MSD
MEDICAL SCIENCE & DISCOVERY
ISSN: 2148-6832
Publisher LuLu Press, USA


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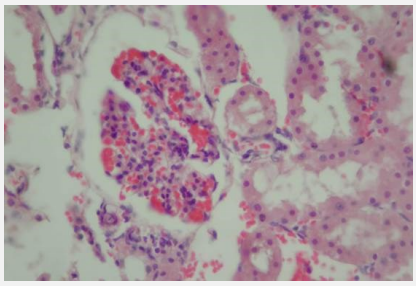

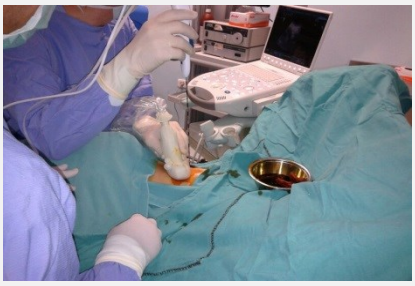

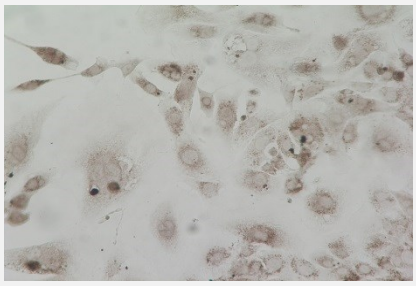

High Lights

RFA Surgery for Small Renal Masses
Thymus Vulgaris and Diabetes Mellitus related nephropathy
Where are we in molecular profiling in breast cancer diagnosis
Electromagnetic Fields and Colon cancer
Congenital Afibrinogemia

International Journal of
Medical Science and Discovery
Open Access Scientific Journal
Jun 2014, Vol.1, No.1,
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Founder: **Independent**

Publisher: **Lulu Press, USA**

Country of Publication: **Turkey**

Sponsor: **TUTKU SAĞLIK GEREÇLERİ TİCARET A.Ş http://www.tutkusaglik.com/**

Adress: Celal Bayar University, Faculty of Medicine Dept. of Biophysics, Uncubozkoy 45080 Manisa-Turkey

Tel: +90-236-2011000/213

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Med Sci Discovery Jun 2014, Vol.1, No.1

ISSN: 2148-6832

MSD Medical Science and Discovery
Jun 2014, Vol. 1, No. 1, p:1

Letter to Editor Doi: 10.17546/msd.84213

Radiofrequency ablation Treatment in Small Renal Masses

Ayhan Karakose[1], Mehmet Bilgehan Yuksel[2]

Sir,

In the recent years, diagnosis of small renal tumors has increased as a result of the development of technology and imaging technique. Number of patients with renal cell carcinoma has been increasing by 2-3% every year [1]. Radiofrequency ablation therapy in the treatment of small renal tumors has been an alternative to other methods of treatment due to high radiological and oncological efficacy [2]. Radiofrequency ablation therapy is applied with success in the treatment of liver, kidney, lung, bone and breast tumors [3]. Radiofrequency ablation therapy can be performed to treat small renal cell carcinoma by both percutaneous and laparoscopic approaches. This modality has some considerable advantages, such as decreased morbidity rate, short recovery time and obtaining reliable oncological outcomes in the long term follow-up [4, 5].

In conclusion, although surgical management is still accepted as the gold-standard treatment for small renal cell carcinomas, Radiofrequency ablation therapy has become a safe and effective treatment alternative especially for T1 stage renal cell carcinomas. Recent progresses in imaging modalities and technological and technical developments have resulted in similar oncological and functional outcomes of Radiofrequency ablation therapy comparable with the surgical treatment of small renal cell carcinomas. Radiofrequency ablation therapy is an increasingly popular ablative treatment modality, which presents some advantages of lower surgical and anesthetically complication rates, shorter recovery time and hospital stay, no renal ischemia, and a curative, nephron sparing treatment choice to the patients who are not appropriate candidate for the surgery or do not prefer the surgical treatment [6].

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Received: 25 Jun 2014**, Revised** 26 Jun 2014**, Accepted** 28 Jun 2014**, Available Online** 30 Jun 2014
[1]Izmir University, Department of Urology, Izmir, Turkey,
[2]Celal Bayar University, Department of Urology, Manisa, Turkey,
*Corresponding Author: Assist. Prof. Dr. Ayhan Karakose drayhankarakose@gmail.com

ISSN: 2148-6832

MSD
Medical Science and Discovery
Jun 2014, Vol. 1, No. 1, p:2-8

Review Article
Doi: 10.17546/msd.70695

Each patient's cancer is like fingerprint: where are we in molecular profiling in breast cancer diagnosis?

Burcak Karaca[1], Harika Atmaca[2], Emir Bozkurt[2], Ruchan Uslu[1]

Abstract

Breast cancer is the leading cause of cancer related deaths in women. Since every patient has unique molecular profile of tumorigenesis, efficient treatment choice should be tailored according to each patient's gene expression profile. Therefore, it is critical to investigate molecular patterns of each patient before choosing the right treatment. For this aim, many commercial breast cancer subtyping methods are available. This review focuses on new developments and methods in molecular profiling of breast cancer.

Keywords: Molecular profiling, breast cancer, tailored therapy

Introduction

Breast cancer is the leading cause of cancer related deaths in women [1]. Traditionally, a thorough evaluation of a breast cancer patient includes determination the dissemination of disease and the assessment of the tumor size, axillary lymph nodes status, histological type, nuclear/histological grade, status of hormone receptor [(estrogen receptor (ER); progesterone receptor (PR)], and Her-2/neu receptors [2-4]. However, tumors with identical histopathology may progress differently, respond to therapy differently, and may result in different disease outcomes. Thus, a new pathological sub-classifications and new molecular diagnostic techniques have been sought in recent years.

By some recent studies it is now well understood that the underlying biological behavior of a tumor reflected in its gene expression is a powerful illustration to define pivotal oncogenic pathways.

Recent trials focusing on gene expression profiling of the tumor indicate that a metastatic risk of a patient is hidden in the gene expression pattern derived from the primary tumor. It should be noted that the metastatic tumor can also be genetically different from the primary tumor, thus a genetic diagnosis of metastatic lesion as well as the primary tumor should also be determined for tailored therapy.

DNA microarray platforms for profiling gene expression in tumors were invented very recently, and breast cancer is the earliest and most intensely studied disease using this technology. The molecular signatures provide diagnostic tools as well as prognostic and predictive gene signatures, and may identify new therapeutic targets. Prospective trials are now underway to determine the value of such tools for clinical decision making in breast cancer.

Received: 25 Jun 2014**, Revised** 26 Jun 2014**, Accepted** 28 Jun 2014**, Available Online** 30 jun 2014
[1]Ege University, School of Medicine, Division of Medical Oncology, Tulay Aktas Oncology Hospital , Bornova, Izmir
[2]Celal Bayar University, Faculty of Science and Arts, Department of Biology, Section of Molecular Biology, Muradiye, Manisa, Turkey
*Corresponding Author: Burcak KARACA burcakkaraca@hotmail.com

Classification of breast tumors based on molecular profiling studies

Breast cancer is a morphologically and genetically heterogeneous disease. Hence, response to treatment as well as the prognosis of two patients with the same stage of breast cancer can be very divergent.

In the very last years, molecular profiling has let us to understand the genotypic characterization of breast cancer and also potentially to discover new molecular biomarkers among cancers with similar histological appearance.

Perou et al. divided the breast cancers into four groups differentiated by expression patterns in several groups of genes; basal-like, Her-2/neu +, normal-breast like, and luminal A and B types [5].

Based on molecular findings, ER status is the most evident classification of breast tumors, since ER status of a tumor has a remarkable impact on the genes expressed by the tumor [5-7]. While the ER positive subtypes included Luminal A and B, the ER negative subtypes included the Her-2/neu+, having expression of Her-2/neu-related genes and basal like subtype with very low expression of Her-2 related genes, but high expression of a group of genes characteristic of normal basal epithelial breast tissue. Luminal subtypes of breast cancer express increased levels of cytokeratin 8 and 18 in addition to those genes associated with ER expression while basal like subtypes of breast cancer express increased levels of cytokeratin 5 and 17 and low levels of ER and genes, whose expression is linked to ER [5, 8, 9]. Similar sub-classification of breast cancer tumors into Luminal and basal like types using different analyses have been done by different investigations [10-12].

From these studies, it is possible to say that all of the luminal groups of breast cancers are ER positive and nearly two thirds of them are of low or intermediate histologic grade, whereas 95% of basal-like cancers are ER negative and most of these tumors are high grade [13]. Although, most of the (80-90%) triple negative tumors (ER, PR and Her-2/neu negative) similar to the basal like genotype, they are heterogeneous and can be divided into multiple additional subgroups [14, 15]. The basal-like tumors have no ER and Her-2/neu expression and feature more frequent overexpression of basal cytokeratins, epidermal growth factor receptor and c-kit [14]. Unlike Luminal B tumors, Luminal A tumors have the highest ER expression level as well as high expression levels of GATA-binding protein 3, X-box binding protein 1, trefoil factor 3, hepatocyte nuclear factor 3, and LIV-1 [8].

