30 days. At the end of the study, all the animals were sacrificed and superoxide dismutase (SOD) and catalase activities for detecting the antioxidant state and malondialdehyde (MDA) levels for definition of the lipid peroxidation state were measured. It was found that MDA levels of deltamethrin and deltamethrin+fungus groups were higher than control group. MDA level of deltamethrin+fungus was lower than deltamethrin group but it wasn't statistically significant ($p>0.05$). SOD activity of deltamethrin group was higher but catalase activity was lower than control group. SOD activity of deltamethrin+fungus group was lower but catalase activity was higher than deltamethrin group, statistically ($p<0.05$). Finally, it is said that treatment with *Funalia trogii* extract helps preventing the living

organisms from the oxidative stress on deltamethrin exposure.

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Poster N° 37

The Antioxidant Effect of *Ocimum Basicilum* on E.coli Exposed to Different Doses of Radiation

M. Cihan Yavaş[1], M.S. Çelik[1], V. Akpolat[1], N. Akpolat[2], S.B. Zincircioğlu[3], M. H. Doğan[3]

[1]Dicle University, Medical Faculty, Dept. of Biophysics, [2]Dept of Microbiology, [3]Dept. of Radiation Oncology, Diyarbakır / Turkey

In this study, the *E.coli* in agar medium and agar + *ocimum basicilum* extracts medium were subjected to different doses of gamma radiation. The antioxidative effects of ethanolic extracts of Ocimum basicilum on the *E coli* exposing to irradiation was investigated.
Ocimum basilicum plant used in the study was dried and powdered by a mechanical chopper of 5 gr, which was later incubated by a mechanical shaker within ethylalcohol for 48 hours at 25 ˚C. After filtration, the ethanol was removed at evaporator at 50 ˚C at 200 rpm.
After preparing the Blood Agar Base by both checking the plant extract and agar's pH, to each plate was added 83 ± 2 µl plant extract. 0,5 cc bacteria culture was added to the medium which has 4, 5 cc pure Mueller Hinton Broth. After it was diluted at the rate of 10^6, 0,5 cc broth culture was planted to agar medium. 100, 200, 500, 1000, 3000, 6000 cGy doses was performed with Co 60 teletherapy machine (exception of control group). After incubated 18 hours at 37 ˚C, colony counts were performed.
The colony numbers of *E.coli* were decreased as the dosage of irradation increased. On the agar medium contaning extracts of *Ocimum basilicum*, irradiated at different doses, the bacterial colonies were grown enormously on the surface of petri plates by spreading all over.
The reason for the increase of the colonies in the presence of the extracts of *Ocimum basilicum* at the irradiated medium is likely due to antioxidant effects of the plant extracts. However, the studies on the subject are being carried out.

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Poster N° 38

The Effect of Prenatally Exposure of a Nonsteroidal Anti-inflammatory Drug on the Optic Nerve of Female Rats in the Late Postnatal Life: A Stereological, Histological and Electron Microscopic Study

S. Kaplan[1], M. Eşrefoğlu[2], A. Aktaş[3], M. Gül[2], Mehmet Emin Önger[1], E. Altunkaymak[1], M. B. Ulkay[3], M. Ç. Rağbetli[4]

[1]Department of Histology and Embryology, Medical School of Ondokuz Mayis University, Samsun

[2]Department of Histology and Embryology, Medical School of Inonu University, Malatya

[3]Department of Histology and Embryology, Veterinary School of Istanbul University, Istanbul

[4]Department of Histology and Embryology, Medical School of Yuzuncu Yil University, Van

Nonsteroidal anti-inflammatory drug (NSAID)s can have adverse effects in both mother and fetus following administration during the prenatal period. Diclofenac sodium (DS), a NSAID, is given during the pregnancy, may also affect development of the central nervous system (CNS) or related structures. In this study, the effects of DS on the developing optic nerve following administration of the prenatal period were investigated using stereological methods.

Pregnant rats were separated into the pure control group (PG), saline group (SG) and diclofenac group (DG). A dose of 1mg/kg of DS and 1 ml/kg saline daily was injected intraperitoneally to the DG and SG respectively at beginning from the 5th day for a 15 day of period. PG received no any treatment. After spontaneously delivery, female offspring were obtained from PG, DG and SG. After at the end of 20th week of postnatal life, the animals (n=6 for each group) were perfused and the right optic nerves were taken from animals. Then, sections were taken for stereological and histological analysis.There were no any significant differences ($p > 0.05$) between PG, SG and DG in respect of myelin thickness, axon cross section area, axon numerical density, total section area of optic nerve and axon number. Altogether, the study results showed that DS have no a toxic effect on the structure and myelinization of optic nerve of female rats if it is administered during the gestational period.
Both histological and stereological results from present study show that DS as well as saline do not cause to undesirable effect on female rat optic nerve development and myelinization in respect of morphology.

Poster N^{o} 39

Effect of estrogens and progesterone on preadipocyte differentiation

Mehtap Pektaş [1], A. H. Kurt[1], İ. Ün[1], K. Büyükafşar[1]

Department of Pharmacology, Medical Faculty, Mersin University, Mersin

Sex hormones are the major regulators of adipocyte development and functions (1). It has been suggested that estrogens has differential effects on adipocyte differentiation (2, 3). However the effect of progesterone has not completely been understood (4). In the present study we investigated the effects of estrogen and progesterone on the differentiation of mouse embryonic fibroblasts, 3T3-L1, to white adipocyte cells. 3T3-L1 cells were cultured in %10 CS/DMEM. After confluency (regarded as the day 0), the cells were incubated with a differentiation medium (0.5 mM IBMX, 0.25 µM dexamethasone and 1µM insulin with 10 % FBS/DMEM) at the day 0-2, with only insulin in 10 % FBS/DMEM at the day 2-4 and with 10 % FBS/DMEM at the day 4-8. 17β-estradiol (10^{-9}-10^{-4} M), estradiol-BSA (10^{-9}-10^{-4} M) or progesterone was applied to the confluent cells for 8 days. Adipocyte differentiation and intracellular lipid deposition were evaluated with Oil Red-O staining at the day 8. For statistical analysis ANOVA and Dunnet *post hoc* test were used. A *P* value of less than 0.05 regarded as significant. Estrogen treatment for 8 days suppressed adipocyte differentiation at the low (10^{-9} and 10^{-8} M) and high (10^{-5} and 10^{-4} M) concentrations. However intermediate concentrations (10^{-7} and 10^{-6} M) had no effects on the differentiation of 3T3-L1 cells to adipocytes. Estradiol-BSA, which is a cell-impermeable conjugate of estrogen almost completely, suppressed adipocyte differentiation, similar to lipophilic estradiol. However, progesterone (10^{-8}-10^{-6} M) did not change adipocyte differentiation. The suppression of adipocyte differentiation by the long-term exposure of estrogenic hormones could be mediated via membranal and/or cytosolic estrogenic receptors.
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Poster N^{o} 40

How to obtain hydrogen peroxide resistant *Saccharomyces cerevisiae* by evolutionary engineering?

Meliha Merve Hız[1, 2], Z.P. Çakar[1]

[1]Department of Molecular Biology, Genetics and Biotechnology, İstanbul Technical University, Istanbul, 34469, Turkey

[2]Department of Biology, Çanakkale 18 Mart University, Çanakkale, Turkey

In this study, the aim was to obtain hydrogen peroxide resistant *Saccharomyces cerevisiae* cells by using an inverse metabolic engineering strategy; evolutionary engineering. The yeast strain CEN.PK113.7D was mutagenized first by ethyl methane sulfonate (EMS) to increase genetic variation. The mutant yeast cell population was then exposed to increasing hydrogen peroxide pulse stress for successive batch cultivations. The sub-lethal levels of H_2O_2 was determined and H_2O_2 resistant generations were obtained by applying increasing levels of pulse stress to each generation for 90 min until the survival rate of the population was below 0.15. Initial experiments showed that EMS mutagenized *S.cerevisiae* population was tolerant up to 5 mM H_2O_2, thus initial stress level was chosen as 5 mM H_2O_2. After that step, the individual mutants were selected randomly from the final populations and tested for their H_2O_2 resistance by using 5-tube MPN method. The survival of the final population increased up to about 11,000 and 2x106-fold of the wild type under continuous 0.1 and 0.2 mM H_2O_2 stress, respectively. The survival of the final population increased up to about 7800-fold of the wild type at pulse 0.1 M H_2O_2 stress level. The results showed that oxidative stress resistant *S.cerevisiae* mutants were successfully obtained by using evolutionary engineering.

Poster N° 41

Do Non-Thermal Electromagnetic Fields cause Nitrosative Stress in male and female rats?

M. Arda Eşmekaya[1], B. Sırav[1], Ç. Özer[2], N. Seyhan[1]

[1]Department of Biophysics, Gazi University, Ankara, Turkey

[2]Department of Physiology Gazi University, Ankara, Turkey

Nitrosative stress occurs when the generations of highly Reactive Nitrogen Species (RNS) such as nitric oxide (NO) exceed the ability of the human body to neutralize and eliminate them. It may lead to nitrosylation reactions that can alter protein structure by inhibiting normal body functions. In this study, we aimed to investigate whether RF Fields at non – thermal level can affect total NO (NOx) and antioxidant levels in plasma thus cause nitrosative stress. Twenty-four male and female Wistar rats were divided into four groups: Group I and Group II: male (n=6) and female (n=6) control groups, Group III and Group IV: male (n=6) and female (n=6) RF radiation exposed groups. The exposed groups of rats were exposed to non - thermal RF radiation 20 min/day for a month. NOx (nitrite/nitrate) levels were measured by Griess assay using Elisa reader. The total sulfhydryl (RSH) levels were determined spectrophotometrically by Kurtel method. The exposure to RF Radiation for a month caused significant increase in NOx plasma levels in male and female rats ($p < 0.05$). Conversely RSH levels were significantly lower in exposed rats compared to control rats ($p < 0.05$). No statistically significant difference was observed in the rectal temperature between control and RF - irradiated rats ($p > 0.05$). In the present study, we showed that plasma NOx level may be increased in both male and female rats due to RF radiation exposure. The results of our study are evident that RF Fields at non – thermal level can induce nitrosative stress by increasing NOx and decreasing antioxidant levels.

Poster N° 42

Effects of Cell Phone Radiation on Oxidant and Antioxidant status in epileptic mouse model

Meric Arda Esmekaya[1], A. G. Canseven[1], E. Yücel[2], Z. Aktuna[3], S. Keskil[4], N. Seyhan[1]

[1]Department of Biophysics, Gazi University, Ankara, Turkey

[2]Department of Neurosurgery, Baskent University, Alanya Training and Research Hospital, Antalya, Turkey

[3]Department of Medical Pharmacology, Kırıkkale University, Kırıkkale, Turkey

[4]Department of Neurosurgery, Bayındır Hospital, Ankara, Turkey

Scientists have been conducting experimental studies on whether Radiofrequency Radiation (RFR) emitted from cellular mobile phones induces oxidative damage on biological tissues or cells. According to the current assumption RFR may initiate oxygen free radical intermediates in cells or tissues. In this study, we investigated the effects of whole body RFR exposure on oxidant and antioxidant levels in epileptic mouse brain. The epileptic seizure was induced by pentylenetetrazole (PTZ) injection. Eight weeks old mice were used in this study. Mice were divided randomly into three groups as follows; Group I: Control group treated with PTZ, Group II: 15 minute mobile phone radiation + PTZ treatment + 30 minute mobile phone radiation, Group III: 30 minute mobile phone radiation + PTZ treatment + 30 minute mobile phone radiation. The RF radiation was produced by a mobile test phone. Lipid peroxidation, which is the indicator of oxidative stress was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS). The Glutathione (GSH) levels were determined by Ellman method. The absorbances of the samples were measured using a spectrophotometer. The accepted level of significance was set at $p < 0.05$. Lipid peroxidation levels of brain tissue increased significantly in group II and III compared to group I. On the contrary, GSH levels were significantly lower in group II and III than group I. However, no statistically significant alterations in any of the endpoints were noted between group II and Group III. These results indicated that exposure to RF fields emitted from cell phones may lead to oxidative stress in mouse brain during epileptic activity by enhancing lipid peroxidation and reducing the antioxidant (GSH) level. Oxidative injury may play a key role in the initiation and progression of epilepsy. Our results showed that mobile phone radiation may increase the oxidative damage during epileptic activity in mouse brain.

Poster N° 43

Quercetin partially improves renal dsyfunction in diabetic nephropathy

Mustafa Edremitlioğlu[1], M. F. Andiç[2], D. B. Sayin[3], O. Korkut[4] and Ü. Kisa[5]

[1]Department of Physiology, Çanakkale Onsekiz Mart University Medical Faculty, Turkey

[2]Department of Physiology, Kirikkale University Medical Faculty, Turkey

[3]Department of Medical Genetics, Kirikkale University Medical Faculty, Turkey

[4]Pharmacology, Kirikkale University Medical Faculty, Turkey

[5]Biochemistry, Kirikkale University Medical Faculty, Turkey

Diabetes mellitus (DM) can cause very serious complications such as nephropathy. One of the factors of diabetic nephropathy pathogenesis is increase of oxidant stress. Another antioxidant agent which has been shown to have beneficial effects in cases of DM is quercetin. The aim of this study is to examine the effect of quercetin on diabetic nephropathy rats and the change in balance of oxidant-antioxidant capacity in this process. Five groups were used: Control, DM 8 wks (DM8), DM 16 wks (DM16), DM 8 wks + quercetin (QUER8), DM 16 wks + quercetin (QUER16) DM was induced by intraperitoneal (IP) injection of streptozotocin. Rats in QUER8 and QUER16 were treated with IP quercetin (15 mg/kg/day) until the end of the experiment. MDA increased in DM8 and DM16 groups. On contrary, it decreased in groups which were administered quercetin. Accordingly, increase in NADPH oxidase activity, and decrease in SOD and CAT activities in diabetic rats were determined. In QUER8 and QUER16 groups, NADPH oxidase activity decreased while antioxidant enzyme activities increased. It is observed that functional kidney parameters were considerably corrupted in diabetic rats. Especially the decrease in reabsorption of sodium and water is remarkable. It is possible to say that kidney dysfunction depends on the increase of oxidant damage. Administration of quercetin totally prevented the increase of oxidant damage. In these groups, partial improvement in kidney functions was observed. The present experiment showed that renal dysfunction in diabetes was attenuated, although not completely prevented, by the use of quercetin.