Germline mutations in BRCA1 and BRCA2 genes, which account for most of the hereditary breast cancers, have been shown to be effective on the genes expressed by tumors [7, 16]. Microarray studies have also been used to classify subgroups of these familial breast cancers, which account for 8-10 % of all breast cancer cases [17]. Tumors with BRCA1 and BRCA2 mutations, each display characteristic gene expression profiles. While most of the BRCA1 tumors are basal-like, BRCA2 tumors make up a more heterogeneous group [9, 18, 19].

Currently available microarray tools

In general, microarray technologies based on the manipulation and interpretation of cDNA arrays generated by converting mRNAs isolated from a variety of tissue types to cDNAs, which are then fixed to a solid substrate that allows quantization of these cDNAs by the degree of fluorescence of each probe is quantitated, and represents the abundance of that specific gene transcript, enumerated as either a ratio to a reference sample or as an absolute intensity value. Currently, many commercially available prognostic breast cancer tests based on gene expression technology are available. There are also research based pathway or disease focused

microarray products which haven't been validated on patients are available.

a) Amsterdam 70 gene MammaPrint assay was the first microarray based multigene assay for breast cancer, which was identified by van't Veer and colleagues [7]. This assay includes 70-genes which are mainly focused on proliferation, genes associated with invasion, metastasis, stromal integrity and angiogenesis. The selection of the most optimal gene set to be included in the assay was performed by comparing the gene expression profile of two distinct patient populations that correlated with clinical outcome [20]. This test is currently designed as a pure prognostic assay and is offered as a prognostic test for women under the age of 60 with either ER-positive or ER-negative, lymph node–negative breast cancer and now available as a commercial laboratory test called MammaPrint (Agendia BV, Amsterdam, The Netherlands). The MammaPrint assay is at its best when identifying cases at the extremes of the spectrum of disease to identify of patients with a very good or very poor prognosis. It has not yet been studied if the assay can also predict sensitivity to various treatment modalities. It is important to note that MammaPrint® is also the first FDA-approved in vitro diagnostic assay for patients with node-negative breast cancers [21].

b) The 21 gene Recurrence Score (Oncotype DX™) is a multiplex prognostic and predictive RT-PCR assay which distinguishes good from bad prognosis following adjuvant tamoxifen for patients, using an analysis of the expression of 21 known genes. These genes are mainly associated with proliferation, HER2 and ER signaling. The original 16 cancer related genes with five reference genes that calculate the recurrence score (RS) were discovered on archived paraffin embedded samples by transcriptional profiling and then converted to RT-PCR assay. Oncotype DX determines the 10-year risk for disease recurrence in patients with ER-positive, lymph node–negative tumors using a continuous variable algorithm and assigning a tripartite RS (≤17, low risk; 18–30, intermediate risk; >30, high risk) [22].

These two tests mentioned above are the most popular ones that have been used for molecular diagnosis of breast cancer. The other available tests are as follows:

c) The H/I™ (Also known as two-gene expression ratio) is a multiplex RT-PCR-based on the ratio of the relative mRNA expression of the homeobox gene-B13 (HOXB13) and the interleukin-17B receptor gene (IL17BR) to predict recurrence in patients with ER-positive, lymph node–negative primary breast cancer. This test requires formalin –fixed and paraffin-embedded tissue for RT-PCR assay [23].

d) Celera Metastasis Score™ prognostic 14-gene multiplex RT-PCR-based assay is also indicated for ER-positive, lymph node–negative tumors treated with tamoxifen. The Metastasis Score™ for breast cancer predicted a 3.5-fold difference in risk between the 20% of women at the highest risk and the 20% of women at the lowest risk for disease recurrence [24].

e) The Rotterdam 76-gene signature was developed to identify the patients with lymph node negative breast cancer that would benefit from adjuvant therapy, independently of the hormone receptor status [25, 26]. This test has mainly consist of proliferation genes and no genes in common with either oncotype DX™ or MammaPrint™, and run on the Affymetrix U-133 GeneChip™ System (Affymetrix, Inc., Santa Clara, CA). It requires fresh/frozen extracted mRNA and, similar to MammaPrint™, has not been validated for use on paraffin embedded tissues or core biopsies.

f) The invasiveness gene signature (IGS) is a prognostic assay which consists of 186 genes related to tumor stem cells that also use the Affymetrix U-133 GeneChip™ System. This assay is used for both node-

negative and node-positive and both ER-negative and ER-positive patients [27].

g) Breast BioClassifier is a qRT-PCR assay which can identify the different subtypes of breast cancer (luminal-A, luminal-B, Her-2, and basal-like) as a prognostic risk assessment tool [28]. The assay consists of 50 classifier genes and five house-keeping genes are measuring simultaneously, using a 384-well format in the LightCycler 480 system. The Breast BioClassifier can be used for different molecular subtypes of ER-negative/positive breast cancer, and determines the patients may benefit from personalized chemotherapy.

The Human Breast Cancer and Estrogen Receptor Signaling RT² Profiler™ PCR Array (SAbiosciences, Frederick, MD, USA) analyses gene expression profiles of 84 genes associated with breast cancer regulation and prognosis, estrogen receptor-dependent signal transduction and response of cancer cells to chemotherapy. Pathway focused DNA microarrays are commercially available. However, these assays have not yet been validated for breast cancer patients. This assay has been studied at our research lab since December, 2008, and we have some preliminary results of Turkish breast cancer patients' genetic signature.

Table 1: mRNA expression levels of genes from Turkish breast cancer patients. Significant changes in mRNA levels of some genes related to breast cancer and estrogen receptor signaling pathway that are grouped according to molecular subtypes in 12 Turkish breast cancer patients (Preliminary findings from Ege University Oncology Research Lab)

Gene	Significant Fold Changes		
	Luminal B (n=7)	Her-2 (+) /ER(-) (n=4)	Basal Like (n=1)
Estrogen Receptor 1 (ESR1)	+11,808	-7,359	-11,437
Keratin 18 (KRT18)	+5,273	+3,011	-8,789
Keratin 19 (KRT19)	+48,946	+7,730	-5,030
Mucin 1 (muc1)	+42,316	+3,581	-5,189
Topoisomerase (DNA) II Alpha (TOP2A)	+12,236	+11,757	-3,483

h) Prediction Analysis of Microarray (PAM50) was designed to determine a risk of recurrence (ROR) score for patients with breast cancer. This test measures the expression of 50 genes to identify the subtypes of breast cancer. It requires formalin-fixed paraffin-embedded tissues for RT PCR method. PAM50 test also provides quantitative determination for proliferation, luminal gene expression, ESR1, PGR, and ERBB2 [29].

Experience of Ege University Medical Oncology research lab on molecular profiling of breast cancer

One of the latest developments of gene expression profiling is a pathway focused PCR Array system which uses SYBR Green-based real-time PCR technology. The fresh tissue samples were obtained from 12 breast cancer patients operated in General Surgery Department at Ege University. Normal and tumor tissues (24 samples) were taken from the same patient during the operation. Samples were stored in RNA stabilizing solution. RNA was isolated by using RNA purification kit (SABioscience, USA). The Human Breast Cancer and Estrogen Receptor Signaling RT² Profiler™ (84 gene) PCR Array (SABioscience, USA) was used to identify differentially expressed mRNA profiles. The conventional clinicopathological data of the patients were compared with the molecular findings.

According to our preliminary findings, seven of 12 patients were Luminal B subtype, 4 of 12 patients were Her2(+)/ER(-) subtype and one patient was

basal like subtype. mRNA levels of cyto keratin 18 (KRT18), cytokeratin 19 (KRT19), mucine 1 (MUC1) and topoisomerase 2 (TOP2) genes were up regulated in luminal B and Her-2/neu (+)/ER(-) subtypes, however, those genes were down regulated in basal like subtypes. As expected, mRNA levels of estrogen 1 (ESR1) gene was down regulated in both Her-2/neu (+)/ER(-) and basal like subtypes, whereas it was up regulated in luminal B subtypes. A strong correlation was observed between conventional pathological data and pathway related mRNA expression profiles of patients (Table 1).

Conclusion

It is found that breast cancer patients belonging to different subclasses had significantly different outcomes from a survival analysis and prognostic factors based on clinical and histopathological variables [8]. Thus, it is needed to identify more accurate prognostic indicators [8, 9, 22, and 26]. ER protein expression status, histological grade, lymph node status, HER-2/neu gene amplification, p53 mutation status, inflammatory breast cancer, and carcinoma-derived stromal signatures have been defined with molecular profiling studies [2-4, 6-8, 30-32].

The advantage of molecular profiling in cancer is providing of individualized treatment for each patient with different stages of the disease and thus, gaining maximal therapeutic benefit from chemotherapy with minimal toxicity. Thereby, it is possible to deliver the appropriate drug to the right patient, and decreasing the use of other unnecessary drugs. Future investigations in oncology are focused on the individualizing cytotoxic therapy, although similar studies for endocrine and biologic therapy are also going on.

Conflict of Interest

The authors declared that they had no conflicts of interest.

Acknowledgements

We thank Ruchan Uslu, M.D., who funded this research.