Functional kidney parameters				
GROUPS	GFR (μ	FENa (%)	FEwater	Plasma

	L/min)		(%)	creatinin (mg/dL)
CONTROL	1525 ± 137	0.81 ± 0.16	1.36 ± 0.14	0.33 ± 0.02
DM8	274 ± 55	3.86 ± 0.63	6.05 ± 1.37	0.87 ± 0.26
DM16	174 ± 27	8.78 ± 0.87	17.54 ± 3.08	1.23 ± 0.03
QUER8	208 ± 44	6.02 ± 1.7	9.93 ± 2.10	0.97 ± 0.19
QUER16	434 ± 76	3.43 ± 0.74	4.44 ± 0.69	0.75 ± 0.13

Poster N° 44

Effects of Vitamins C and E Combination on Element Levels in Blood of Smoker and Nonsmoker Radiology X-Ray Technicians

Mustafa Kayan[1], M. Nazıroğlu[2], C. Barak[3]

[1]Department of Radiology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

[2]Department of Biophysics, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

[3]Department of Chemistry, Science Institute, Balıkesir University, Balıkesir, Turkey

Cigarette paper and cigarette smoke contain toxic elements, whereas vitamins C and E (VCE) may have regulator effects on the elements. We investigated effects of VCE administration on X-ray-induced element changes in blood of smoker and nonsmoker X-ray technicians.
Twenty technicians and 30 healthy age-matched control subjects were used in the study. 10 of the X-ray technicians and 15 of the control were smokers. Blood serum samples were taken from the control. Oral vitamins C (500 mg) and E (150 mg) were supplemented daily to the smoker and nonsmoker X-ray technicians for 5 weeks. Serum samples were taken from the X-ray technicians before and after 5 weeks. Copper, zinc, selenium, aluminum, iron, magnesium, and calcium levels were investigated in control and X-ray technicians, both smokers and nonsmokers. Copper, zinc, and selenium levels were lower in the total X-ray group and smoker X-ray group than in control and nonsmoker X-ray group, although iron, magnesium, and calcium levels were higher in X-ray group than in control. The copper, zinc, selenium, and aluminum levels were higher in the VCE treatment group than those in X-ray group, although magnesium and calcium levels were decreased by the treatment. The serum zinc, copper, selenium, and magnesium levels were lower in smoker control group when compared to nonsmoker control group. The serum zinc levels were lower in smoker X-ray group than nonsmoker X-ray group, although iron level was higher in smoker X-ray group than in nonsmoker X-ray group.
VCE prevents the smoke and X-ray-induced selenium, zinc, magnesium, and copper decrease to strengthen the antioxidant trace element levels in the serum of the technicians.

Poster N° 45

Vitamin C and E combination modulates oxidative stress induced by X-ray in blood of smoker and nonsmoker radiology technicians

Mustafa Kayan[1], M. Nazıroğlu[2], Ö. Çelik[2], K. Yalman[2], H. Köylü[3]

[1]Department of Radiology, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

[2]Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

[3]Department of Physiology, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

X-ray radiation is detrimental to human cells. Cigarette smoke contains chemicals that include oxidant compounds whereas vitamin C and E (VCE) have scavenger effects on the compounds. We investigated effects of VCE administration on X-ray-induced oxidative

toxicity in blood of smoker and nonsmoker X-ray technicians.
Twenty technicians and 30 healthy age-matched subjects control were used in the study. Ten of the X-ray technicians and 15 of the control were smokers. Oral vitamin C (500 mg) and E (150 mg) were daily supplemented for 5 weeks. Blood samples were taken from the X-ray technicians after and before 5 weeks. Plasma and erythrocytes lipid peroxidation (LP), reduced glutathione (GSH) levels, erythrocytes glutathione peroxidase (GSH-Px), and plasma antioxidant vitamin concentrations were investigated.
Plasma and erythrocyte LP levels were higher in the total X-ray group and smoker X-ray group than in control and nonsmoker X-ray group, respectively although the LP level was decreased by the VCE treatment. The plasma vitamin C, A, E, and ß-carotene concentrations were lower in the X-ray group than in control although their concentrations were increased by the treatment. The erythrocyte GSH level and GSH-Px activity were found to be higher in the treatment group than in the X-ray group. Plasma GSH level was not found to be different in all groups.
Free radicals are highly unstable substances produced in body through the metabolism of oxygen. They also result from exposure certain environmental factors. GSH, GSH-Px, and antioxidant vitamins protect cells from oxidative stress.9, 10 study clearly shows that smoking induced oxidative stress increase effect in smoke exposure to X-ray technicians. VCE combination may play an antioxidant role against X-ray-induced oxidative injury.
X-ray and cigarette smoke induces oxidative stress by augmenting LP and diminishing the antioxidant vitamin levels. VCE combinations recover LP damage probably through its free radical scavenging in blood.

Poster N^{o} 46

Testicular apoptosis and histopathological changes induced by a 2.45 GHz electromagnetic field

Mustafa Saygin[1], S.Caliskan[1], N.Karahan[2], A. Koyu[1], N. Gumral[1], A.C. Uguz[3]

[1]Department of Physiology, [2]Department of Pathology, [3]Department of Biophysics, Faculty of Medicine Suleyman Demirel University, Isparta, Turkey

There is a growing public concern about the potential human health hazard caused by exposure to electromagnetic radiation (EMR). The objective of this study is to investigate the effects of 2450 Mhz electromagnetic field on apoptosis and histopathological changes on rat testis tissue. Twelve weeks-old male Wistar Albino rats were used in this study. Eighteen rats equally divided into three different groups which were named Group I, II and III. Cage control (Group I), sham control (Group II) and 2.45 GHz EMR (Group III) groups are formed. Group III were exposed to 2.45 GHz EMR, at 3.21 W/kg Specific Absorption Rate (SAR) for 60 minutes/day for 28 days. There was no difference among the groups for the diameter of the seminiferous tubules, pyknotic, and karyolectic and karyotic cells. However, the number of Leydig cells of testis tissue of the rats in group III was significantly reduced comparing with the group I ($p<0,05$). Estimation of spermatogenesis using the Johnsen testicular biopsy score revealed that the difference between groups is statistically significant.
The level of TNF-α, Caspase-3 and Bcl-2 were compared, and no significant difference was found between the groups. When Bax apoptosis genes and Caspase-8 apoptosis enzyme were compared, there were significant differences between the groups ($p<0,05$). Electromagnetic field affects spermatogenesis and causes to apoptosis due to the heat and other stress related events in testis tissue.

Poster N^{o} 47

Protective effects of L-carnitine and Selenium on 2450 MHz Electromagnetic radiation exposed Pancreas Tissue

A.Koyu[1], N. Gumral[1], A. Gokcimen[2], Mustafa Saygin[1], H. Vural[3], M. Ozgocmen[2]

[1]Department of Physiology, [2]Department of Histology and Embriyology, [3]Department of Biochemistry, Faculty of Medicine Suleyman Demirel University, Isparta, Turkey

In this study, we aimed to research protective effects of L-carnitine and selenium on rat's

pancreatic biochemical and structural changes induced by 2.45 GHz electromagnetic radiation.
In this study thirty male Wistar Albino rats were used. Rats divided five groups randomly and groups were named as cage control, shame control, electromagnetic radiation (EMR), EMR+Selenium [(1.5 mg/kg/ every other day-intraperitoneal (ip)], EMR+L-carnitine (100 mg/kg/day-ip). Except for cage and shame control groups all of the other groups were exposed to 2450 MHz EMR 60 min per day during 28 days. At the end of the study blood samples were obtained. Then amylase, lipase, alkaline phosphatese (ALP) and gamma glutamil transferase (GGT) enzyme activities were measured from the blood serums. After fixation of pancreas tissue by using 10% neutral formaldehyde solution the pancreas tissue samples were embedded in paraffin, cross-sections were cut in 4–5 µm thickness and stained with hematoxylin-eosin. Preparations were examined with light microscope. The data were analyzed by using a commercially available statistics software package *(SPSS for Windows)*. Basing on blood serum amylase, lipase, ALP and GGT enzyme activities significantly increased in EMR group as compared with cage and shame control groups ($p<0.05$). Basing on blood serum amylase, lipase, ALP and GGT enzyme activities significantly decreased in EMR+Selenium and EMR+L-carnitine groups as compared with EMR group ($p<0.05$). In respect to the structural appearances of pancreas tissues there was no difference between control and the other groups. It is observed that the radiation generated by 2450 MHz causes some biochemical changes on pancreatic enzyme activities. These changes were not at structural levels. Basing on amylase, lipase, ALP and GGT enzyme activities, a significant difference was determined between EMR exposed group and treatment groups. It can be an evidence of Selenium and L-carnitine have protective role on EMR exposed pancreas tissue.

Poster N^{o} 48

The Role of Free Radicals in Retinitis Pigmentosa

Mustafa Tümay[1], F. R. Hatungil[1], M. Berköz[2], S. Yalın[3]

Mersin University, [1]Faculty of Medicine, Department of Physiology, Faculty of Pharmacy, [2]Department of Pharmaceutical Technology, [3]Department of Biochemistry, 33169, Mersin

Mutations in humans are associated with several forms of inherited retinal dystrophies, such as Retinitis Pigmentosa which lead to retinal cell death and irreversible loss of vision. In retinitis pigmentosa cases, photoreceptors can get lost as a result of apoptosis in retina. Reactive oxygen products can have a role on retina cells apoptosis and trigger the retinitis pigmentosa. For this aim, venous blood samples of patients and controls were collected and analyzed for plasma malondialdehyde (MDA) level as a marker of lipid peroxidation and catalase, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities for determining the antioxidant statue in a group of 30 patients with retinitis pigmentosa and compared with 45 age and sex-matched healthy control subjects without retinitis pigmentosa. Statistical evaluation of data was performed SPSS v10.0 programme and independent t-test for the comparison of the groups. The levels of MDA showed a significant increase ($p<0.05$) in the patients when compared to control. SOD, catalase and GSH-Px activities were lower in retinitis pigmentosa than in control group ($p<0.001$). As a result of this study, it can be suggested that lipid peroxidation increases and antioxidant enzyme activities decrease in retinitis pigmentosa cases. In conclusion, it is assumed that being exposed to free radicals or insufficient level of antioxidant enzymes can be the considerable reason of retinitis pigmentosa.

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Poster N^{o} 49

17β-estradiol and vitamin E decrease oxidative stress in brain cortex of diabetic ovariectomized rats

Mustafa Ulas[1] and M. Cay[2]

[1]Department of Physiology, Faculty of Medicine, Firat University, Elazig, Turkey

[2]Department of Physiology, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey

In the current study, the effects of 17β-estradiol (E_2) and vitamin E on lipid peroxidation and antioxidant levels in brain cortex of diabetic ovariectomized rats were investigated. Forty-two rats were equally divided into seven groups: (1) Control; (2) ovariectomized (OVX); (3) OVX+E_2; (4) OVX+E_2+vitamin E; (5) OVX+diabetic; (6) OVX+diabetic+E_2 and (7) OVX+diabetic+E_2+vitamin E. E_2 (40µg kg^{-1}/day) and vitamin E (100 mg kg-1/day) were administered. The activities of glutathione peroxidase (GSH-Px) and catalase (CAT), superoxide dismutase (SOD) and levels of glutathione (GSH), vitamin A and beta-carotene were significantly reduced in brain ($p<0.05$) compared to control in OVX but level of MDA in brain and level of glucose in plasma were significantly increased ($p<0.05$). The activities of antioxidant in brain ($p<0.05$) increased although MDA ($p<0.05$) in brain and glucose ($p<0.05$, $p<0.01$) in plasma decreased in OVX after E_2 and E_2+vitamin E supplementation. The activities of GSH-Px ($p<0.001$, $p<0.01$), CAT ($p<005$, $p<0.001$) and SOD ($p<0.001$) and the levels of GSH ($p<0.001$), vitamin A and beta-carotene ($p<0.001$, $p<0.05$) were lower in the brain of OVX diabetic rats, while MDA in the brain and glucose in the plasma were higher ($p<0.001$). The antioxidant enzymes ($p<0.05$), GSH, vitamin A and beta-carotene ($p<0.01$, $p<0.001$) in brain increased while MDA in brain and glucose in plasma decreased ($p<0.01$, $p<0.001$) in diabetic OVX after treatments. In conclusion, the E_2 and E_2+vitamin E supplementation to diabetic OVX and OVX rats may strengthen the antioxidant system by reducing lipid peroxidation.

Poster N° 50

17β-estradiol and vitamin E supplementation restore blood trace element and antioxidant enzyme to control levels in ovariectomized rats

Mustafa Ulas[1] and M. Cay[2]

[1]Department of Physiology, Faculty of Medicine, Firat University, Elazig, Turkey

[2]Department of Physiology, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey

In the present study, effect of 17β-estradiol (E_2) alone and separately vitamin E treatment on trace element status of rats following an ovariectomic operation were investigated. Forty rats were equally divided into four groups: (1) control; (2) ovariectomized (OVX) rats, which were ovariectomized under general anesthesia. (3) OVX+E_2 rats, which received subcutan E_2 at a dose of 40µg kg^{-1} per day. (4) OVX+E_2+vitamin E rats, which received intraperitoneal vitamin E at a dose of 100 mg kg^{-1} as well as subcutan E_2 at a dose of 40µg kg^{-1} per day. At the end of the 30-day treatment, the rats were sacrificed and their blood was collected for biochemical measurements. The levels of zinc, copper, iron, phosphorus, selenium, calcium, chromium and manganese and activities of copper-zinc superoxide dismutase (SOD), manganese-superoxide dismutase (Mn-SOD), glutathione peroxidase (Se-GSH-Px) and catalase (CAT) were lower in the OVX when compared to control group, but magnesium level was unaffected. However, zinc, copper, iron, phosphorus, selenium, calcium, chromium and manganese levels and SOD, Mn-SOD, Se-GSH-Px and CAT activities were higher in E_2–treated and E_2+vitamin E–treated groups. There was no significant difference between the treated-OVX groups the OVX group in the level of magnesium. In conclusion, E_2 treatment has an ameliorating effect on the trace element status in OVX, and this effect may be enhanced with the addition of vitamin E.

Poster N° 51

Apoptotic and antiproliferative effects of single and combined use of alpha tocopherol and antifibrotic agents, on human endothelium cells:

a comparative evaluation

M. Beyza Çetin[1], S. Karadenizli[1], U. Serbes[2], K. Akgün- Dar[2], G. Ö. Arıcan[2], K.N. Engin[3], S. E. Kuruca[1],

[1]Istanbul University, Istanbul Faculty of Medicine, Department of Physiology,

Istanbul, Turkey

[2]Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey

[3]Bağcılar Education and Research Hospital, Department of Ophthalmology, Istanbul, Turkey

In this study, we investigated the apoptotic and antiproliferative effects of α-tocopherol on ECV304 human endothelial cells, combined antiproliferative agents frequently used in glaucoma surgery. Side effects of this current antifibrotics on endothelium, limits their use in glaucoma surgery. Vitamin E is an important natural antioxidant and its most common and biologically active form α-tocopherol, prevents tenon's fibroblast proliferation and it also effects similar pathways with other antifibrotics as well. Antiproliferative and apoptotic effects of α-tocopherol were comperatively evaluated, both solely and in combination with antifibrotic agents for long and short time applications. Mitomycin-C, 5FU and paclitaxel were used as antifibrotic agents in vitro. Apoptotic indexes found with DAPI staining in fluorecence microscope. Apoptotic cells were evaluated in molecular level with RNA isolation, bcl2 and bax gene expression were evaluated with PCR and also determined with caspase3 immunohistochemical staining. Mitotic indexes were also determined. Mitomycin-C and paclitaxel groups' demostrated a significant AI increase which were augmented by α-tocopherol addition. Bcl2/Bax ratio decrease or caspase activity was found only in mitomycin group and further augmention was achieved with α-tocopherol addition. Mitotic cells were observed in α-tocopherol groups. We observed noticable decreased in mitosis for short and long term applications of mitomycin-C and 5FU groups. As a result, combination with α-tocopherol did not reduce the apoptotic effects of antifibrotics on human endothelial cells. 5FU and α-tocopherol have been found to be safe in glaucoma surgery for therapy because of little apoptotic and antimitotic effects.

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Poster Nº 52

The effect of smoke and smokeless tobacco use as Maraş powder on saliva adenosine deaminase, xanthine oxidase, and malondialdehyde levels

Naciye Kurtul[1], E. Gökpınar [1]

[1]University of Kahramanmaraş Sütçü İmam, Faculty of Science Department of Chemistry, Division of Biochemistry, Kahramanmaraş

The use of smokeless tobacco (ST) is common worldwide. Also, in Kahramanmaraş, a city Southern Turkey, ST used as Maraş powder (MP) has been consumed widely instead of cigarette smoking for a long time. The habit is becoming increasingly popular, especially due to banning smoking in our country. MP is prepared from a tobacco of species *Nicotiana rustica* L. In this study, xanthine oxidase (XO), adenosine deaminase (ADA), and lipid peroxidation levels as malondialdehyde (MDA) were measured in saliva samples obtained from smokers (Group II), MP users (MPU; Group III), and healthy control subjects (Group I) who were nonsmokers and nonusers of ST

and the results were compared. The salivary XO, ADA, and MDA levels were found to be significantly higher in smokers and MPU than that of control subjects and also in MPU than that of smokers ($p<0.001$). In Group III, statistically important correlation was found between MDA levels and ADA, and XO activities. We have also observed that as the number of cigarettes and MP amount increases, the salivary XO, ADA, and MDA levels increase. We have concluded that salivary XO, ADA, and MDA levels are increased by smoking and MP use. Results obtained from this study can help evaluate harmful effects of various use of tobacco. It is important to point out that bigger change in the measured parameters observed for MP use. This finding may be an indication of harmful effects of ST use as MP and draw attention to a significant potential public health hazard.

Poster N^{o} 53

Evaluation the nitric oxide level and lipid peroxidation in the serum of migraine patients

Naciye Kurtul[1], A.Seyhan[1], A. Akçalı[2]

[1]University of Kahramanmaraş Sütçü İmam University, Faculty of Science Department of Chemistry, Division of Biochemistry, Kahramanmaraş

[2]University of Gaziantep University, Faculty of Medicine, Department of Neurology, Gaziantep, Turkiye

Migraine a common and disabling disorder. It has suggested that the release of nitric oxide (NO) is an important trigger mechanism in migraine. In this study, NOlevel and lipid peroxidation (LPO) as malondialdehyde (MDA) were measured in the serum samples obtained from the 22 healthy controls without migraine (mean age: 30.40±6.28 years) and 26 migraine patients (mean age: 31.21±6.55 years) in a crisis-free period. Also, migraineurs were groupped as with and without aura. Mean MDA level was found to be significantly higher in migraine group than that of control subjects ($p<0.001$). Even though migraine patients group had NO values higher than that of control group's, this difference wasn't statistically important ($p>0.05$). At these parameters there wasn't a statistically difference between migraine groups with aura and without aura. As a result it was observed that migraine had effect on the levels of the measured parameters in serum. While NO normally functions as a physiological neuronal mediator, excess production of NO mediates brain injury. As a free radical, NO mediates cellular toxicity and reacts with superoxide to form peroxynitrite which an even more potent oxidant. Thus the findings of the present study suggest that NO and LPO are possibly important factors in the migraine pathogenesis.

Poster N^{o} 54

Attenuation of Ischemia-Reperfusion Injury by Iloprost on Apoptosis and Antioxidant Capacity in Kidney as a Distant Organ

Necmiye Canacankatan[1], N. Sucu[2], S. Şahan-Fırat[3], A. Görür[1], Ş.E. Antmen[4], B. Korkmaz[3], B. Tunçtan[3], OE. Gül[1], L. Tamer[5], L. Ayaz[5], Ö. Vezir[2], A. Kanık[6]

[1]Mersin University, Faculty of Pharmacy, Department of Biochemistry, Mersin, Turkey

[2]Mersin University, School of Medicine, Department of Cardiovascular Surgery, Mersin, Turkey

[3]Mersin University, Faculty of Pharmacy, Department of Pharmacology, Mersin, Turkey

[4]Mersin University, Medical Services Vocational School, Mersin, Turkey

[5]Mersin University Medical Faculty, Department of Biochemistry, Mersin, Turkey

[6]Mersin University, Medicine Faculty, Department of Biostatistics, Mersin, Turkey

Activation of apoptotic cascade is crucially involved in post-ischemic inflammation which causes tissue damage and organ deficiency. In this context, we investigated the cytoprotective and antioxidant effects of iloprost, a prostacyclin analogue in kidney as a distant organ after ischemia-reperfusion (I/R) injury. For this purpose; apoptosis was evaluated by measurement of caspase -3, -8 and -9 enzymes, antioxidant capacity was evaluated by measurement superoxide dismutase (SOD) enzyme activity, and nitric oxide (NO) production was evaluated by measurement of nitrite content in kidney tissues. Wistar albino rats were randomized into five groups as sham, ischemia, I/R, iloprost (10 μg/kg) and I/R with iloprost (10 μg/kg). 12 rats were included in each group. A 4 h reperfusion procedure was carried out after 4 h of ischemia. 10 μg/kg iloprost was administered to the rats in 1ml of saline from the tail vein in iloprost (10 μg/kg) group. Rats in I/R with iloprost (10 μg/kg) group received 10μg/kg iloprost in the same way 10 min before reperfusion. There were statistically significant increase in caspase -3, -8 and -9 enzymes in both ischemic and I/R groups to the sham group. Iloprost prevented these increase in caspase activities. SOD enzyme activity and the nitrite levels also increased in ischemic rats compared to the sham group; but only SOD activity decreased by iloprost. As a conclusion iloprost may be considered as a cytoprotective agent to apoptosis. However, further studies are needed with different doses of iloprost to emphasize these results.

Poster N° 55

Influence of grape seed oil on paraoxonase/arilesterase activities of the erytroleukemia cell line treated with methotrexate

Nefise Özlen Şahin[1], E.D. Eker[1], M. Berköz[2], S. Yalın[3], B. Pomierny[1,4], K. Przejczowska[1,4]

Mersin University, Faculty of Pharmacy, [1]Department of Pharmaceutical Biotechnology, [2]Department of Pharmaceutical Technology, [3]Department of Biochemistry, Yenisehir Campus, Mersin 33169, Turkey. [4]Jagellonian University, Faculty of Pharmacy, Krakow, Poland

There is a balance between a free radical formation and endogenous antioxidant defense mechanisms. Disturbance of this balance results in oxidative stress. This, in turn, causes injury to all the important cellular components like proteins, DNA and membrane lipids which can cause cell death. Recently, significant evidence in experimental and clinical data was provided to indicate the involvement of oxidative stress in carcinogenesis. On the other hand, research conducted on toxicity of anticancer drugs indicates compelling evidence on oxidative stress as well. The most significant drug with cytotoxicity is methotrexate (MTX), which has been widely used in treatment of various types of cancer. Additive effect of oxidative damage caused by MTX to oxidative stress induced by cancer makes the situation dramatically bad. Oxidative stress has been reported responsible for the adverse reaction mechanism of MTX in liver, kidney, intestine, CNS, and hematopoietic cells. In order to reduce the damage, several medical approaches have been suggested. The use of products with antioxidant activity (e.g. melatonin, vitamin E, methionine) can reduce the influence of MTX-induced tissue damage. Grape seed is one of the most significant profilactic agents due to its antioxidant and bioflavonoids composition. However, there is no study regarding the protective effect of grape seed oil on the MTX-induced oxidative stress. Therefore, the aim of this study was to investigate the protective effect of grape seed oil against MTX-induced oxidative stress in K-562 human erythroleukemia cell lines. Paraoxanase and aryl esterase enzyme activities indicating antioxidant properties in cell lines were investigated by the method of Eckerson et al. Protein assay was conducted by Lowry method at the 24th and the 48th hour following the treatment. The difference between groups was statistically evaluated using SPSS v10.0 program and ANOVA test. It was determined that MTX treatment reduced both PON and ARE activities at both time points. Involvement of grape seed oil administration did not change PON and ARE activities for 24 hours. However, a significant increase was observed at 48th hour. In conclusion, grape seed oil administration for 48th hours following MTX

treatment gives rise to paraoxanase and aryl esterase activities in erythroleukemia cells. Thus, it may be suggested that use of grape seed oil along with MTX in cancer patients may provide a protective effect against oxidative stress and xenobiotics that are the substrates of these enzymes.

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Poster N° 56

Effects of caloric restriction on brain antioxidant redox system, microsomal Ca^{2+}-ATPase activity and nitric oxide levels in rats

M. Nazıroğlu[1], A. C. Uğuz[1], Ö. Çelik[1], Nigar Yılmaz[2], M. Yılmaz[3], H. Vural[4]

[1]Department of Biophysics, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

[2]Department of Biochemistry, Medical Faculty, Mustafa Kemal University, Hatay, Turkey

[3]Department of Neurology, Antakya State Hospital, Hatay, Turkey

[4]Department of Biochemistry, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

Reactive oxygen substances (ROS) and lipid peroxides cause injury by reacting with biomolecules as well as by depleting enzymatic and/or nonenzymatic antioxidants, plasma membrane dependent microsomal Ca^{2+}-ATPase in the brain. Caloric restriction rescues neuronal cells from mitochondrial redox impairment and ROS because ROS produce in mitochondria by metabolism of proteins, lipids and carbohydrates. The mode of action on neuron protection by caloric restriction is unclear. We investigated effects of caloric restriction on Ca^{2+}-ATPase and antioxidant redox system in rat brain. Thirhty eight rats (four month) were divided into four groups. The first, second, third and fourth groups were used for the control, caloric restriction, obese and obese+caloric restriction groups, respectively. Initial body weight of control and obese groups were 250±20 g and 400±20 g, respectively. Second and fourth groups were exposed to 60% caloric restriction for 10 weeks. After 12 hours fasting, brain samples were taken from the four groups and brain microsomal samples were obtained by ultracentrifugation. Lipid peroxidation levels of brain cortex were increased in second group although lipid peroxidation levels were decreased in fourth groups by caloric restriction. Brain vitamin A and C concentrations, and brain microsomal Ca^{2+}-ATPase activities were decreased in second groups although Brain vitamin A, C and E concentrations, and brain microsomal Ca^{2+}-ATPase activities were increased in fourth groups by caloric restriction. Brain glutathione peroxidase and reduced glutathione values did not change in the four groups.
In conclusion, caloric restriction in obese rats seems to have protective effects on the oxidative stress-induced brain toxicity by inhibiting free radicals and supporting the antioxidant redox system.

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Poster N° 57

Investigation of the Effects of Electromagnetic Field at 2.45 GHz on Rat Skeletal Muscle Tissue with Protective Effects of Selenium and L-Carnitine

D. Bayram[1], N. Gumral[2], A. Gokcimen[1], S. Calıskan[2]

[1]Department of Histology and Embryology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

[2]Department of Physiology, Medical Faculty, Süleyman Demirel Univesity, Isparta, Turkey

The hazard of microwave electromagnetic fields (EMF) to humans and animals has been intensively studied during the last 20 years, because large professional groups of people are subjected to microwave exposure. Several studies have suggested that biological systems would exhibit sensitivity to 2.45 GHz of EMF emitted from wireless settings. This study was conducted to investigate the histopathological effects of electromagnetic field at 2.45 GHz on rat skeletal muscle tissue and protection by Selenium (Se) and L-Carnitine (L-Car).
There were five study groups each consisting of six animals. Group I: Controls, Group II: Shame- controls, Group III: 2.45 GHz EMF exposed, Group IV: 2.45 GHz EMF exposed + Se treated (1.5 mg/kg/over day) and Group V: 2.45 GHz EMF exposed + L-Car treated (100 mg/kg/day). 2.45 GHz EMF was applied to Groups III, IV and V for 28 days (1h/day). At the end of the 4th week, tissue samples were taken for histological examination.
Control and shame groups were observed as normal. There were slight changes in skeletal muscle tissues of L-Car and Se groups but these differences were not statistically meaningful ($p>0.05$). Some structural changes observed in 2.45 GHz exposed group, including skeletal muscle fibrillary degeneration and increased of muscle fibers diameter when compared with other groups ($p\leq0.05$).
Exposure to the 2.45 GHz EMF caused some degeneration in skeletal muscle while these changes were fixed in L-Car and Se given groups in our study. Antioxidant effects of these agents may be useful for degeneration skeletal muscle.