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ISSN: 2148-6832

MSD

Medical Science and Discovery
Jun 2014, Vol. 1, No. 1, p:9-15

Original Article
Doi: 10.17546/msd.91847

Examination of P300 in Veteran Males: Aging, Physical Activity and Cognitive Processing

Necip Kutlu[1], Gurbuz Buyukyazi[2], Gulten Kaptan[3], Deniz Selcuki[4], Rengin Artug[4]

Abstract

Aim: In the recent years, the effects of long term exercise on physiological systems have been thoroughly investigated. The aim of this study was to investigate the effect of long-term physical exercise on cognitive processing in elderly male athletes.

Materials and Methods: Master athletes who had been exercising regularly (EME), healthy sedentary elderly volunteers (HSE) and healthy sedentary middle-aged volunteers (HSMA) were included in the study. Indirect maximum oxygen uptake capacity (VO2max) was determined by Astrand Test. Cognitive function (P300) was recorded at the same time of the day and in the same setting in all subjects

Results: Mean latency of P300 was 320.50±20.4 ms in EME, 344.70±24.48 ms in the HSE and 303.20±33.79 ms in the HSMA group. Mean amplitudes of P300 were 11.03 ±7.60 mV, 10.39±5.48 mV and 23.90 ±9.56mV in the EME, HSE and HSMA groups, respectively. Mean indirect maximum oxygen uptake capacity was 32.18±5.7, 18.07±5.0 and 15.8±5.0 ml/min/kg in the HSE, EME and HSMA groups, respectively

Conclusion: Results of this study indicate that long-term regular exercise affected cognitive functions positively. Even though the difference in P300 latency and amplitude between EME and HSMA groups was not statistically significant, that between HSE and HSMA was. We conclude that long-term exercise slows down the age-related decline in physical and cognitive performance

Keywords: P300, Aerobic exercise, Aging, Male

Intruduction

Several studies have suggested that long-term exercise may have facilitating effects on general cognitive functions. Physical training
has been proposed to attenuate age-related decline in cognitive performance [1-5]. Numerous studies investigating age-related changes in performance on some cognitive tasks showed that subjects who engaged in high, as opposed to low-physical exercise performed better [5, 6, 7], although significant differences were not always observed [8]. Exercisers at all ages are superior to sedentary individuals on tests of various types of cognitive functions, especially those cognitive functions that require faster information processing. Although, findings on the effects of aerobic fitness on mental functioning are widely more contradictory for young than for old people, a positive influence of aerobic fitness also has been reported in young men [9, 10]. Similar to physical training, acute exercise is assumed to have facilitating effects on mental functioning, but previous studies are not conclusive because of the lack of consistency in the method of testing chosen. Effects of acute exercise on cognitive performance are classically explained by the activation of

Received: 16 Jun 2014**, Revised** 24 Jun 2014**, Accepted** 24 Jun 2014**, Available Online** 30 Jun 2014
1Celal Bayar University, Medical School, Department of Physiology, Manisa, Turkey
2Celal Bayar University, School of Physical Education, Department of Sport, Manisa, Turkey
3Arel University, School of Nursing, Department of Medical Nursing, Istanbul, Turkey
4Celal Bayar University, Faculty of Medicine, Department of Neurology, Manisa, Turkey
*Corresponding Author: Necip Kutlu, nkutlu@cbu.edu.tr

the central nervous system via exercise-induced physical arousal leading to narrowing of attentional focus [11]. However, relations between exercise and arousal are yet to be clarified [12]. Moreover, the terminology of arousal and related states, as well as the uni-dimensionality of the relationship between arousal and performance is disputed [13]. To better understand the effects of chronic exercise on brain functioning, electrophysiological studies have been performed. P300 and mental reaction time are considered as valuable tools for the measurement of cognitive function because they are thought to reflect neural activity underlying basic aspects of cognition. The use of P300 as a clinical evaluation tool should be revisited with contemporary theory, methods, and analysis procedures because a reliable neuroelectric measure of mental function would redefine the assessment of cognitive disorders [14, 15]. P300 latency is sensitive to neural changes in development and aging. Meta-analysis of P300 in normative aging studies by Polich suggested that P300 latency can provide useful information about cognitive aging, and that relative proportions of male and female subjects across samples could readily affect P300 latency aging correlations. During childhood and adolescence, P300 latency is inversely related to age, perhaps reflecting processes such as myelination and cognitive development [16].

Numerous studies have investigated the effects of aerobic fitness and exercise on electroencephalographic activity, which can be considered an index of cortical arousal. There is evidence that physical training may modify electrophysiological data [9, 17]. Similarly, Polich and Kok demonstrated a positive effect of physical training on P300 [16]. Dustman reported that fit elderly men showed shorter P300 latencies compared to unfit elderly men [9]. Polich and Lardon found that P300 amplitude was higher in individuals who performed high amount of exercise [18]

The P300 component and mental reaction times are considered to reflect basic cognitive processes [14]. There is evidence that aerobic physical activities which improve cardiorespiratory fitness are beneficial for cognitive function in healthy older adults, with effects observed for motor function, cognitive speed, auditory and visual attention [19]. Physical activity appears to attenuate the decline of cognitive functions typically observed in older men. The P300 component of the event-related potential (ERP) is particularly affected by aging and allows for basic neurobiological assessment of cognitive functions [20].

The aim of this study was to investigate the effect of long-term physical exercise on cognitive processing P300 event- related potential in elderly male athletes.

Table 1: Patient demographics and aerobic capacity of the subjects (mean ± SD).* p< 0.05, 2-tailed value-student t test, n=10.

Groups	Age (year)	Height (cm)	Weight (kg)	VO_{2Max} (min/l/kg)
HSMA	35±12	168±5	68±5	15.08±5
EME	67±6	167±8	71±4	18.07±5
HSE	65±5	169±5	81±11*	32.18±5.7*

Material and Methods

A total of 30 subjects were included in the study and they were allocated into one of three groups: Elderly veteran athletes who exercised regularly (Group EME, n=10), elderly veteran athletes who led a sedentary life (Group HSE, n=10) and middle aged subjects who lived sedentarily (Group HSMA, n=10). P300 was recorded at the same time of the day and in the same setting. Neurological, physical and mental examinations were carried out in each subject. All subjects were reviewed for history of migraine, psychological disorder, diabetes, hypertension, epilepsy, cancer and alcohol and smoking habits. Amplitude (size) and latency (timing) of P300 event-related potential (ERP) were measured. The study was approved by the Ethics Committee of the Celal Bayar

University Hospital, and all subjects provided written informed consents.

Table 2: P300 amplitudes and latencies of the groups (mean ± SD). * p< 0.05, 2-tailed value-student t test.

Groups	P300 Amplitude(mV)	P300 Latency(ms)
HSMA (n=10)	23.9±9.6	303.2±33.8
ME (n=10)	11.0±7.6	320.5±20.4
SE (n=10)	10.4±5.5*	344.7±24.5*

Electrodes were arranged in five columns, each with seven antero-posterior sites (Fig.1). Amplitude (μV) is defined as the voltage difference between a pre-stimulus baseline and the largest positive-going peak of the ERP waveform within a latency window (e.g., 250-400 ms, although the range can vary depending on stimulus modality, subject age, task conditions, etc.). Latency (ms) is defined as the time from stimulus onset to the point of maximum positive amplitude within the latency window. In addition, P300 scalp distribution is defined as the change in component amplitude across the midline recording sites (Fz, Cz, Pz), which typically increases in magnitude from the frontal to parietal electrodes. Scalp distribution effects are of considerable importance, since variation in amplitude from the manipulation of task or subject variables has been used to infer information about P300 neural generators. This task has been used to study a wide variety of information processing issues [21, 22]. A strand test is given to these groups but before the test, groups were instructed not to exercise heavily. The VO2max measurement was conducted on a calibrated bicycle ergometer (Morark 860,