Poster N° 58

Oral zinc supplementation protects rat kidney tissue from oxidative stress in diabetic rats

N.Özsoy[1], A. Can[1], Ö. Mutlu[2], Nuriye Akev[1], R. Yanardağ[2]

[1]Department of Biochemistry, Faculty of Pharmacy, Istanbul University, 34116, Istanbul, Turkey

[2]Department of Chemistry, Faculty of Engineering, Istanbul University, 34320, Istanbul, Turkey

Various substances are investigated nowadays for the prevention of diabetic complications. During long-life diabetes, persistent high blood glucose levels cause the destruction of small blood vessels in the nephrons, leading to diabetic nephropathy and chronic kidney disease. Zinc is a trace element possessing a wide range of functions and antioxidant properties. This study was undertaken in order to illuminate the conflicting data on the status of zinc in diabetes, present in literature. Female Swiss albino rats were randomly divided into 4 groups: Group I, control; Group II control+zinc sulfate; Group III, STZ-diabetic; Group IV, STZ-diabetic+zinc sulfate. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ, 65 mg/kg body weight). Zinc sulfate was given daily by gavage at a dose of 100mg/kg body weight every day for 60 days to groups II and IV. At the last day of the experiment, rats were sacrificed, kidney tissue was taken, homogenized by means of a glass homogenizer in cold saline to make a 10% (w/v) homogenate. Antioxidant enzymes, catalase, glutathione reductase, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase activities were determined in tissue homogenates. The results were evaluated statistically. It was shown that antioxidant enzyme activities were significantly decreased in zinc treated diabetic groups in comparison with untreated diabetic group, thus showing the beneficial effect of Zn treatment in diabetes.

Poster N° 59

Activity paraoxonase and arylesterase in sportsmen and sedentary controls

E. Eren[1], A. Yeğin[2], A. Kurtoğlu[2], Nurullah Ay[2], N. Şimşek[2], N. Yılmaz[2]

[1] Antalya Atatürk Hospital, Biochemistry Laboratory, Antalya, Turkey

[2]Antalya Education and Research Hospital, Clinical Biochemistry, Antalya, Turkey

Physical activity aids the human body antioxidant defense system via obscure mechanisms. Paraoxonase (PON) and arylesterase (ARE) are enzymes that own antioxidant characteristics. The studies investigate a possible beneficial relationship, that increased levels of these enzymes due to physical activity might help our understanding of the issue. In this study we focus on healthy young subjects unlike previous ones that have prefered middle aged or older participants. Serum paraoxonase and arylesterase activities were evaluated in 31 well-trained young sportsmen and 30 sedentary age-matched controls. Paraoxonase activity measurements were performed in the absence of salt (basal activity). The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 10.5, which was 18,290 $M^{-1}cm^{-1}$. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol, $1310 \cdot mol^{-1} \cdot L^{-1} \cdot cm^{-1}$. Although the sportsmen were not receiving any special diet or vitamin supplementation, they showed a slightly improved antioxidant status, mainly represented by increased paraoxonase ($p<0.01$), arylesterase levels ($p<0.05$), and in addition, elevated ratio of paraoxonase/HDL-C ($p<0.01$). In biological systems, cells respond to mild oxidative stress by inducing their antioxidant defenses and other protective systems. Increased paraoxonase and arylesterase activities, in young sportsmen might indicate eliminated oxidative stress. It proves once again that physical activity provides disease prevention.

Poster N° 60

Investigation of the role of oxidative stress in experimental gentamycine induced nephrotoxicity and effect of Caffeic Acid Phenethyl Ester on possible oxidative stress in rats

Fadime Özge Aygün[1], F.Z. Akçam[1], O.Kaya[1], B.M.Ceyhan[2], R.Sütçü[2]

[1]Department of Infectious Diseases, [2]Department of Biochemistry and Clinical Biochemistry [2], Medical Faculty, Süleyman Demirel University, Isparta, Turkey

Forty female Wistar rats were randomly divided into four groups. Each group consisted of 10 rats. Control group received 1 ml/kg serum physiologic (SF) intraperitoneally (i.p.) once a day, CAPE and GM+CAPE groups received 10 µmol/kg/day CAPE i.p. for 12 days. GM group; injected i.p. with 1 ml/kg SF for 2 days before GM treatment and after the GM and afterwards i.p with 100 mg/kg GM for 8 days, continued only injected i.p. with 1 ml/kg SF for 2 days. GM+ CAPE treated group; injected i.p with 10 µmol/kg CAPE for 2 days before GM treatment and daily during GM treatment and after the GM treatment for 2 days. On the twelfth day of the study all rats were sacrificed and then blood samples and kidneys were taken. Left kidneys were used for hystopathological evaluation. Malonyldialdehyde (MDA) and nitric oxide (NO) levels, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) enzyme activities were determinated in right kidneys of rats. Levels of BUN and creatinin were studied in serum.
Hystopathologic evaluation showed nephrotoxicity findings in GM and GM+CAPE groups. Serum BUN and creatinin levels of GM group were significantly higher than control group. In GM group, SOD, GSH-Px, CAT enzyme activities were lower than the control group. NO and MDA levels were higher than the control group. In GM+CAPE group, it has been observed that the oxidative stress amount was decreased in renal tissue. According to these findings, it could be concluded that oxidative stress may be a critical mechanism in GM nephrotoxicity and using of CAPE maybe an effective treatment option in prevention of oxidative stress due to GM nephrotoxicity.

Poster N° 61

The role of SAG as a prognostic factor on radio/chemotherapy in patients with advanced colorectal cancer

Oya Orun[1], S. Özden[1, 2], H. Özyurt[2], Z. Özgen[2], M. Öncel[2], O. Kılınç[1], B. Kan[3]

[1]Department of Biophysics, Marmara Univ. School of Medicine, Haydarapasa, İstanbul

[2]Dr.Lütfi Kırdar Kartal Education and Research Hospital, Kartal, İstanbul

[3]Department of Biophysics, Acıbadem Univ. School of Medicine, Maltepe, İstanbul

Colorectal cancer is one of the most malignant disorders worldwide and show nearly 50% local recurrence posing a serious problem in treatment (Folkesson 2005, Adell 1999). Preoperative radiotherapy with or without chemotherapy is widely accepted to improve local control and overall survival rates.
Resistance of cancer is one of the main causes for treatment failure. Predictive indicators will be highly valuable estimating the response of a patient. SAG (sensitive to apoptosis gene) protects cell from apoptosis induced by redox agents like hydroxyl radicals and irradiation. Prognostic value of apoptosis has been described at various reports.
To assess the relation between SAG expression levels and sensitivity to radiation, 31 patients with rectal cancer were examined in this study before and after treatment.
Our results indicate that expression levels of apoptosis related genes can be a regulatory factor for resistance against exposure and can considerably affect the therapeutic results, sensitivity against radiation and survival rates. This small group under study has revealed that SAG expression have correlated with 1 and 2 year survival rates and could have predictive value in progression of disease.

Poster N^{o} 62

Serum vitamin levels in young basketball players and sedentary controls

E. Eren[1], G. Aykal[2], A. Yeğin[2], N. Ay[2], Özgür Aydın[2], N. Yılmaz[2]

[1] Antalya Atatürk Hospital, Biochemistry Laboratory, Antalya, Turkey

[2]Antalya Education and Research Hospital, Clinical Biochemisty, Antalya, Turkey

Physical activity is known to increase the antioxidant defense system and reduce exercise-induced oxidative stress. Nevertheless, the precise mechanisms by which regular training is responsible for this protection are not completely clear. The cohort studies that have indicated benefits of physical activity have all focused on middle aged or older participants. The current study focuses on 10 year old young basketball players. Antioxidant profiles α-tocopherol, retinol, pyridoxal-5-phosphate, bilurubin, and uric acid were evaluated in 31 well-trained young basketball players and 30 sedentary age-matched controls. Although the sportsmen were not receiving any special diet or vitamin supplementation they showed a slightly improved antioxidant vitamin status, mainly represented by an increased antioxidant status. The latter was evidenced by an increment in higher serum retinol, pyridoxal-5-phosphate and α-tocopherol ($p<0.05$) concentrations. In biological systems, cells respond to mild oxidative stress by inducing their antioxidant defenses and other protective systems. Increased vitamin levels in young basketball players might indicate eliminated oxidative stress. Physical activity once again promises a prolonged and healthier life.

Poster N^{o} 63

Evaluation of the Effect of Carnosine and Melatonin on Acetaminophen Induced Acute Liver Toxicity

Ozgur Fedakar[1], M. Oncu[1], F. Gultekin[2]

[1]Department of Histology and Embryology, University of Suleyman Demirel, Isparta, 32100, Turkey

[2]Department of Biochemistry, University of Suleyman Demirel, Isparta, 32100, Turkey

Acetaminofen is the most common using pain and fever reducing medical agent worldwide. But there is a lot of study, suggesting this medicine makes suffering of liver via liver damage. Melatonin, which is famous as a member of most powerful antioxidants, is a hormone. Carnosine is a powerful antioxidant dipeptide. In our study, our goal was to reveal to compare melatonin and carnosine for their effects on acetaminophen mediated damage on rat liver tissue.48 Wistar Albino female rats were randomly divided into six equal groups. Groups planned as : Control. Acetaminophen (1 g/kg, ip, single dose), Melatonin (10 mg/kg, ip, single dose), Carnozine (250 mg/kg, ip, single dose), Acetaminophen plus Melatonin and Asetaminofen plus Carnozine, at the same

doses. At the end of the study; Serum tolal oxidan status (TOS) and total antioxidant status (TAS) levels measured and oxidative stres index (OSI) values were calculated. Liver tissue samples examined histologically and liver tissue inducible nitric oxide sentase (iNOS) receptor range examined immunohistochemically. In histological examination; damage in the rat liver tissues and sentrolobuler necrozis caused by Acetaminophen markedly decreased in the liver tissues of Melatonin and Carnosine plus groups ($p<0.05$). In the only Acetaminophen given group, iNOS receptor range increased; as seen more stained immunohistochemically. In Acetaminophen plus Melatonin and Acetaminophen plus Carnosine groups, a little more staining were present when compared to the control group. The increased values of TOS and OSI parameters in the Acetaminophen group, significantly decreased in the Acetaminophen plus Carnosine and Acetaminophen plus Melatonin groups, especially in the Acetaminophen plus Carnosine group ($p<0.05$). We have concluded that; in preventing high doses of Acetaminophen's toxic effects in the rat liver tissues, melatonin and especially carnosine has powerful effects.

Poster N^{o} 64

Cellular telephone use and cancer risk

Ozlem Coskun[1], S. Comlekci[2]

[1]Department of Biomedical Device Technology, University of Suleyman Demirel University, Isparta, Turkey

[2]Department of Electronics and Communication Engineering, University of Suleyman Demirel University, Isparta, Turkey

The cellular phones and base stations are getting more into our lives. The effects of this fast development in technology have been creating a great concern and curiosity on public and science community. In the last two decades, many studies have been published in the media and in the scientific world on the effect of radiofrequency (RF) emissions distributed from cellular phones on cancer. There are contradictory results on this topic in the literature. The assessment of these studies from the biophysical point of view revealed that RF energy in general did not cause a significant biological effect. However, there are also reports suggesting a direct or indirect effect of thermal RF energy on cancer progression. Studies performed in humans mainly concentrated on brain cancer, leukemia and intraocular melanoma. Although no solid association was found between cellular phone use and cancer risk, there are some studies showing an increased risk in acoustic neuroma and glioma due to long term (≥ 10 years) cellular phone use. Animal studies could not provide consistent evidence that RF energy exposure at non-thermal intensities caused cancer. Therefore, planning of detailed research on this subject, definition and application of legal limitations, pursue of studies are vital for this issue. Additionally, it is important to inform public about the possible adverse effects of electromagnetic radiation and get them take the precautions about the use of the equipment generating radiation.

Poster N^{o} 65

Health effects of radiation exposure during pregnancy

Ozlem Coskun[1], S. Comlekci[2]

[1]Department of Biomedical Device Technology, University of Suleyman Demirel University, Isparta, Turkey

[2]Department of Electronics and Communication Engineering, University of Suleyman Demirel University, Isparta, Turkey

Imaging modalities that do not use ionizing radiation (ie, ultrasonography and magnetic resonance imaging) are preferred for diagnosis of maternal and fetal illnees during pregnancy. However, radiographic imaging procedures with ionizing radiation sometimes may be required to diagnose. Ionizing radiation can directly affect fetal development in several ways. Radiation dose and the time of exposure are critically important in the development of these effects. Radiation can cause fetal death or congenital anomalies, growth deficiencies or several structural and/or functional development abnormalities. The purpose of this study is to discuss the potential adverse effects of radiologic imaging modalities on pregnancy and fetus.

Poster N° 66

The protective effects of ghrelin on stomach tissue of neonatal diabetic rats

Ozlem Sacan[1], N. Türk[2], A. Karatug[3], S. Bolkent[3], R. Yanardag[1], S. Bolkent[2]

[1]Department of Chemistry, Faculty of Engineering, Istanbul University, Avcilar, Istanbul, Turkey

[2]Department of Medical Biology, Cerrahpasa Faculty of Medicine, Istanbul University Cerrahpasa, Istanbul, Turkey

[3]Department of Biology, Faculty of Sciences, Istanbul University, Vezneciler, Istanbul, Turkey

Diabetes mellitus is associated with an increased production of reactive oxygen species and a reduction in antioxidant defenses. Ghrelin is a novel 28 amino acid gastric peptide, which controls the acid release and is produced abundantly in the stomach. Effects of ghrelin treatment in diabetes are still unknown exactly. In the present study, we aimed to demonstrate the effect of ghrelin on stomach damage in streptozotocin (STZ)-induced diabetic rats. In this study, Wistar albino newborn rats were divided into four groups. Group I: Saline was administered intraperitonally (i.p.). Group II: Ghrelin dissolved in saline was given to newborn rats from day 3 after birth, 100 µg/kg daily, subcutaneously (s.c), during four weeks. Group III: Second day after birth, 100 mg/kg STZ was administered i.p. as a single dose to newborn diabetic rats (n2-STZ) group. Group IV: Diabetic animals were given ghrelin for four weeks. Stomach tissue was taken from animals, homogenized in 0.9% saline to make up to 10% homogenate. The homogenate was used for protein level and enzyme activities. Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GP_x) and glutathione reductase (GR) activities were determined. Stomach CAT, SOD, GP_x and GR activities were decreased in diabetic rats. Administration of ghrelin to diabetic rats increased stomach CAT, SOD, GP_x and GR activities. As a result, we can propose that ghrelin could be a potentially beneficial agent in reducing stomach damage in neonatal diabetic rats, probably by decreasing oxidative stress.