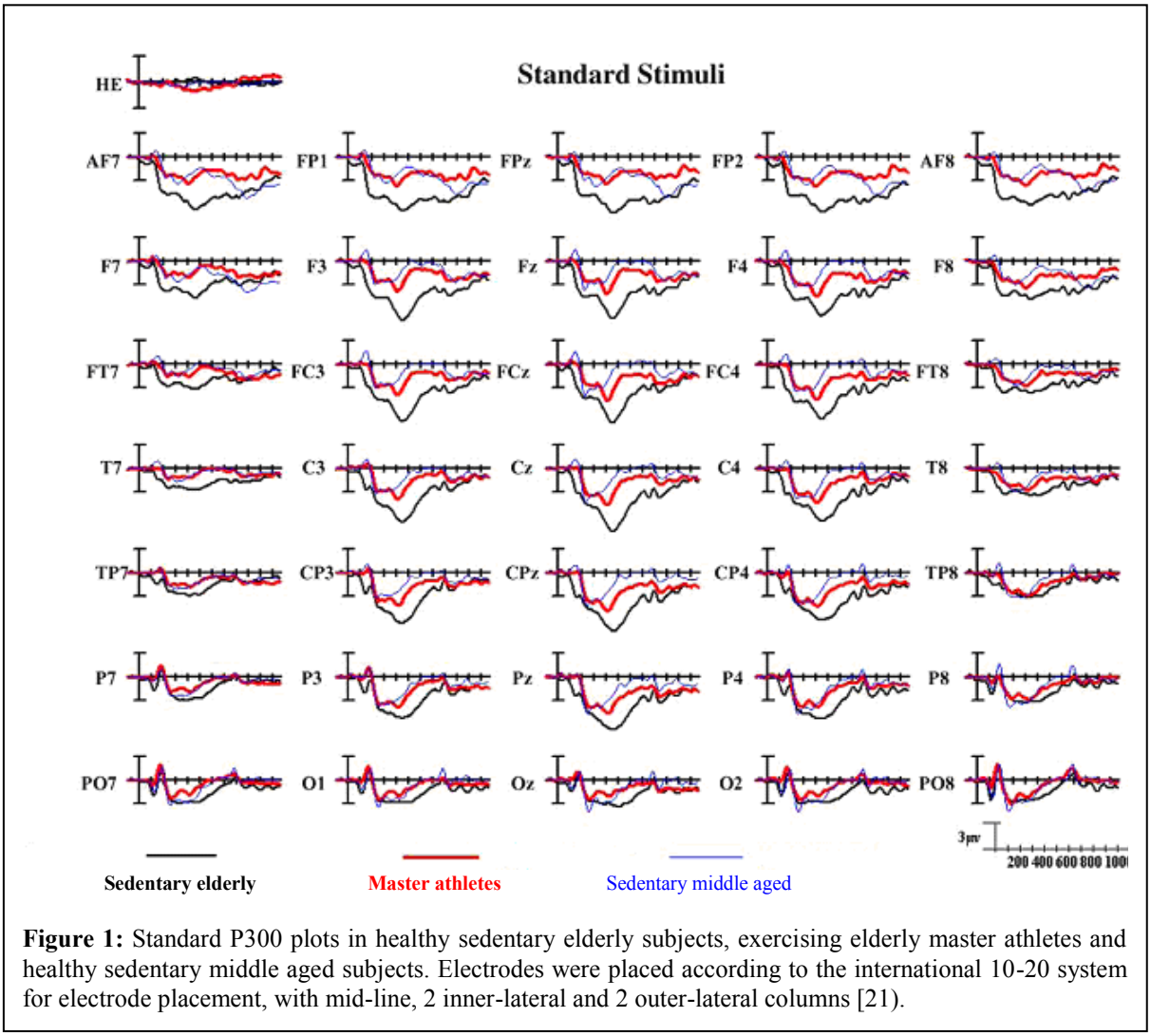

Figure 1: Standard P300 plots in healthy sedentary elderly subjects, exercising elderly master athletes and healthy sedentary middle aged subjects. Electrodes were placed according to the international 10-20 system for electrode placement, with mid-line, 2 inner-lateral and 2 outer-lateral columns [21).

Varberg, Sweden) using Astrand protocol [23].

Statistical methods

Kruskall Wallis and Mann-Whitney U tests were employed for the statistical analysis of the data, using SPSS v11.0 statistical software.

Results

Table 1 shows the P300 amplitudes and P300 latencies in the EME, HSE and HSMA groups. The statistical analyses revealed that the mean amplitude and latency of P300 of the HSE group were significantly different from the EME and HSMA groups.

Figure 1 illustrates standard P300 plots in EME, HSE and HSMA. Mean latency of P300 was 303.20±33.79 ms in the HSMA, 320.50±33.79 ms in the EME and 344.70±24.48 ms in the HSE group (see Figure 2).

There was a significant difference in mean P300 latency between the HSE and HSMA groups ($p<0.05$), while the difference between EME and HSMA was not statistically significant ($p>0.05$).

Mean amplitude of P300 was 23.90 ±9.56mV, 11.03 ±7.60 mV and 10.39±5.48 mV in the HSMA, EME and HSE groups, respectively (Figure 3). The difference in the amplitude of P300 between the HSE and HSMA groups was significant ($p<0.05$).

Table 2 shows the patient demographics and aerobic capacities of the subjects in three groups. Mean age, height and weight of the subjects in the HSE were 65±5 years, 169±5 cm, and 81±11 kg, respectively. Mean age, height and weight of the subjects in the EME group were 67±6 years, 167±8 cm and 71±4 kg, respectively while those in the HSMA group were 35±12 years, 168±5 cm and 68±5 kg, respectively.

Mean VO2max in the EME and HSE groups were 18.8±5.0 ml/min/kg and 32.18±5.7 ml/min/kg, respectively, whereas it was found to be 15.8±5.0 ml/min/kg in the HSMA group. Statistical analyses indicated that the VO2max in the HSE group was significantly higher than the EME and HSMA groups ($p<0.05$):

Conclusion

The results of the present study suggest that long-term exercise affects the P300 event-related potential, which is an indicator of cognitive functions. Even though the effects of long-term exercise on

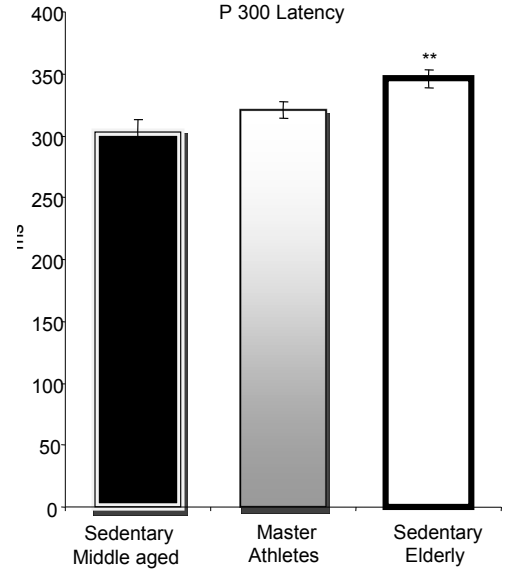

Figure 3: P300 latency in healthy sedentary elderly subjects, exercising elderly master athletes and healthy sedentary middle aged subjects

the body as a whole are well established, those on the brain need to be elucidated. We can infer information on the effects of cognitive functions by the latency and amplitude of P300 event-related potential (ERP). The theoretical interpretation of the P300 is based on: (1) neurophysiological investigations of the brain mechanisms that underlie its generation, (2) evidence from experimental studies that manipulate psychological variables, and (3) biological influences on central nervous system (CNS) function. After consideration of methodological issues, the major findings concerning P300 neural origins and psychological theory are summarized, with

biological determinants reviewed subsequently [24, 25, 26]. Two main findings emerged in the present study. First, there were significant differences in P300 parameters between HSE and HSMA groups; whereas the differences between EME and HSMA were not. Second, there was an inverse relation between ERP amplitude and latency though the difference between the EME and HSMA groups did not reach the level of statistical significance.

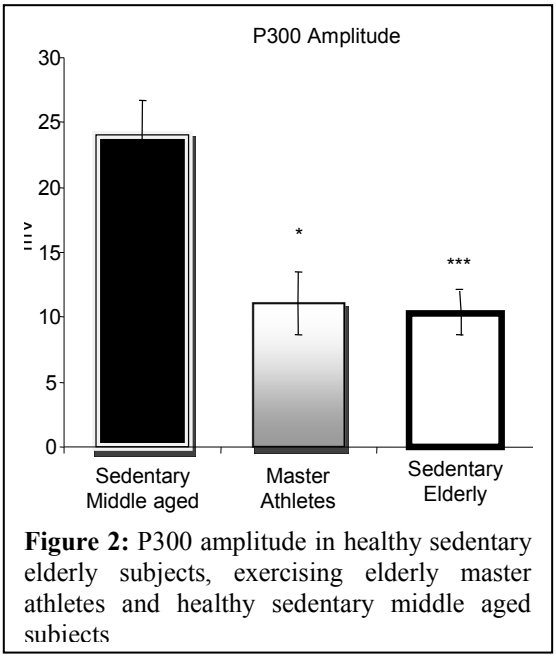

Figure 2: P300 amplitude in healthy sedentary elderly subjects, exercising elderly master athletes and healthy sedentary middle aged subjects

Individual fluctuations of ERP measures can occur spontaneously, or can be induced by environmental factors that exert their influence in at least two ways: a general state effect operates on the entire organism, and is assumed not to be restricted to the population of neurons influenced by a particular ERP task variables: a specific state effect means that biological conditions influence the same population of neurons as engaged by the task variables crucial for the elicitation of this particular ERP component. General and specific state factors can be organized into categories defined by the origins of the determinant: natural, induced, and constitutional. It is well known that an appreciable proportion of normal P300 variation is caused by factors related to the subject's level of arousal [27]. Many studies have been conducted to elucidate the effects of P300 biological determinants, both natural and environmental [15, 16, and 27]**.**

Physical training can affect brain functions via several mechanisms, including promotion of cerebral blood flow, improvement in cerebral neurotransmitter function and balance, and enhancement of neuroendocrine and autonomic tone. Because some authors explained this relationship with increased circulatory capacity, it would be particularly interesting to see how the level of fitness related to an aerobic physical activity, such as cycling. It has been argued that fitness-induced effects on ERPs might originate from fundamental changes in baseline EEG that are produced by aerobic fitness [18]. Many studies assessed EEG activity before and after exercise in a variety of subject sport-populations [9, 20, 21, and 28].