Poster N° 67

Effects of Curcumin and Dithioerythritol on Post-thawed Bull Sperm

N. Başpınar[1], Pınar Peker Akalın[2], M. N. Bucak[3], P. B. Tuncer[3], S. Sarıözkan[4], K. Coyan[5]

[1]Selcuk University, Faculty of Veterinary Medicine, Department of Biochemistry, Konya, Turkey

[2]Ministry of Agriculture and Rural Affairs, Central Veterinary Control and Research Institude, Ankara, Turkey

[3]Ministry of Agriculture and Rural Affairs, Lalahan Livestock Central Research Institute, Lalahan, Ankara, Turkey

[4]Erciyes University, Safiye Cikrikcioglu Vocational College, Kayseri, Turkey

[5] Selcuk University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Konya, Turkey

The aim of this study was to determine the effects of curcumin and dithioerythritol added into bull semen extender on sperm parameters, LPO (lipid peroxidation), total glutathione (total GSH) and antioxidant potential (AOP) levels of bull sperm following the freeze-thawing process. Nine ejaculates obtained from three bulls were included in this study. Each ejaculate which was splitted into seven equal groups and diluted in a Tris-based extender containing Curcumin (0,5 and 2 mM), dithioerythritol (0,5 and 2 mM) and no additive (control) was cooled to 5°C, and frozen in 0.25 ml French straws. Frozen straws were thawed individually at 37°C for 20 s in a water bath for evaluation.

The extender supplemented with 0,5 mM dose of curcumin led to lower percentage of total abnormality (20.40±2.36%), when compared to the control (30.60±1.47%, $p<0.05$). Curcumin and dithioerythritol at 0,5 mM provided a greater protective effect in the membrane functional integrity (54.40±2.09% and

50.00±2.68%), in comparison to control (37.20±1.77%, p<0.001). While curcumin and dithioerythritol at 0,5 mM led to higher percentages of post-thaw motilities, when compared to the control groups, these increases seemed to be insignificant. No significant differences were observed in sperm acrosome abnormalities among the groups (p>0.05). Supplementation with antioxidants did not significantly affect the LPO and AOP levels, compared to the control groups. The maintenance of total GSH level in curcumin 0.5 mM was demonstrated to be higher than that of control, following the freeze-thawing (p<0.05).

Poster N° 68

The Effect of Cysteine and Glutathione on Sperm Parameters, Malondialdehiyde and Glutathione Peroxidase Activities of Post-Thawed Bull Semen

Pürhan Barbaros Tuncer[1], M. N. Bucak[1], S.Sarıözkan[2], S. Büyükleblebici[1], H.Kinet [1], O. Büyükleblebici [3]

[1] Ministry of Agriculture and Rural Affairs, Lalahan Livestock Central Research Institute, Lalahan, Ankara, Turkey

[2] Erciyes University, Safiye Cikrikcioglu Vocational Collage, 38039, Kayseri, Turkey

[3]Ankara University, Faculty of Veterinary Medicine, Department of Biochemistry, Ankara, Turkey

The aim of this study was to determine the effects of antioxidants reduced glutathione (GSH) and cysteine in Laiciphose® extender on semen parameters, malondialdehyde (MDA) and glutathione peroxidase (GPx) of post-thawed bull semen. Five groups, namely; GSH (0.5 and 2 mM), cysteine (5 and 10 mM) and control group, were conducted to test the effects of antioxidants in Laiciphose®. Insemination doses were processed so that each 0.25-ml straw contained 15 x 10^6 sperm. The addition of antioxidants did not provide any significant effect on the percentages of post-thaw sperm morphology (acrosome and total abnormalities), subjective, computer assisted sperm motility analysis (CASA) and progressive motilities, as well as sperm motility characteristics (VAP, VSL, VCL, LIN and ALH), compared to the control groups (p>0.05). However, cysteine at 10 mM dose gave rise to a slight higher percentage of membrane integrity assessed by HOST than those of the other groups. With respect to fertility results based on 59-day non-returns, the supplementation of GSH at 2 mM gave a lower rate (p<0.05). For MDA level, cysteine at 10 mM dose gave the highest level (4.99±0.44 nmol/L) (p<0.001). GPx activity was demonstrated to be higher upon the addition of 5 mM cysteine, when compared to the other groups (p<0.05).

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Poster N° 69

Effects of dietary zeolite on serum contents and feeding performance in rats

Ramazan Demirel[1], B. Yokuş,[2] D. Şentürk Demirel,[1] M. A. Ketani,[3] M. S. Baran [4]

[1]Department of Animal Science, Feeds and Animal Nutrition, Faculty of

Agriculture, Dicle University, Diyarbakir, Turkey

[2]Department of Biochemistry, Faculty of Veterinary, Dicle University, Diyarbakir, Turkey

[3]Department of Histology and Embryology, Faculty of Veterinary, Dicle University, Diyarbakir, Turkey

[4]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary, Dicle University, Diyarbakir, Turkey

This study was conducted to determine the effects of dietary natural clinoptilolite on serum contents and feding performance. This research was arranged according to Randomized Plots Design by using 4 groups with 3 replicates. Adult male Sprague–Dawley rats (n=24) were randomly divided into 4 groups with three replicates, including a control group (without zeolite) and 3 doses of natural zeolite (2, 4 and 6 %) in the diets. All rats were fed the above concentrates during the experimental period for 56 days. Blood samples were collected from each animal at the end of the experiment. Dieatary clinoptilolite increased serum albumin, triglyceride and VLDL levels. However the differences among treatment groups were not significant for serum minerals (Ca, P, Mg, K, Na, Cl, Fe), urea, Fe binding, LDL, ALP, glucose, uric acid, total Fe, total protein, globulin, cholesterol, HDL cholesterol, creatinin; metabolizable energy and crude protein consumption for 1 g live weight gain of rats.

According to diets, serum parameters were ranged for urea, Fe binding, LDL, alkaline fosfatase, glucose, uric acid, total Fe, total protein, globulin, triglyceride, cholesterol, HDL cholesterol, albumin, VLDL, creatinine and Fe levels as follows (42.65 – 46.08, 314.17 – 325.50, 15.33 – 19.00, 662.90 – 816.44, 159.72 – 197.35, 1.56 – 1.73, 523.83 – 529.00, 6.53 – 6.82, 3.50 – 3.62, 40.78 – 58.40, 80.32 – 84.35, 54.44 – 57.32, 3.03 – 3.20, 8.16 – 11.67, 0.54 – 0.58 and 198.33 – 214.33), respectively. Average serum macro mineral contents were ranged between for Ca (10.28 – 10.70 mg/dl), K (5.56 – 6.25 mg/dl), Na (143.93 – 144.74 mg/dl), P (8.48 – 9.87 mg/dl), Mg (2.62 – 2.83 mg/dl) and for Cl (101.87 – 103.73mEq/L). Metabolizable energy and crude protein consumptions for 1 g live weight gain of rats were ranged between 59.91 - 69.34 (ME, kcal / LWG, g); 3.48 – 4.88 (CP, g / LWG, g), respectively.

The highest blood serum urea, Fe binding and LDL values were obtained from control group; alkalin fosfatase, Ca and Na from group II (2%); glucose, uric acid, total Fe, total protein, albumin, globulin, triglyceride, cholesterol, HDL cholesterol, VLDL, P, Mg, Cl and protein consumption from group III (4%); creatinin, K, Fe levels and energy consumption were obtained from group IV(6%). The results showed that the supplementation of clinoptilolite did not have positive effect on serum concentrations of investigated parameters, however at the same time did not effect negatively health status of animals.

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Poster N° 70

Investigation of the cellular mechanisms of actions of digoxin and monensin by using guinea-pig papillary muscles

Ramazan Üstün[1], İ. Meral[2]

[1]Department of Nurse, School of Healty, Karamanoğlu Mehmetbey University, Karaman, Turkey

[2]Department of Physiology, School of Medicine, Yuzuncu Yıl University, Van, Turkey

This experiment was carried out to determine the possible mechanism of actions of monensin and digoxin by using guinea-pig papillary muscles. A total of 120 papillary muscles obtained from 60 guinea-pigs were used. Three treatment groups; control (0,1% ethanol), monensin (10 µmol/l) and digoxin (3µ mol/l) were compared in normal, Na ($^{+}$) - free, Ca ($^{2+}$)- free and thapsigargin-added Krebs solutions. It was found that both digoxin and monensin caused apositive inotropic effect in normal and Ca ($^{2+}$) - free Krebs solutions. Although monensin produced a positive inotropic effect in thapsigargin-treated papillary muscles, digoxin did not show a positive inotropic effect. Because papillary muscles in Na ($^{+}$) - free Krebs solution showed spontaneous contractions, trusted results were not obtained in Na ($^{+}$) - free medium. It is concluded that monensin produces a positive inotropic effect by depending on both extra cellular Ca ($^{2+}$) and Ca ($^{2+}$) released from the sarcoplasmic reticulum (SR). Digoxin, on the other hand, produces its effect by depending on the Ca ($^{2+}$) released from SR.

Poster N^{o} 71

Combined effects of niacin and chromium on the heart tissue of hyperlipidemic rats

M. M. Doger[1], B. B. Sokmen[2], Refiye Yanardag[1]

[1]Istanbul University, Faculty of Engineering, Department of Chemistry, Istanbul, Turkey

[2]Giresun University, Faculty of Arts and Sciences, Department of Chemistry, Giresun, Turkey

Atherosclerotic vascular disease, including hyperlipidemia is associated with endothelial dysfunction. Therefore, it is important to improve endothelial dysfunction for treatment of atherosclerotic disease. In this study, female Swiss albino rats were used. They were divided into four groups. The animals of the first group (group I) were fed with pellet chow. The rats (group II) were fed with a lipogenic diet consisting of 2% cholesterol, 0.5% cholic acid and 20% sunflower oil added to the pellet chow, and given 3% alcoholic water for 60 days. The rats (group III) were fed with the same lipogenic diet and treated by gavage technique $CrCl_3$ $6H_2O$ to a dose at 250 µg/kg and 100 mg/kg niacin for 45 days, 15 days after experimental animals were done hyperlipidemic. Group IV was fed with pellet chow and treated with 250 µg/kg $CrCl_3$ $6H_2O$ and 100 mg/kg niacin for 45 days. On the 60th day, the heart tissue and blood samples were taken from animals. Heart lipid peroxidation levels (LPO), serum gama glutamyl transferase (GGT) activity and serum protein carbonyl levels were increased, serum paraoxonase (PON) activity and heart glutathione (GSH) levels were decreased in hyperlipidemic rats. Treatment with combined niacin and chromium reversed these effects. In conclusion, the present study revealed that the combined treatment with niacin and chromium to hyperlipidemic rats might induce a protective effect on heart GSH and LPO levels and serum PON, GGT activities and protein carbonyl levels.

Poster N^{o} 72

The effect of smoke and smokeless tobacco use as Maraş powder on erythrocyte nitric oxide level

N. Kurtul[1], Saniye Böyükkara[1], E. Gökpınar[1]

[1]University of Kahramanmaraş Sütçü İmam, Faculty of Science Department of Chemistry, Division of Biochemistry, Kahramanmaraş, Turkey

The use of smokeless tobacco (ST) is common worldwide. Also, in Türkiye, especially in Kahramanmaraş and other Southern cities, a kind of ST prepared from a tobacco of species *Nicotiana rustica* L. and known as Maraş powder (MP) has been consumed widely as alternative to smoking. The habit is becoming increasingly popular due to banning smoking in our country. The ST is causatively associated with an increased risk for cardiovascular diseases and cancers of the oral cavity, larynx, and pharynx. In this study, erythrocyte nitric oxide (*E*NO) concentrations were measured by Griess reaction in blood samples obtained from

smokers (Group I), MP users (MPU; Group II), and healthy control subjects (Group III) who were nonsmokers and nonusers of ST and the results were compared. The *E*NO concentrations were found to be lower in smokers and in MPU than that of control subjects and also in MPU than that of smokers. There was significant difference between MPU and control subjects. In Group II, important correlation was found between consumed daily MP amount and *E*NO levels. Also, there were positive correlations between the number of cigarettes and duration of smoke or MP use and *E*NO levels. Erythrocytes represent an important compartment for NO metabolism. As a free radical, NO mediates cellular toxicity and reacts with superoxide to form peroxynitrite which an even more potent oxidant. It was concluded that smoke and ST use possibly affected oxidant and antioxidant balance of erythrocytes and changes may be related with harmful effects of ST.

Poster N° 73

Effects of the exposed to biomass and cigarette smoke on antioxidant defense system, GST activity and GSH levels in rat liver

Serap Sahin Basak[1], Y. Silig[2], O. T. Dogan[3]

[1]Department of Chemistry, Faculty of Science and Art, [2] Department of Biochemistry, Faculty of Medicine, [3] Department of Respiratory Disease, Faculty of Medicine, University of Cumhuriyet, Sivas, Turkey

The harmful effects of tobacco smoke have been well-known for years. Moreover, these effects have been demonstrated by experimental trials. Free radicals and reactive oxygen species (ROS) are produced during cigarette smoking. In this study, we investigated effects on antioxidant defense system, GST and GSH levels in liver of rats exposed to biomass (dried dung) and cigarette smoke. Adult male Wistar-strain albino rats weighing about 300 g each and nourished under normal conditions were used in this study. Rats were divided into five groups each of which contains seven animals. Animals were exposed to biomass smoke (group 1), to cigarette smoke (group 2) and to both cigarette smoke and biomass together (group 3), for an hour daily for three months. Whereas the control group animals was not subjected to any application. It has been found out GSH level, the activities of GST and antioxidant enzymes in experimental groups were significantly elevated than control group. On the other hand, when compared to controls, MDA level in all groups were significantly increased (group 1:1.51-fold, group 2:1.36 fold, and group 3:1.41.fold). It has been determined that antioxidant defense system was induced in all groups associated to an increase in free radicals, however this induction could not protect the liver cells from the damage caused by free radical which was understate by detecting the increase in lipid peroxidation. On the other hand, it could be suggested that an increase GST enzyme activity could be the result of the induction of detoxification system.