Generally similar results for P300 latency from a complex visual stimulus task in a comparable young subject group have been reported [1], although the present study also showed increased P300 amplitudes for the exercise relative to control subjects. Thus, it is reasonable to conclude that exercise does affect EEG and P300 values, but that the amount of exercise as well as the electrophysiological measurement parameters employed is important factors to consider when these variables are evaluated.

The underlying causes for the influence of physical exercise on the P300 ERP are far from clear, although speculation on the sources of these effects can be made. For example, it is straightforward to assume that physical exercise promotes cerebral blood flow (CBF) that could affect EEG measures [3, 9, 29], but why such physiologic changes would affect specific EEG bands is uncertain [30, 31].

However, when a decrease in CBF occurs because of anoxia or hypoxia, an increase in delta and decrease in alpha and beta activities typically are observed [32, 33].

If physical exercise promotes increased CBF, an EEG spectral pattern opposite that of poor CBF might be obtained. Given that such EEG changes contribute to P300

components measures, the present study is findings can be viewed as suggestive support for the hypothesis. The relationship of P300 latency to age and amplitude was tested.

The fact that VO_{2max}, which shows indirect O_2 consumption, while doing physical activity was higher in the HSE than EME and HSMA indicates that higher energy is spent by the subjects in HSE group while doing the same exercise [2, 3, 6]. In the present study, VO2max measurements were comparable to those reported in the literature.

Results of the present study showed that the amplitude of P300 increased with age. However, even though the difference between HSE and HSMA was significant, that between EME and HSMA was marginally significant. The difference in P300 latency between EME and HSMA was not significant despite a significant difference between HSE and HSMA. These results suggest that long-term exercise affects P300.

Even though numerous studies showed a decrease in the latency and amplitude of P300, this is one of the first studies which showed that exercise alleviated the deterioration effects of aging on brain functions. Taken together, the present results suggest that exceptional amounts of physical exercise can alter the P300 ERP component from simple auditory and visual stimuli, but that these effects are variable across subjects and most evident only with very high amounts of weekly aerobic activity [34]. Given the links between background EEG and cognitive ERPs outlined above [35,36,18], it seems likely that the effects of exercise observed for the present ERP data might originate from fundamental changes in baseline EEG that are produced by aerobic activity [17, 18, 35, 36, 37].

From this point of view, extended exercise helps to contribute to increased amounts of alpha band activity and, therefore, increased P300 amplitude and decreased peak latency [36, 38]. Long-term exercise may ameliorate or protect against cognitive aging for simple stimulus discriminations [37].

Presence of statistically significant differences between the subjects in the HSMA and HSE groups as opposed to non-significant differences between the subjects in the HSMA and EME groups suggests that long-term exercise might reduce the negative effects as reflected by the less reduction of neural effort.

In conclusion, adverse effects of aging on cognitive performance may be alleviated by long time physical exercise.

Conflict of Interest

The authors declared that they had no conflicts of interest.

Acknowledgements

We would like to thanks to Dr. Sıddık Keskin for statistical editing.

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MSD Medical Science and Discovery
Jun 2014, Vol. 1, No. 1, p:16-21

Original Article Doi: 10.17546/msd.98361

Effects of Thymus Vulgaris L. and Thymbra Spicata L. on diabetes mellitus associated cognitive impairment and neuropathy: Thymus Vulgaris and Cognitive Function Improvements

Zafer Akan[1], Melek Dikilidal[2], Hulya Ozdemir[2], Gokhan Oto[2], Ahmet Yilmaz[3]

Abstract

Aim: Diabetes mellitus (DM) is a metabolic disease due to increased blood glucose, with multiple organ involvement. Although various oral drugs are used to treat DM, they do not prevent the development of DM related diseases such as cognitive disorder, neuropathy and vascular diseases. Thus novel strategies for the prevention and treatment of DM are urgently needed. This research aimed to reveal the effects of Thymus Vulgaris Lamiaceae (TVL) and Thymbra Spicata Lamiaceae (TSL) on the damaging effects of DM.

Materials and Methods: Therefore, prepared TVL and TSL aqueous extracts were studied in the streptozocin induced experimental diabetic rat model. Blood glucose, body weight, and cognitive functions were examined. Morris water maze test was used to define the effect of TVL and TSL on DM related cognitive dysfunction.

Results: Briefly, impaired blood glucose, and cognitive dysfunction of Diabetic rats were significantly improved by TVL in dose dependent manner ($P<0.01$). Impaired Blood Glucose significantly improved and adjusted to the control group values ($P<0.01$)

Conclusion: Our findings strongly recommend the usage of TVL treatments for DM control by the DM patients

Keywords: Diabetes mellitus, Streptozocin, Diabetic Rat Model, Thymus Vulgaris L., Thymbra Spicata L., Morris Water Maze, Cognitive Dysfunction

Introduction

Diabetes mellitus (DM) is the common metabolic disease referring to hyperglycemia due to the corruption of insulin secretion, insufficient insulin sensitivity, or both. DM can affect almost every organ system in the body, and the level of the damage is particularly related to the severity and duration of the disease [1, 2]. Type 1 DM (T1D) is an autoimmune disease characterized by the destruction of pancreatic ß-cells.

Exposure of environmental harmful substances during neonatal period is accused of leading to initiation of immune process underlying the destruction of ß-cells and the development of disorder [3].

Patients with Type 1 Diabetes (T1D) are presented with absolute insulin deficiency, and multiple types of insulin formulations have been developed for the treatment of T1D in the last three decades [4]. Type 2 Diabetes (T2D), non-insulin-dependent type of DM, is mainly an adult disease and associated with insulin resistance. The incidences of T2D rapidly increase due to the decrease in physical activity, sedentary lifestyle, and aging population.

Received: 16 Jun 2014, **Revised** 24 Jun 2014, **Accepted** 24 Jun 2014, **Available Online** 30 Jun 2014
[1]Celal Bayar University, School of Medicine, Department of Biophysics, Manisa-Turkey
[2]Yuzuncu Yil University, School of Medicine, Department of Pharmacology, Van-Turkey
[3]Dicle University, School of Medicine, Department of Family Medicine, Diyarbakir-Turkey
*Corresponding Author: Zafer Akan, zafer.akan@cbu.edu.tr

T2D is characterized by insulin insensitivity as a result of insulin resistance, decreased insulin production, and finally pancreatic ß-cell failure. Patients with T2D should receive lifestyle alteration recommendations and diet modification.

Karabas Kekik or Zahter) (TSL) is also a member of the Lamiaceae family, and the leaves of this plant have recently gained much popularity as a remedy to combat hypercholesterolaemia [8].

TVL and TSL have been arbitrarily and widely used in the west region of Turkey

Table 1: The effects of different doses of aqueous extract of TVL and TSL on fasting blood glucose level (mg/dl) in streptozocin induced diabetic rats. Values given represent the Mean±SD; One-way Analysis of Variance (ANOVA) was applied to results *P<0.05, **P<0.01 and ***P<0.001.

	Blood glucose (mg/dl)				
	1st week	2nd week	3rd week	4th week	5th week
CRs	86,70±10,82	94,40±14,72	94,70±14,11	96,00±16,81	94,80±11,22
DRs	425,22±133,01	409,56±80,43	423,67±108,99	436,43±68,62	416,63±110,64
DRs+Gli	340,50±89,37	410,00±118,89	341,00±107,59	332,33±191,85	345,11±140,73
DRs+TVL100	312,10±53,71	286,11±66,04	298,78±85,85	237,56±100,37	322,75±182,56
DRs+TVL200	299,10±83,55	293,70±52,23	278,40±118,16	288,40±116,48	261,67±144,76
DRs+TSL100	303,50±76,57	431,40±91,14	367,00±140,82	345,50±115,92	425,33±156,48
DRs+TSL200	406,80±81,68	395,89±110,72	355,22±113,39	359,88±166,87	348,25±143,99
CRs/versus	P value				
DRs	***	***	***	***	***
DRs+Gli	***	***	***	**	**
DRs+TVL100	***	***	**	*	*
DRs+TVL200	***	***	**	**P>0.05**	**P>0.05**
DRs+TSL100	***	***	***	***	***
DRs+TSL200	***	***	***	***	**

Although novel drugs are being developed, no cure is currently available for DM related diseases [5, 6]. DM may lead to the microstructural complications, such as neuropathy, retinopathy. Diabetic neuropathy is characterized by distal symmetric polyneuropathy. The most common symptoms and findings are fatigue, paresthesia, plantar burning, loss of sense and pain. As a result, some pathology, such as diabetic foot and a morbid condition, may occur due to diabetic neuropathy [7]. Thymus vulgaris L. (Local name: Izmir Kekigi) (TVL) is a species of flowering plant in the mint family Lamiaceae and native to the west of Turkey. Thymbra Spicata L (Local name:

for the treatment of various diseases, such as diabetes mellitus, diabetes dependent retinopathy, neuropathy, urinary system disorders, and cardiovascular diseases, in folk medicine. However, no previous scientific report is available regarding the influences of TVL and TSL on DM related damage and impairment in cognitive systems. Therefore, the present study was created to examine the possible effects of TVL and TSL on the DM associated neuropathy and cognitive improvements.