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Poster N° 74

Influence of Various Antioxidants on Microscopic-Oxidative Stress Indicators and Fertilizing Ability of Frozen-Thawed Bull Semen

Serpil Sarıözkan[1], P. B. Tuncer[2], M. N. Bucak[2], P. A. Ulutaş[3]

[1]Erciyes University, Faculty of Medicine, Hakan Çetinsaya Experimental and Clinical Research Center, Kayseri, Turkey

[2]Ministry of Agriculture and Rural Affairs, Lalahan Livestock Central Research Institute, Lalahan, Ankara, Turkey

[3]Adnan Menderes University, Faculty of Veterinary Medicine, Department of Biochemistry, Aydın, Turkey

Cryopreservation is associated with the production of reactive oxygen substances (ROS), which lead to lipid peroxidation of sperm membranes, resulting in a loss of sperm

motility, viability and fertility. The aim of this study was to determine effects of the antioxidants of oxidized glutathione (GSSG), reduced glutathione (GSH) and bovine serum albumin (BSA) on standard semen indicators (motility, acrosome and total abnormalities, HOST), endogenous antioxidant enzyme activities and fertilizing ability of frozen-thawed bull semen. Eighteen ejaculates from each of 3 Holstein bulls were collected using an artificial vagina and 9 replicates of the ejaculates were diluted in Bioxcell extender supplemented with antioxidants, including BSA (5 mg/ml), GSH (2 mM), GSSG (2 mM), and an extender containing no antioxidants (control). Insemination doses (1.5 × 107 sperm/0.25 ml straw) were prepared for the insemination of cows at observed oestrus. Supplementation with antioxidants led to lower percentages of acrosome damage and total abnormalities, compared to the controls ($p< 0.01$). Pregnancy rate after insemination was highest (72.2%) in the group which was given BSA ($p< 0.05$). There were no significant differences among groups in GSH and glutathione peroxidase (GSH-PX) enzyme activities. Superoxide dismutase (SOD) activities in all of the experimental groups with antioxidants were lower than the control group ($p< 0.001$). Furthermore, BSA increased ($p< 0.001$) the activity of catalase (CAT), following the freezing-thawing process.

Poster N^o 75

The protective effects of N (1)-2,4-dihydroxybenzylidene-N (4)-2-hydroxybenzylidene-S-methyl-thiosemicarbazidato-oxovanadium (IV) on brain injury in streptozotocin-induced diabetic rats

Sevim Tunali[1], R. Yanardag[1], T. Bal-Demirci[2], B. Ülküseven[2]

[1]Istanbul University, Faculty of Engineering, Department of Chemistry, Biochemistry Division, Avcilar, Istanbul, Turkey

[2]Istanbul University, Faculty of Engineering, Department of Chemistry, Inorganic Division, Avcilar, Istanbul, Turkey

Diabetes is usually accompanied by increased production of free radicals or impared antioxidant defences. Oxidative damage contributes to biochemical abnormalities in brain tissue. In the present study, we aimed to demonstrate the effect of N (1)-2, 4 Dihydroxybenzylidene-N (4)-2-hydroxybenzylidene-S-methyl-thiosemicarbazidato-oxovanadium (IV) (VOL) on brain damage in male rats with streptozotocin (STZ)-induced diabetes. Male, 3-3.5 months old, Swiss albino rats were randomly divided into four groups. Experimental diabetes was induced by intraperitoneal injection of STZ in a single dose 65 mg/kg. Group I: control (intact) animals. Group II: control animals given VOL. Group III: STZ-induced diabetic animals. Group IV: STZ-induced diabetic rats treated with VOL. VOL of 0.2 mM/kg daily dose were administered by gavage technique to rats for 12 days, after the experimental animals were made diabetic. On day 12, the brain tissues were taken from animals. Brain tissues were homogenized in 0.9 % saline with a glass homogenizer to make up to 10 % homogenate (w/v). The homogenates were centrifuged and the clear supernatants were used for protein levels and enzyme activities. Brain xanthine oxidase, alanine transaminase, aspartate transaminase activities, nonenzymatic glycosylation and lipid peroxidation levels were increased in diabetic rats. Also brain catalase, acetylcholinesterase activities and glutathione levels were decreased in diabetic rats. Treatment with VOL reversed these effects. As a result it might be concluded that treatment with VOL has a protective effect on damage of brain of STZ-induced diabetic rats.

Poster N^o 76

The effect of deuterium depleted water consumption on blood and liver biochemical parameters in healthy rats

Sevinç Özgür[1], A. Yarat[1], T. Yardımcı[2]

[1]Department of Basic Medical Sciences, Biochemistry, Faculty of Dentistry, Marmara University, Istanbul, Turkey

[2]Department of Biochemistry, Faculty of Pharmacy, Marmara University, Istanbul, Turkey

Deuterium (D) is the only stable and non-radiating isotope of Hydrogen (H). D concentration varies in human fluids depending on deuterium contents of drinking water and diet. Also D/H ratio is not constant in tissues and body fluids. The effect of the replacement of H with D in biological systems is well documented, however, the possible role of naturally occurring D has not completely elucidated. Recent studies have shown that reduction of D concentration in drinking water diminishes the growth rate of the tumors and induces apoptosis (1-3). In this study, the effect of deuterium depleted water (DDW) (85 ppm) consumption on the blood and liver parameters were investigated in healthy rats. Creatinine kinase, creatinine, glucose, protein, bilirubin, urea, uric asid, total lipid, cholesterol, and triglyceride levels were determined in blood samples; lipid peroxidation (LPO) and glutathione levels, tissue factor activity (DFa), carbonic anhydrase, sodium-potassium ATPase, superoxide dismutase, catalase (CAT), glutathione-S-transferase and glutathione peroxidase enzyme activities were also measured in liver tissue. No significant differences on these parameters were found between either tap water or DDW consumption for 30 days. On the other hand, consumption of DDW for 40 days decreased protein levels and increased LPO, CAT and DFa in liver and increased creatinine kinase activity in blood significantly, compared to tap water drinking group. As a result, the application duration and probably the concentration of DDW consumption are important factors. It is necessary that more comprehensive research has to be done to enlighten the mechanism of this issue.

Poster N^{o} 77

Coupling of Ca^{2+} microdomains to spatially and temporally distinct cellular responses by the tyrosine kinase Syk

Siaw W. Ng [1], C. Nelson [1], A.B. Parekh[1]

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, Parks Road, Oxford OX1 3PT, United Kingdom

Communication between the cell surface and the nucleus is essential for regulated gene expression. In immune cells, excitation-transcription coupling is thought to involve global Ca^{2+} signals. The current study investigates whether excitation-transcription coupling can be driven by Ca^{2+} microdomains arising from open CRAC channels. A combination of Ca^{2+} imaging, whole cell patch clamp and reverse transcription-PCR experiments were used to address this in rat basophilic leukemia-1 cells, using thapsigargin as a stimulus. We find that in mast cells, Ca^{2+} microdomains from store-operated Ca^{2+} release-activated Ca^{2+} channels activate expression of the transcription factor c-fos. Local Ca^{2+} entry is sensed by the tyrosine kinase Syk. Syk clusters at the cell periphery in resting cells and is retained here after stimulation. Syk signals to the nucleus through the transcription factor STAT5 in a protein kinase C- and MEK/ERK-independent pathway. Ca^{2+} microdomains following CRAC channel activation also activate Ca^{2+}-dependent phospholipase A_2, followed by synthesis and secretion of cysteinyl leukotrienes. However, unlike c-fos expression, this is mediated via the MEK/ERK pathway. In conclusion, Syk therefore couples Ca^{2+} microdomains to the activation of two spatially and temporally distinct cellular responses, revealing the versatility of local Ca^{2+} signals in driving cell activation.

Poster N^{o} 78

Effects of short-term hyperglycemia on the contractile responses of rat aorta

Soner Dönmez[1], O. Gökalp[2], M. Doğan[3], H. Vural[4], B. Yiğit[4]

[1]Department of Chemistry, Biochemistry Division, School of Art and Science, University of Nevsehir, Nevsehir, Turkey

[2]Department of Pharmacology, School of Medicine, Dicle University, Diyarbakir, Turkey

[3]Department of Pharmacology, School of Medicine, Suleyman Demirel University, Isparta, Turkey

[4]Department of Biochemistry, School of Medicine, Suleyman Demirel University, Isparta, Turkey

There are a lot of studies on vasodilatation of the effect of hyperglycemia, but there is a poor knowledge of the effect of hyperglycemia on vascular contractile responses. Furthermore chronic exposure to high concentrations of has generally been studied. So the aim of this study was to investigate whether short-term hyperglycemia affected vascular contractility. Non-diabetic rat aorta rings were incubated in normal (11 mM) (NG) and high (22 mM and 44 mM) (HG) glucose ambient for four hours. Responses of vasoconstriction to serotonin (10^{-5} M), phenyleprinhe (10^{-6} M) and KCl (60 mM) compared to the ambient including different glucose concentration. In addition, MDA (malondialdehyde, a product of lipid peroxidation) levels of aorta tissue was assessed by high-performance liquid chromatography in different glucose concentration (NG and HG).Responses of vasoconstriction to KCl were increased in the presence of Krebs' solution with high glucose (p<0,05). Although there are slightly increasement of respones observed with serotonin, no statistically significant changes of responses to phenyleprinhe and serotonin. Besides, MDA levels increased significantly during the hyperglycemia (p<0,05).Short-term hyperglycemia may lead to augment contractile response in aort rings through several mechanisms and our results showed that oxidative stress is most probably one of them.

Poster Nº 79

T-type calcium currents and intracellular calcium concentration are modulated by PKA in mice Leydig cells

R.R.Costa, R.I. Reis, J.F. Aguiar and Wamberto A. Varanda

Department of Physiology, School of Medicine of Ribeirão Preto/USP Brazil

Luteinizing hormone (LH) is known to activate both adenyl cyclase and phospholipase C and also to increase intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in Leydig cells. Here we investigated the effects of modulators of PKA and PKC on T-type Ca^{2+} currents and on the overall $[Ca^{2+}]_i$ transients induced by LH. Treatment of the cells with 1 µg/ml LH increased the amplitude of the peak current at -20 mV, from -2.4 ± 0.7 to -3.8 ± 0.8 pA/pF (n = 22, p = 0.0381) and 1 µM PMA increased the current to -3.6 ± 0.4 pA/pF, (n = 6, p = 0.00033). Only minor changes were observed in the voltage dependence of activation, inactivation or deactivation of the currents. Inhibition of both PKC and PKA with 400 nM staurosporine blocks the $[Ca^{2+}]_i$ changes induced by LH (n = 37 cells). A similar effect was seen with 10 µM H89, a specific inhibitor of PKA. Although PMA by itself slowly increased the fluorescence, the subsequent addition of 1 µg/ml LH still triggers the typical transients in $[Ca^{2+}]_i$. Chelerythrine, a PKC inhibitor, also does not avoid the Ca^{2+} transients.
Taken together our results show that PKA plays a leading role in mediating the LH induced changes in $[Ca^{2+}]_i$, a necessary step in the process of testosterone production and secretion by Leydig cells.

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Poster Nº 80

The effect of nicotine consumption & deprivation on damage of multi-organ failure caused by sepsis: Role of Neutrophils

Zarife Nigâr Özdemir[1], D. Özbeyli[1], A. F. Özdemir[1], M. B. Karaaslan[1], K. Kaytaz[1], M. F. Kara[1], O. E. Tok[2], F. Ercan[2], B. Ç. Yeğen[1]

[1]Marmara University School of Medicine, Department of Physiology, Istanbul, Turkey

[2]Marmara University School of Medicine, Department of Histology and Embryology, Istanbul, Turkey

Sepsis is a severe clinical condition causing multi-organ failure. Nicotine decreases the severity of many diseases by inhibiting pro-inflammatory cytokines. In a sepsis model, we aimed to show the protective effects of nicotine on two target organs. Male Wistar albino rats

received tap water with (50mg/ml) or without nicotine for 14 days prior to surgical procedures. Under ketamine anesthesia, sepsis (n=45) was induced by ligation and punction of the cecum, while sham group (n=8) had only laparotomy. In a subgroup of rats, nicotine drink was withdrawn for 5 days before sepsis induction, while in the acute nicotine group rats were administered a single injection of nicotine (30mg/kg, ip) before sepsis, but had no oral intake. Rats were decapitated 24 hours after surgery to obtain lung, liver and kidney tissues to determine malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activities. Data were analyzed by ANOVA and Tukey multiple comparison tests. Sepsis resulted in increased pulmonary and hepatic MPO activities as compared to control group ($p<0.001$), but in the chronic nicotine group this increase was abolished ($p<0.001$). However, in the withdrawal and single-dose nicotine groups pulmonary and hepatic MPO activities were not different than the sepsis group. Histologically observed damages were ameliorated by all nicotine treatments at varying degrees, but MDA and GSH levels were not different among experimental groups. The findings of the present study raise the possibility that long-term nicotine consumption reduces sepsis-induced organ damage, which appears to involve the inhibition of neutrophil recruitment to the inflamed tissues.

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Poster N^{o} 81

Evaluation of buccal mucosa epithelium in type 1 diabetic patients by an exfoliative cytology method and clinical findigs

Zehra Safi Oz [1], F. Battal [2], H. Atmaca [3], B.Ermis [2]

[1] Zonguldak Karaelmas University, School of Medicine, Department of Medical Biology

[2] Zonguldak Karaelmas University, School of Medicine, Department of Pediatrics

[3] Ondokuz Mayıs University, School of Medicine, Department of Endocrinology, Samsun, Turkey

Diabetes mellitus is one of the most common endocrine metabolic disorders. It is basically characterized by chronic hyperglycemia, associated with disturbances in the metabolism of carbohydrates, lipids, and proteins. Further, diabetes damages tissue repair processes and causes stomatologic problems of the dental interest. We therefore decided to evaluate cellular changes in buccal epithelial cells using an exfoliative cytology method and clinical findings in type 1 diabetes. All patients were asked to fill in the questionnaire focusing on detailed history and symptoms. In 30 patients with type 1 diabetes (age mean 15,5 ± 7,9) and 30 control individuals (age mean 15,3 ±6.5), buccal smears were obtained and stained with Papanicolaou technique. Cellular changes were evaluated in each slide by light microscope. All analyses were performed SPSS software version 13 for windows. The chi-square and Fisher's exact tests were used for the statistical analysis. The difference between of type 1 diabetes and binucleation, nuclear membrane irregularity, and also clinical data such as sensitivity of buccal mucosa and xerostomia was statistically significant ($p<0.05$). Type 1 diabetes can cause cellular changes in the buccal epithelium that are noticeable with this exfoliative cytology method. Exfoliative cytology is useful as an additional tool to aid in the diagnosis of the diabetes mellitus.