Material and Methods

Animals and experimental groups

Male Wistar albino rats weighing 305.2± 4.33 g, 60 days old, were obtained from the Yuzuncu Yil University experimental animal unit. Rats were maintained on 12-h dark/light cycle at 22 °C, housed in groups of seven, and fed with a standard commercial rodent chow. The care of the animals and this experimental animal study were conducted with the approval of the Institutional Animal Care and Use Committee of the Yuzuncu Yil University Experimental Animal Unit and Ethic Committee (YUHADYEK).

DM induction

To examine the effects of TVL and TSL treatment on DM, fifty six Wistar albino rats were divided into seven equal groups. Group 2, 3, 4, 5, 6 and 7 were induced to DM. Severe DM was induced in the animals by intra-peritoneal injection of Streptozotocin (STZ; Sigma–Aldrich, St. Louis, MO) that was dissolved in 0.1 M citrate buffer solution (0.1 M, pH 4.5) at the dose of 50 mg/kg body weight (BW). Animals were fasted overnight for 12 h prior to STZ administration. Water and food were available immediately after dosing. The development of DM was determined by observing hyperglycemia (>300 mg/dl) [10] as measured by an Accu-Chek Go glucometer (Roche, Mannheim, Germany). Body weights and blood glucose levels were recorded once a week throughout the study.

Extraction of TVL and TSL:

The species of TVL and TSL were collected from Aegean region (Aydın and Izmir cities). Taxonomic identification was performed by Associate Prof. Dr. Fevzi Ozgokce. The collected plants were dried in an oven at 40 °C and then ground into a powder. For extraction, the decoction method and distilled water as solvent were used [8]. For the decoction method, 20 g of dried powder was extracted with 100 ml of distilled water at 100 °C for 30 min in a water bath. Subsequently, it was filtered, and the water was evaporated to dryness. The residue was weighed to obtain the extractive yield, and it was in air tight bottle at 4 °C. The yield of dried extract were found % 5.9 and 5.85, respectively. The extracts were prepared daily. Resolved in 100 and 200 mg/ml distilled water and orally administered to the rats daily.

The creation of groups and the assessment of the effects of TVL and TSL treatment on blood glucose level (BGL) and body weight changes:

Diabetic rats were treated by the agents of Glibenclamide, TVL and TSL aqueous extracts. Both BGL and body weight alterations were compared with the result of control group. The groups were created as; (1) no additive; (2) 50 mg/kg STZ only; (3) 50 mg/kg STZ plus 5 mg/kg glibenclamide; (4) 50 mg/kg STZ plus 100 mg/kg TVL; (5) 50 mg/kg STZ plus 200

Table 2: Effects of different doses of aqueous extract of TVL and TSL on spatial learning latency (minute) of streptozocin induced diabetic rats. Values given represent the mean and S.E.M. One-way Analysis of Variance (ANOVA) was applied to results.

	1st day	2nd day	3rd day	4th day
CRs	0,60±0,080	0,19±0,02	0,22±0,050	0,14±0,02[a]
DRs	0,80±0,089	0,55±0,082**	0,37±0,064*	0,34±0,064*
DRs+Gli	0,80±0,081	0,47±0,073	0,52±0,074	0,29±0,055
DRs+TVL100	0,71±0,089	0,36±0,066	0,36±0,072	0,14±0,037[a]
DRs+TVL200	0,78±0,084	0,50±0,075*	0,33±0,054	0,12±0,017[aa]
DRs+TSL100	0,91±0,085	0,52±0,078*	0,50±0,094*	0,30±0,076
DRs+TSL200	0,76±0,088	0,47±0,086	0,36±0,064	0,22±0,046

[a]P<0,05 and [aa]P<0,01 compromising between DRs versus CRs, TVL100 and TVL200

mg/kg TVL; (6) 50 mg/kg STZ plus 100 mg/kg TSL; (7) 50 mg/kg STZ plus 200 mg/kg TSL. Body weight and were measured once in a week through 5 weeks. Rats were treated with glibenclamide/TVL/TSL single daily dose.

The assessment of TVL and TSL treatment on spatial learning of diabetic rats by Morris water maze test:

The Morris water maze (MWM) is a test for spatial learning examination for rodents that relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform [9]. We tested spatial learning of control, diabetic, and Glibenclamide/TVL/TSL treated rats.

Statistical Methods

For spatial learning tests; a total of 56 rats including 8 rats in each group were subjected to the study. Each experiment was repeated at least 3 times, and the results of a representative experiment were shown. One-way Analysis of Variance (ANOVA) and Tukey-Kramer Multiple Comparisons tests were applied to the variables, and each result was reported as mean ± S.E.M. and a, p value less than 0.05 was accepted as statistically significant. (*P<0.05, **P<0.01 and ***P<0.001)

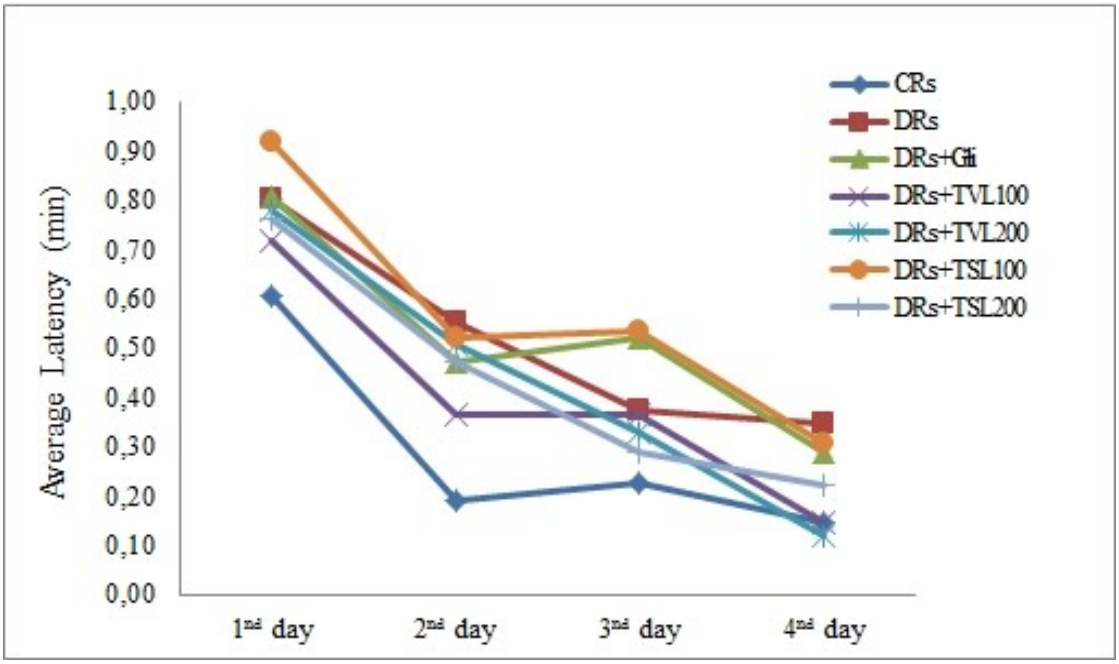

Figure 1: Time elapsed in the Morris water maze by different groups of animals (n=8). Diabetic Rats (DRs) needed the longest time to locate the platform versus the Normal Rats (CRs: Control) ($P < 0.01$). TVL improved the maze navigation in dose manner both 100 mg/ml and 200 mg/ml concentrations ($P < 0.01$). Each point represents data (mean ± SEM)

Present protocols for performing variants of the MWM test, from which results can be obtained from individual animals in as few as 4 days end of the study.

All tissue and blood samples were collected to search for different tissue involvements under anesthetic conditions (200 U/kg Ketalar).

Results

Control group blood glucose levels were measured in an average of 86.7 mg/dl. BGL were chronically elevated to the average of 422.3 mg/dl by the single (Acute) dose STZ (50 mg/kg) administration. BGL remained stable in both Control and STZ groups for 5 weeks.