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Poster Nº 82

Effects of propolis on tyrosine hydroxylase activity and blood pressure in nitric oxide synthase inhibited hypertensive rats

A. Gogebakan[1], Zeliha Selamoglu Talas[1], I. Ozdemir[2], E. Sahna[3]

[1]Department of Biology, Faculty of Arts and Science, Nigde University, Nigde, Turkey

[2]Department of Chemistry, Faculty of Arts and Sciences, Inonu University, Malatya, Turkey

[3]Department of Pharmacology, Faculty of Medicine, Firat University, Elazig, Turkey

Reduction in the synthesis or bioavailability of nitric oxide plays a significant role in the development of hypertension. Propolis is a resinous product collected by honeybees from various plant sources. Tyrosine hydroxylase (TH) is the rate limiting enzyme in the biosynthesis of the catecholamines. The aim of this study was to examine the effect of propolis on blood pressure (BP), TH and total RNA levels in the adrenal medulla, heart and hypothalamus tissues in chronic nitric oxide synthase (NOS) inhibited rats by *N*G-nitro-L-arginine methyl ester (L-NAME). Rats received NOS inhibitor (L-NAME, 40 mg/kg, intraperitoneally) for 15 days to produce hypertension and propolis (200mg/kg, by gavage) the last 5 days. TH activity and total RNA levels significantly increased in adrenal medulla, heart and hypothalamus tissues in L-NAME-treated groups ($P<0.05$). TH activity and total RNA levels of L-NAME + propolis-treated rats significantly reduced ($P<0.05$) as compared with L-NAME-treated groups. TH activity in propolis-treated rats was reduced to the control group values. L-NAME led to a significant increase in BP as compared with control group. Propolis administration to L-NAME treated rats reduced BP but this was not statistically significant. These results suggest that propolis decrease TH activity and thereby may modulate the synthesis of catecholamine and BP.

Poster Nº 83

Changes in tyrosine hydroxylase activity and adrenomedullin level by treatment of organoselenium compounds in rat hypothalamus exposed to 7,12-dimethylbenzanthracene

I. Ozdemir[1], Zeliha Selamoglu Talas[2], Y. Gok[1], B. Ates[1], I. Yilmaz[1]

[1]Department of Chemistry, Faculty of Arts and Sciences, Inonu University, Malatya, Turkey

[2]Department of Biology, Faculty of Arts and Science, Nigde University, Nigde, Turkey

The effects of synthetic organoselenium compounds (Se I and Se II) on the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis as well as adrenomedullin (ADM) level were determined in the hypothalamus of adult rats exposed to DMBA (7,12-dimethylbenz[a]anthracene). DMBA, an organic environmental pollutant, is a polycyclic aromatic hydrocarbon that can induce a range of toxic effects and stress in rats. Selenium is an essential trace element, which interacts with antioxidants, and has anticancer and antihypertensive properties. TH is an aromatic amino acid hydroxylase whose activity is elevated in response to a range of stress inducers. TH activity is normally regulated by negative feedback in catecholamine biosynthesis. ADM is an abundantly present peptide in a broad range of normal tissues including adrenal medulla, lungs, kidneys and brain. Plasma ADM levels are elevated in a number of diseases including essential hyptertension and chronic renal failure. The antioxidant properties of ADM offer protection against organ damage induced by high blood pressure, ischemia and aging. DMBA treatment increased the TH activity and ADM level in the hypothalamus. These increases were found to be inhibited by Se I and Se II treatments.These studies demonstrate that synthetic organoselenium compounds can suppress DMBA-induced

stress related changes in the rat hypothalamus. Therefore, the antioxidant and antihypertensive effects of Se I and Se II may have important effects in the maintainance of homeostasis.

Poster Nº 84

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], S.Uğur[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.

Poster Nº 85

Determination of GST activity with CDNB of *Orthrias angorae*

Cem Ozic[1, 2], S.Gül[2]

[1, 2] Department of Biology, University of Anadolu, Eskisehir, Turkey
[2] Department of Biology, University of Kafkas, Kars, Turkey

Glutathione S-transferases, GSTs (EC 2.5.1.18) are a family of diverse multifunctional proteins mainly involved in detoxification of xenobiotics and antioxidant defense .GSTs have been found in virtually all plants, vertebrates, insects, yeasts, and bacteria. GSTs detoxify herbicides, organic pollutants, and naturaltoxins, in addition to less characterized role in oxidative stress tolerance. In most of the organisms, GST expression is modulated in response to exposure to prooxidant xenobiotics. Overall, it is believed that the induction of GSTs is an evolutionary conserved response of the organisms towards exposure to prooxidants. Under certain conditions, the interaction between glutathione and CDNB is totally dependent on the presence of active GST. The GST-catalyzed formation of GS-DNB produces a dinitrophenyl thioether which can be detected by spectrophotometer at 340 nm. One unit of GST activity is defined as the amount of enzyme producing 1 µmol of GS-DNB conjugate/min under the conditions of the assay. In brief, the incubation mixture of GST toward CDNB activity assay consisted of 0.1M phosphate buffer, 1 mM of GSH and 1 mM of CDNB solution. After preincubation at assay temperature (25 °C), the reaction was initiated by the addition of 10 µL of liver subcellular fraction. GST activity (toward CDNB) (µmol/mg protein/min) in homogenate was found to be 0.71±0.04. CDNB in GST activity was determined be used Orthrias angorae. **GST** activity with 1-chloro-2,4-dinitrobenzene (**CDNB**) was determined by the method of Habig et al.

Poster Nº 86

Oxidative and antioxidative parameters in the eyes of rats exposed to extremely low frequency magnetic fields

Mehmet Zulkuf Akdag[1], S. Dasdag[1], H. Cakir [2], O. Erel[3]

[1]Department of Biophysics, Medical Faculty, Dicle University, Diyarbakir, Turkey

[2]Department of Biochemistry, Medical Faculty, Harran University, Urfa, Turkey

[3] Department of Biochemistry, Ataturk Education Hospital, Ankara, Turkey

Most of the scientific concerns arise from several studies, which suggest possible interaction mechanisms between extremely low frequency magnetic fields (ELF-MFs) and living organisms. However, the molecular mechanism through which ELF-MFs can influence cellular behavior is still unclear. On the other hand, it has been suggested that the interaction between living organisms and ELF-MFs could involve interferences with reactive oxygen species (ROS) and free radical production and half-life extensions. Therefore, the present study aimed to investigate the effect of ELF-MF on oxidative and antioxidative parameters in rat eyes. In this study, 30 male Sprague–Dawley adult male rats were used. Rats in two experimental groups were exposed to 100 and 500 μT ELF-MF, which are the safety standards of public and occupational exposure for 2 h/day for 10 months. Same procedure applied to the rats in sham group except ELF-MF exposure. The levels of catalase (CAT), malondialdehyde (MDA), myeloperoxidase (MPO), total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were measured in rat eye. The decrease of CAT levels in exposure groups were found significant compared to sham group ($p<0.05$).However, no significant alterations were found in other endpoints such as MDA, MPO, TAC, TOS and OSI levels. In conclusion, CAT levels were affected by long-term exposure of both 100 and 500 μT ELF-MF in rats' eye. However, further and more researches need to be performed to explain interaction mechanisms between ELF-MF exposure and eyes.

Poster N^{o} 87

Colchicine modulates oxidative stress and Ca^{2+} release in polymorph nuclear leucocytes of remission (but not unremission) of patients with familial Mediterranean fever

H. Demirkan[1], Mehmet Şahin[1], A. C. Uğuz[2], İ.S. Övey[2], M. Nazıroğlu[2], E. Tunç[1]

[1]Department of Internal Medicine, Division of Rheumatology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

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

It has been suggested that there is an abnormality in the oxidative stress of chronic inflammatory patient with familial Mediterranean fever (FMF) (1). A pharmacological characterization of Ca^{2+} influx pathways in polymorph nuclear leucocytes (PMN) is problematic because of lack of specific inhibitors. It was reported that colchicine induced antioxidant and cation channel blocker effects in heart cells (2, 3). We aimed to investigate effects of colchicine on oxidative stress and Ca^{2+} release in plasma and polymorph nuclear leucocytes of attack, remission and unremission patients with family Mediterranean fever. Eighteen FMF patients and six age-matched health control within four groups were used in the study. First group used was control. Second group was patients with active FMF. Third and fourth groups were patients with patients with remission and remission, respectively. Colcichine (1,5 mg/day) was given to consisting second, third and fourth groups for one month. Lipid peroxidation (LP) and cytosolic Ca^{2+} release levels in PMN of FMF patients within attack and unremission periods were higher than in control although their levels were higher in FMF attack patients than in remission group. Vitamin E and □-carotene levels were higher in patients with remission period than in control and patients with attack period. LP levels were also higher in serum of patients with attack period than in control although serum LP levels were lower in patients with remission period than in patients with attack period. However, PMN and serum LP levels and Ca^{2+} release concentrations were further increased in

patients with unremission period. In conclusion we observed that colcichine induced protective effects on oxidative stress by modulating vitamin E, β–carotene and Ca^{2+} release levels in FMF patients with remission period. Colcichine did not affect on the values in patients with unremission period.

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Poster N° 88

Ameliorating effects of the caffeic acid phenethyl ester on diazinon poisoning

E. Oğuzhanoğlu[1], Osman Gökalp[2], L. Yavuz[1], C. A. Andaç[2]

[1]Medical School, Department of Anesthesiology and Reanimation, Suleyman Demirel University Isparta, Turkey

[2]Medical School, Department of Pharmacology, Dicle University, Diyarbakır, Turkey

The caffeic acid phenethyl ester (CAPE) is a compound structurely similar to the flavonoids and is found in the bee gums produced by honeybees. CAPE possesses antiinflammatory, antioxidant and immune regulatory effects. We aimed to investigate whether CAPE has an ameliorating effect on the diazinon poisoning on rats. Rats were divided into 4 groups. The control (the first) group of the rats were not treated with any substance. The second group of the rats were orally given 200 µg/kg subletal doze of diazinon. The third group of the rats were given interperitoneally (ip) 10 µmol/kg of CAPE one day before 200 µg/kg of diazinon was applied orally. The fourth group of the rats were ip injected 10 µmol/kg of CAPE half an hour after 200 µg/kg of diazinon was orally administered. Blood samples were obtained from the aorta of the rats under anesthesia at 48th hour. After analysis of the blood samples, it was found both in the third and fourth groups of the rats that CAPE diminished the inhibiting effect of diazinon on the butyrylcholine esterase enzyme by half. The mechanism of the action of the ameliorating effect of CAPE was further computationally investigated by Autodock_Vina.[2] Three binding conformations of CAPE with binding energies ΔG^o1= -8.2 kcal/mol, ΔG^o2= -7.9 kcal/mol and ΔG^o3= -7.2 kcal/mol, respectively, and one binding conformation of diazinon with a binding energy of ΔG^o= -6.7 kcal/mol were determined in the crystal structure of the active site of butyryl choline esterase (PDB ID: 2WID).[1] Therefore, it was concluded that CAPE binds to butyryl choline esterase with roughly 2-10 times greater affinity than that of diazinon.

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Poster N° 89

The effects of extracts obtained from *Nepeta Italica L.* species on the antioxidant enzymes

Sercan Özbek[1], F. Özdemir[2], Ş. Konak[1], İ. Özmen[b], T. İ. Büyükoğlu[1], H. Genç[4]

[1] Health Vocation Training School, Mehmet Akif Ersoy University, Burdur, Turkey

[2] Faculty of Arts and Sciences, Süleyman Demirel University, Isparta, Turkey

[3] Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey

[4] Faculty of Education, Mehmet Akif Ersoy University, Burdur, Turkey

We aimed to examine the effects on the antioxidant enzymes of the extracts obtained from the N*epeta italica L.*.

The *Nepeta italica* extracts were obtained by using ethanol and hexane solutions and administered orally to rats. The rats were separated into 3 groups (Group I; normal saline, Group II; ethanol, group III; hexane). The extracts were administered single dose (1.0 g/kg). The blood samples were taken 3 hours later. In all groups the superoxide dismutase (SOD), catalase (CAT), glucose-6-phosphate dehydrogenase (G6PD) activities and the malondialdehyde (MDA) levels were determined.

SOD and CAT activities decreased and G6PD activity increased in the group treated hexane extract ($p<0.05$). SOD and CAT activities increased while there is not a significant difference at the G6PD activity in the group treated Ethanol extract. MDA levels were not observed in all groups administered extract ($p>0.05$).It is known that the organisms are adapted to the high free radical levels by increasing the antioxidant enzyme activities (Stephensen et al..2002). However, it is suggested that the decrease at the SOD and CAT enzymes is caused by the accumulation of free superoxide radical and the H_2O_2 by inhibiting the protein synthesis (Husain et al..2001; Santhakumarı et al, 2003).It was reported that the extract administration caused increasing at the SOD and CAT enzyme activities and was suggested that it was related to the increased antioxidant state (Karuna et al..2009). In our study, MDA levels were not alter for all groups. This situation shows the bad effects of free radicals may be eliminated without causing any cell damage.

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Poster N^{o} 90

The effects of the extracts obtained from the *Nepeta Cilicia Boiss* on the antioxidant enzymes

Sercan Özbek[1], Fevziye Özdemir[2], İsmail Özmen[2], Şevkinaz Konak[1], Tülay İleri Büyükoğlu[3], Hasan Özçelik[2]

[1] Health Vocation Training School, Mehmet Akif Ersoy University, Burdur, Turkey

[2] Faculty of Arts and Sciences, Süleyman Demirel University, Isparta, Turkey

[3] Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey

It was to determine the effects on some antioxidant enzymes of the ethanol and hexane extracts obtained from the *Nepeta cilicia*.The *N. cilicia* extacts obtained with ethanol and hexane organic solvents were given to the rats. Rats were separated into 3

groups of 8 animals each. The extracts were given orally and single dose (1,0 g/kg). The blood samples were taken from at 3 h after the administration. Some antioxidant enzyme activities and MDA levels were determined.The ethanol extract caused an important decrease ($p<0.05$). The hexane extract increased the glucose-6-phosphate dehydrogenase (G6PD) activity, but not significantly ($p>0,05$). Catalase (CAT) activities increased in the group given ethanol extracts ($p<0.05$). It was not observed any change at superoxide dismutase (SOD) activities and MDA levels in all groups. NADPHs produced by G6PD are necessary for nucleotides in the formation of reduced glutathione in erythrocytes. In our study, G6PD activity was increased insignificant in group given hexane extract, this situation might be explained by the high value of reduced glutathione against the oxidative stress. G6PD enzyme activities decreased in the group given ethanol extract. It is reported that the decrease of G6PD activity shows a decrease at NADPH production (Özmen et al., 2005). Also the increase at CAT activity is related to the antioxidant activity (Kocabaş, 2008). It is known the MDA level is one of the most sensitive indicators of lipid peroxidation also oxidative stress (Knight, 1988), therefore our findings show that the bad effects of free radicals may be decreased.

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Poster N° 91

Effect of memantine on brain injury and oedema after cerebral ischemia

Siğnem Eyuboğlu[1], G. Büyükgüçlü[1], M. Uğur[1], T. Gençol[1], E. Piranen[1], Ü. Kılıç[2], B. Yılmaz[1], E. Kılıç[1]

[1] Department of Physiology, Faculty of Medicine, University of Yeditepe, Istanbul, Turkey

[2] Department of Medical Biology, Faculty of Medicine, University of Yeditepe, Istanbul, Turkey

In vitro data suggested that rt-PA (recombinant tissue plasminogen activator) may aggravate neuronal injury by enhancing N-methyl- D-aspartate (NMDA) receptor signalling, possibly due to cleavage of its NR1 receptor subunit. It was subsequently shown that rt-PA treatment also potentiated NMDA toxicity in vivo when NMDA was directly applied into the brain. Based on these data, it was hypothesized that increased NMDA receptor transmission may be responsible for the rt-PA induced aggravation of injury after stroke (Kilic et al., 2001; 2005). We hypothesized therapeutically delivered t-PA might increase ischemic injury, which may be reversed by an NMDA antagonist memantine.

In this study, male C57/BL6 mice were used. The animals were divided into 4 groups, submitted to 90 mins of focal cerebral ischemia and 24 hours reperfusion. The animals were treated with (1) vehicle, (2) rt-PA (10 mg/kg, IV), (3) memantine (20 mg/kg, IP) and (4) memantine add on to rt-PA, just after stroke onset. Twenty-four hours after ischemia, brain injury was evaluated.rt-PA delivered immediately after reperfusion onset increased infarct volume and brain oedema at 24 hours after focalcerebral ischemia. Memantine did not decrease infarct volume significantly, when administrated alone. In addition, memantine did not reverse rt-PA induced brain injury.

We provide evidence that rt-PA increases brain injury. However, the NMDA antagonist memantine does not reverse rt-PA induced

toxicity, indicating memantine is not attractive as an add-on treatment with thrombolytics.

Poster Nº 92

The effect of long-term 900 MHz RF exposure on Rat Eyes in Terms of Oxidative and Antioxidative parameters

Suleyman Dasdag*, M.Z. Akdag*, O. Erel**

*Department of Biophysics, Medical Faculty, Dicle University, Diyarbakir, Turkey

** Department of Biochemistry, Ataturk Education Hospital, Ankara, Turkey

In the last decade, public opinion has been focused on hazards of radiofrequency (RF) radiation emitted from mobile phones. Recently, most of studies, which are investigate the effects of mobile phone exposure concentrated to the oxidative process in the cells. Although many of studies performed on effects on mobile phone exposure on brain, the studies on the effects of mobile phone exposure on eyes relatively are limited. Whereas, the eyes also one of the most important organs exposed to radiofrequency radiation emitted from mobile phones because of the position of them in the head. However, the studies on the effects of mobile phones recently focused on the oxidative process in cells. Therefore, the aim of this study was to investigate the effect of 900 MHz radiofrequency radiation on the oxidative parameters in the eyes of rats. The study was carried out on 19 Wistar Albino adult male rats. The rat heads in a carousel exposed to 900MHz radiofrequency radiation emitted from a generator, which simulate mobile phones. For the study group (n: 12), rats exposed to the radiation 2 h per day (7 days in a week) for 10 months. For the sham group (n: 7), rats were placed into the carousel and the same procedure was applied except that the generator was turned off. The levels of catalase (CAT), malondialdehyde (MDA), myeloperoxidase (MPO), total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were measured in rat eye. TAC, TOS and OSI levels were found higher in the eyes of rats exposed to 900 MHz radiofrequency radiation ($p<0.05$). In conclusion 900 MHz radiofrequency radiation emitted from mobile phone can induce oxidative process in the eyes of rats. More performance is necessary.

Poster Nº 93

N-acetylcysteine modulates doxorubicin-induced liver Damage in Rats

M. Cem Koçkar[1], M. Nazıroğlu[2], O.Celik[2], H. T. Tola[3], D. Bayram[4], A.Koyu[5]

[1]Department of Internal Medicine, Division of Gastroenterology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

[2]Department of Biophysics, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

[3]Department of Pediatrics, Şanlıurfa State Hospital, Şanlıurfa, Turkey

[4]Department of Histology and Embryology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

[5]Department of Physiology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

Doxorubicin (DOX) is a chemotherapeutic agent, which is widely used in cancer treatment. The most common side effect of DOX is on cardiovascular system. There are some studies suggesting oxidative stress-induced toxic changes on liver related to DOX administration. Various methods have been tried to prevent DOX-induced the toxic effects. In this study, we aimed to investigate whether

N-acetylcysteine (NAC), a fatty acid co-factor, has a protective effect on hepatic tissue against a possible DOX-induced damage. Twenty-four male Wistar albino rats weighing between 200-250 grams were included in this study. All animals were divided into three equal groups. First group was used as control. Second group received single dose of intraperitoneal DOX (2.5 mg/kg BW). Intraperitonel NAC (10 mg/kg BW) for 10 days was given to consisting third group after giving one dose of DOX. After ten days, liver tissues removed to study antioxidant values and histologically examined.Lipid peroxidation levels were higher in DOX group than in control whereas lipid peroxidation levels were lower in NAC group than in control. Vitamin C and E levels were lower in DOX group than in control whereas vitamin C and E levels were higher in NAC group than in DOX group. Glutathione peroxidase, reduced glutathione, vitamin A, □-carotene values did not change by DOX and NAC administrations. In liver tissue samples of DOX group, there were mononuclear cell infiltrations, vacuolar degeneration, hepatocytes with basophilic nucleus and sinusoidal dilatations. The histological findings were decreased by NAC administration.In conclusion, DOX caused increase in lipid peroxidation levels of liver in rat although antioxidant values decreased. The changes were recovered by NAC administration.

Poster N° 94

A study about the effect of pomegranate extract on the serum paraoxanase activity of cigarette smokers

Mehmet Berköz[1], N. Ö. Şahin[2], D. Battal[3], Ö. Şahin[1], A. Yüksel[1]

Mersin University, Faculty of Pharmacy, [1]Department of Pharmaceutical Technology, [2]Department of Pharmaceutical Biotechnology, [3]Department of Toxicology, Yenisehir Campus, Mersin 33169, Turkey.

Paraoxanase enzyme (PON1) with antiatherogenic activity exhibits in high density lipoproteins (HDL) and plays a major role in protecting lipoproteins from oxidation. Activity of PON1 enzyme is regulated by genetic and environmental factors. Cigarette consumption is one of the important reasons reducing PON1 enzyme levels. It's reported in literature that use of antioxidants can change PON1 enzyme levels. The research on pomegranate juice and extract showed that they have significant antioxidant activity. Although there are some nutraceuticals bearing pomegranate extract in the market, biochemical effect and pharmacological activity of them on the antioxidant levels of smokers has not been investigated in detail. With this in mind, the goal of this study was set to determine whether pomegranate extract can change serum PON1 enzyme levels of cigarette smokers or not. 29 subjects (15 cigarette smoker and 14 nonsmoker subjects) were included to the study. Both groups were asked to take pomegranate extract bearing capsules once a day for fifteen days. Blood samples were collected from all subjects in the beginning and the end of the study. Serum PON1 and arylesterase (ARE) activities were investigated. All the data were statistically analyzed using SPSS v10.0 program and Independent t-test. PON1 and ARE activities of smokers were found to be higher in smokers ($p<0,05$). Furthermore, pomegranate consumption increased serum PON1 and ARE activities ($p<0,05$). In conclusion, pomegranate extract show antioxidant effect on both cigarette smokers and non-smokers. It leads to an increase in PON1 and ARE activities. Thus, pomegranate may be used for profilaxy against cardiovascular diseases

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atherosclerotic mice and in humans. Drugs Exp Clin Res. 2002; 28:49-62.

Poster Nº 95

Effects of apple cider vinegar on blood lipid profiles, liver lipid peroxidation and antioxidant levels in streptozotocin induced-diabetic and cholesterol-fed mice

Osman Kanatsız[1], Z. Yozgat[1], Ö. Ören[1], İ.S. Övey[2], E. Sözbir[2], C. Özgül[2], M. Küçükayaz[2], B. Çig[2], B. Kale Köroğlu[3], M. Nazıroğlu[2]

[1]Student of Medical Faculty, Suleyman Demirel University, Isparta, Turkey

[2]Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

[3]Department of Internal Medicine, Division of Endocrinology, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

Apple is relatively poor in lipid-soluble protectants such as vitamin E and carotenes although it contains vitamin C and phenolic antioxidant compounds (1). Apple could also reduce atherogenecity of lipoproteins by protecting these from oxidative stress. Atherosclerosis and lipid profile anormalities are main problem in diabetes (2). Apple vinegar could be beneficial on blood lipid profiles, liver lipid peroxidation and antioxidant levels in streptozotocin (STZ)-induced-diabetic mice. We aimed to investigate effects of apple vinegar on blood lipid profiles, liver lipid peroxidation and antioxidant levels in STZ-induced diabetic mice. Forty female mice weighing between 36-40 grams were included in this study. All animals were divided into four equal groups. First group was used as control. Second group received single dose of intraperitoneal STZ (45 mg/kg BW) (3). Apple vinegar (0.6% of feed) was intragastrically giving to consisting third group mice for 28 days. Apple vinegar for 28 days was intragastrically given to consisting fourth group after giving one dose of STZ. All groups except control group were fed cholesterol rich ration (5% cholesterol). After 28 days, serum and liver tissues removed to study lipid profile and antioxidant values, respectively. Liver lipid peroxidation, total cholesterol and triglycerides levels were higher in diabetes group than in control whereas their levels were lower in apple vinegar and apple vinegar+diabetes groups than in control. Liver vitamin E and β-carotene levels were lower in diabetes group than in control and liver vitamin E and β-carotene levels were further decreased in apple vinegar and apple vinegar+diabetes. Glutathione peroxidase, reduced glutathione, vitamin A and C values did not change by diabetes and apple vinegar. In conclusion, apple vinegar caused decrease in serum total cholesterol, triglycerides, and liver lipid peroxidation, vitamin E and β-carotene levels in diabetic and hypercholesterol fed mice.

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Poster Nº 96

Apple cider vinegar reduces blood lipid profiles, liver lipid peroxidation and vitamin E and β-carotene levels in ovariectomized and streptozotocin-induced diabetic mice fed cholesterol

Mustafa Güler[1], G. Saydam[1], İ. S. Övey[2], C.Özgül[2], M. Küçükayaz[2], E. Sözbir[2], B.Çig[2], M. O. Özkaya[3], M. Nazıroğlu[2]

[1]Student of Medical Faculty, Suleyman Demirel University, Isparta, Turkey

[2]Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

[3]Department of Obstetrics and Gynecology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Apple is relatively poor in lipid-soluble protectants such as vitamin E and carotenes although it contains vitamin C and phenolic antioxidant compounds (1). Apple could also reduce atherogenecity of lipoproteins by protecting these from oxidative stress. Atherosclerosis and lipid profile abnormalities are main problem in postmenopausal period due to estrogen deficiency as well as diabetes (2). Apple vinegar could be beneficial on blood lipid profiles, liver lipid peroxidation and antioxidant levels in ovariectomized (OVX) and streptozotocin (STZ)-induced-diabetic mice. We aimed to investigate effects of apple vinegar on blood lipid profiles, liver lipid peroxidation and antioxidant levels in OVX and STZ- induced diabetic mice. Sixty female mice weighing between 36-40 grams were included in this study. All animals were divided into six equal groups. First group was used as control. Second group was used OVX. Apple vinegar (0.6% of feed) was intragastrically giving to consisting third group mice for 28 days. Fourth group received single dose of intraperitoneal STZ (45 mg/kg BW) (3) after OVX. Apple vinegar for 28 days was intragastrically given to consisting fifth group after OVX Apple vinegar for 28 days was intragastrically given to consisting sixth group after giving one dose of STZ and OVX. All groups except control group were fed cholesterol rich ration (5% cholesterol). After 28 days, serum and liver tissues removed to study lipid profile and antioxidant values, respectively. Liver lipid peroxidation, total cholesterol and triglycerides levels were higher in OVX nad OVX+diabetes group than in control whereas their levels were lower in apple vinegar and apple vinegar+OVX+diabetes groups than in control. Liver vitamin E and β-carotene levels were lower in OVX+diabetes group than in control and liver vitamin E and β-carotene levels were further decreased in apple vinegar and apple vinegar+OVX+diabetes.Glutathione peroxidase, reduced glutathione, vitamin A and C values did not change by OVX, diabetes and apple vinegar. In conclusion, apple vinegar caused decrease in serum total cholesterol, triglycerides, and liver lipid peroxidation, vitamin E and β-carotene levels in OVX+diabetic and hypercholesterol fed mice.

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