BGL were slightly decreased by the Glibenclamide treatment in the Glibenclamide group. The most significant results over the increased BGL were obtained with TVL100 mg/kg and TVL 200 mg/kg treatments ($p<0.01$) (Table 1). The mean body weight was 305.2 gr, and it was decreased to 208.3 gr by the administration of STZ at the end of 5th week. TVL treatment reduced body weight loss in STZ administrated diabetic rats ($p < 0.01$). As to spatial learning MWM test results; figure 1 and table 2 showed that the escape latency to locate the hidden platform was statistically significantly shorter in the groups of control, TVL 100 and 200 mg/kg treated diabetic rats compared with the diabetic vice/versa glibenclamide, TSL100 and 200 mg/kg treated groups ($*p < 0.05$, $**p < 0.01$). The performance at fourth day of the control, TVL 100 and 200 mg/kg groups was not statistically different ($p > 0.05$). Spatial learning performances of diabetic rats were improved by the TVL 100 and 200 mg/kg treatments (Figure 1)

Discussion

TVL and TSL are known with their antimicrobial and antiviral effects, thus the use is common among the population for the treatment of DM and various diseases. They are characterized by the large scale component of free radical scavenger essential oils such as thymol, carvacrol, 8-terpinene, p-ceymene and α-pinene. In addition, the chemical composition of TVL and TSL essential oils varies in a wide range. Carvacrol and thymol are the main components of the mint family Lamiaceae plants. The rate of these components is due to the environment and ecology [8]. The aim of the present study was to analyze the impact of TVL and TSL in terms of blood glucose, vascular, renal and cognitive systems in diabetic rats. Streptozotocin is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. It is used in medical research to produce an animal model for Type 1 DM in single large dose as well as Type 2 DM with multiple low doses. In a long period of time, diabetic rats are characterized by high blood glucose and body weight loss. In our study, we produced Type 1 DM by a single large dose of STZ administration (50 mg/kg) in the Wistar albino rats. The diabetic rats were treated by glibenclamide, TVL and TSL aqueous extracts. It was seen that TVL was significantly effective in the avoidance of the increase in blood glucose, weight loss, the decline in cognitive functions, and also in the improvement of them.

DM is associated with slowly progressive end-organ damage in the brain. Mild to moderate deteriorations of cognitive functions have been declared in patients with both type 1 and in type 2 DM [10]. Many of DM related clinical complications are commonly caused by the vessel disorders. The peripheral microvascular complications of DM occurring outside the brain seem to be associated with corresponding microvascular changes in the brain. For instance, diabetic retinal microvascular abnormalities were associated with various MRI findings including small focal white matter hyper intensities and lesions [11]. In the present study, it was determined that a significant improvement in cognitive functions of diabetic rats was provided by the TVL treatment. It was supposed that this positive impact of TVL on the cognitive function was due to its positive effects on blood glucose level and microvascular system.

In conclusion, it was supposed that both TVL and TSL had dose dependent protecting and healing effects against the damaging effects of DM in terms of blood glucose cognitive systems moreover, diabetic neuropathy.

Our finding strongly recommends the usage of TVL treatments for DM control by the DM patients

Conflict of Interest

The authors declared that they had no conflicts of interest.

Acknowledgements

This project was supported by Yuzuncu Yil University Scientific Research Project Funding (YYU-BAP/2013-SBE-YL051). Thanks to Associate Prof. Dr. Ozgokce F for herbal taxonomy and Associate Prof. Dr. Keskin S for statistical analysis

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ISSN: 2148-6832

MSD Medical Science and Discovery
Jun 2014, Vol. 1, No. 1, p:22-26

Original Article Doi: 10.17546/msd.88603

Effect of extremely low frequency electromagnetic fields on α-Lactalbumin and Sulindac treated colon cancer cells: ELF-EMF and colon cancer

Kivanc Gorgulu[1], Burak Gokce[2], Isil Aydemir[1], M. Kemal Ozbilgin[1], H. Seda Vatansever[1]

Abstract

Aim: In this research, we aimed to investigate the effects extremely low frequency electromagnetic fields (ELF-EMF) on proliferation and apoptosis during treatment of primary and metastatic colon cancer cell lines.

Material and Methods: Colon cell lines; COLO-320, COLO-741 and as control mouse fibroblast (STO) cells were cultured in 24 wells of tissue culture plate. Both COLO-320 and COLO-741 cells were treated with α-lactalbumin, sulindac and α-lactalbumin + sulindac. The cells from all groups were exposed to 60 Hz ELF-EMF for 48 hours. For cytotoxicity analyses, the cells were collected and analyzed with ELISA. The cells were fixed in 4% paraformaldehyde for 30 minutes. Cell proliferation was analyzed by evaluating of anti-Ki-67 distribution using indirect immunohistochemistry, cell death were evaluated using TUNEL assay.

Results: After TUNEL assay, the TUNEL positive cells were detected in all treated and control groups. However, the number of apoptotic cells was increased after treatment with α-lactalbumin and EMF exposure on COLO-320 cells. The apoptotic cells were less in STO cells. The distribution of Ki-67 was also detected in all groups, but, there were more Ki-67 immunoreactivity in COLO-741 cells than other groups. The cell cytotoxicity was also increased after EMF exposure in all groups.

Discussion: Our results suggested that the EMF exposure may increase the effects of α-lactalbumin on primary colon cancer cell lines. However, the proliferation of both cancer and control cells were not affected. The EMF exposure may trigger apoptotic pathway in primary colon cancer cell lines.

Keywords: Electromagnetic Fields, Apoptosis, Proliferation, Colon cancer, α-lactalbumin, sulindac, Tunnel assay

Introduction

Electric and magnetic fields (EMF) are produced when electric power is generated, transmitted, and used. Worries about even if EMF could negatively affect human health were raised at the beginning by epidemiologic studies reported in the late 1970s, and since the 1980s, data's on EMF have been widely announced in the popular press. Electromagnetic fields (EMF) such as those from electric power transmission and distribution lines (50/60 Hz) have been associated with increased risk of childhood leukemia, cancer of the nervous system, lymphomas and other cancer types. At the same time humans are daily exposed to electromagnetic field (EMF) resources. In 2002, the International Agency for Research on Cancer (IARC, 2002) categorized extremely low frequency (ELF-EMF) (including the power frequencies of 50 and 60 Hz) magnetic fields as "possibly carcinogenic" [1]. Some parts of studies have marked possibly contrary effects induced by EMF [2]. On the other hand some researchers have showed beneficial effects of EMF in biological aspects [3].

Received: 25 Jun 2014, **Revised** 26 Jun 2014, **Accepted** 28 Jun 2014, **Available Online** 30 Jun 2014
[1]Celal Bayar University faculty of Medicine, Department of Histology and Embryology, Manisa, Turkey
[2]Ege University, Science Faculty, Department of Biology, Zoology Section, Izmir, Turkey
*Corresponding Author: H. Seda VATANSEVER sedavatansever@yahoo.com

Due to colon cancer is the second most frequent reason in the cancer-related deaths in the world, in this study; we have studied effects of electromagnetic fields during in vitro treatment of colon cancer cells. It is reported that colon cancer is the 4th most frequent cancer in males and the 5th most frequent cancer in females [4]. There are a lot of studies about colon cancer which are trying to find new therapeutic approach by various combinations with drugs or other treatment manners. We used two drugs for combination therapy which are Sulindac and alpha Lactalbumin. Researchers have showed that non-steroidal anti-inflammatory drugs and highly selective COX-2 inhibitors hold promise as anticancer agents. Giardiello et al showed that Sulindac effectively inhibits growth of adenomatous polyps and cause regression of existing polyps in patients with the unusual hereditary condition familial adenomatous polyposis (FAP) [5]. In the 2008, Researchers published that Sulindac may be more effective than Celecoxib which is Celecoxib, highly selective COX-2 inhibitor, when it is combined with DMFO in FAP patients who has colectomy operation [6]. On the other hand α-lactalbumin has been used for combination therapy. α-lactalbumin is the most generous protein in human milk and is renowned as a coenzyme in lactose synthesis. Researchers have found that when α-lactalbumin and oleic acid are used together as a complex which is called as HAMLET, they have effects on tumor cell viability. This molecular complex induces apoptosis in cancer cells but normal differentiated cells are resistant to HAMLET [7-9].

Living systems are under pressure of a lot of factor, which some of these factors compels to cells to cancer formation such as air, water pollutants and electromagnetic fields. Response to cancer therapy is also dependent to environmental factors. For this reason, in this study, effects of ELF-EMF on the α-lactalbumin and sulindac treatments were investigated

Material and methods

Cell Culture

Semi-adhesive, Human Primary colon cancer cell line Colo-320 (HTL95027, Interlab Cell Line Collection, Genova, Italy) and Adhesive, Metastatic human colon cancer cell line Colo-741 (HTL95008, Interlab Cell Line Collection, Genova, Italy) were incubated in %5 CO_2, 37 ºC, Humidified Atmosphere conditions.

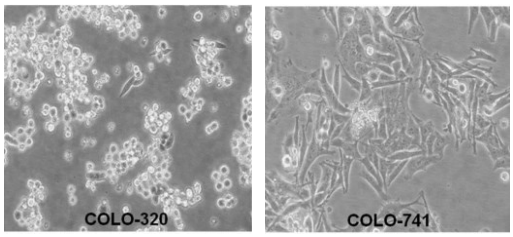

Figure 1. Semi-adhesive, Human Primary colon cancer cell line: Colo-320 (HTL95027, Interlab Cell Line Collection, Genova, Italy) . Adhesive, Metastatic human colon cancer cell line: Colo-741 (HTL95008, Interlab Cell Line Collection, Genova, Italy)

Extremely Low Frequency Electromagnetic Field ELF-EMF

For generating electromagnetic field, Helmholtz coils (Leibold Elektrotechnik, Germany) and signal generator (Philiphs) equipment was used. Diameter of Helmholtz coil measured as 20 cm and distance between two coils measured as 10 cm. Configuration of field is designed by using non-pulsed alternative current at 60 Hz and 15 volt.

Field that generated by method described above, was measured and characterized by

Figure 2. Helmholtz Coils and Power supply system for ELF-EMF

using Gauss meter measuring equipment. According to measurements, electric field value was 10 v/m and magnetic field value was 15 mT in the middle of the coils where culture flask was placed during exposure time (Figure 2).

Results

ELISA Results

There are certain implications of cellular responses influenced by EMF *in vitro*.

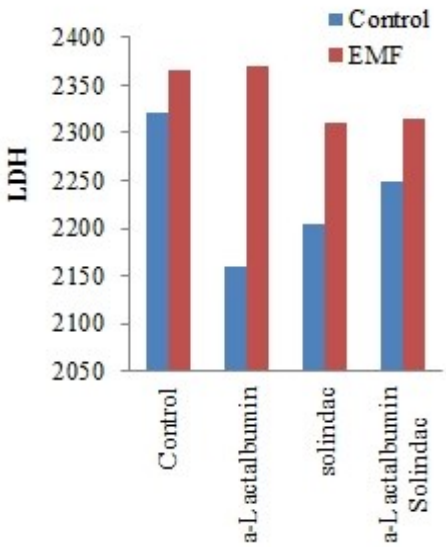

Figure 3a. Cytotoxicity evaluation of treatment has been elucidated by serum LDH levels. COLO-320 ELISA Results

A tremendous number of cellular components, cellular signals and cellular systems can probably be affected by EMF. However, EMFs are contrary to cause DNA damage directly because it has not been showed any data like that; most studies have been developed to investigate its effect on the cellular membrane, several gene expression, and signal transduction pathways. In our study, firstly we tested cytotoxicity of combination drug treatment and EMF effect on primary, metastatic colon cancer cells and mice STO fibroblast cells as a normal cell group by ELISA. We have clearly seen that EMF treated all groups have more cytotoxic effect than other groups (Figure 3).

Cells were cultured in 24 wells of tissue culture plate. After sub-culturing of cells, they were cultured 24 hours. Both COLO-320 and COLO-741 cells were treated with α-lactalbumin, sulindac and combined treatment (α-lactalbumin + sulindac). For control, we used untreated COLO-320, COLO-741 and STO cells. The cells from all groups were exposed to 60 Hz power line EMF for 48 hours. Every 2 hours, EMF was exposure for 15 minutes, during 48 hours, 8 exposures were applied. The TUNEL positive cells (apoptotic cells) were detected, which were identified with

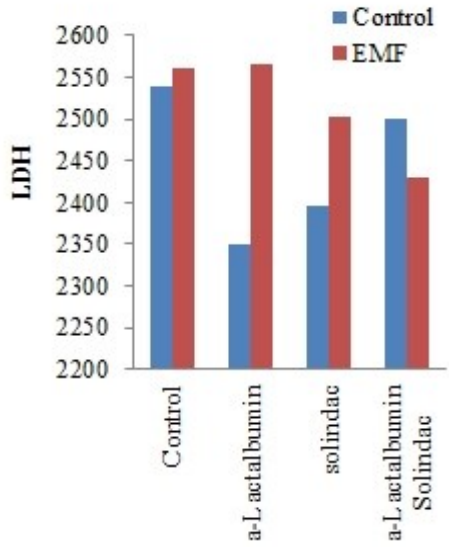

Figure 3b. Cytotoxicity evaluation of treatment has been elucidated by serum LDH levels. COLO-741 ELISA Results.

brown nucleus, in all groups. However, there were more apoptotic cells in COLO-320 treated with α-lactalbumin and EMF exposure applied group when we compare with other groups (Figure 4). Apoptotic cells were less in STO cells (Figure 5, 6). There was some brown looking cells observed but they were death cells in the culture.

Ki-67 Immunoreactivity

Ki-67 is a cancer antigen that is found in growing and proliferating cells. The distribution of Ki-67 was also detected in all groups, but, there were more Ki-67 immunoreactivity in COLO-741 cell line which is characterized metastatic behavior (Figure 7).

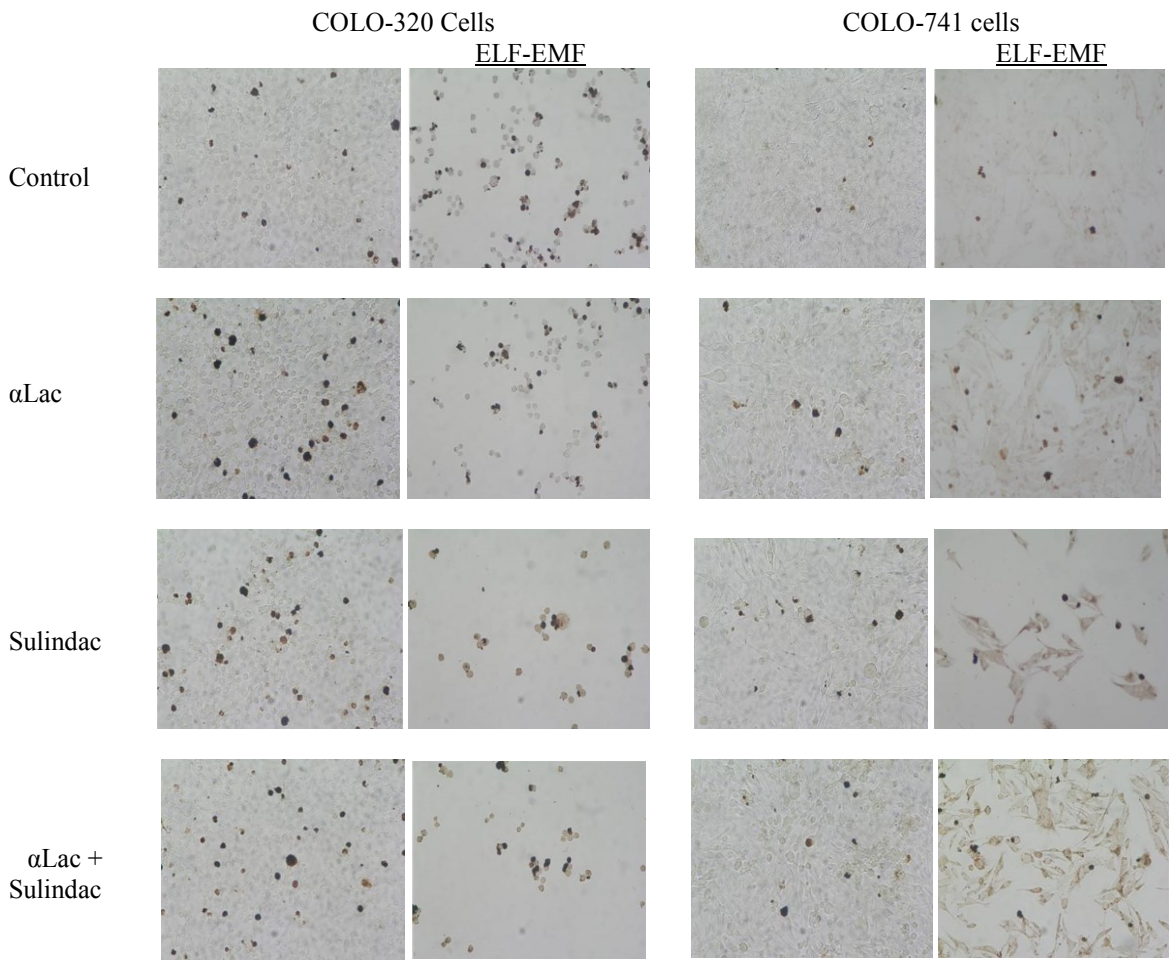

Figure 5. TUNEL Assays of COLO cells (x200)

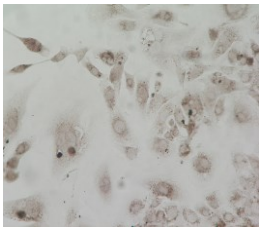

Figure 6. TUNEL Assay in STO Cells

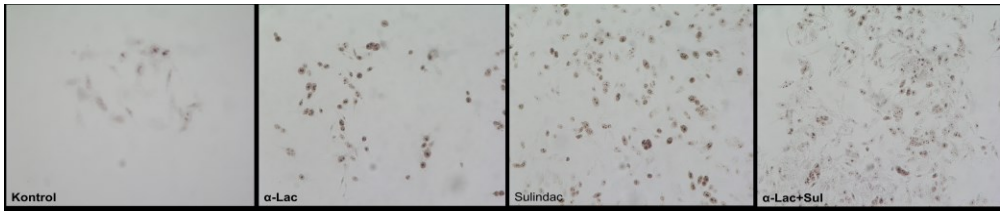

Figure 7. Ki-67 Immunoreactivity in COLO-741 cell line

Discussion

Effects of electromagnetic field in normal and cancer cells are under investigation by researchers. But still there are a few studies about EMF effect combined with chemotherapeutics and other neo-adjuvant agents. Especially, in this study the behavior of primary or metastatic tumor cells may be different; therefore, the effective treatment can be important to cure for cancer. Our results suggest that combination treatment of EMF and α-lactalbumin is more effective when we compare with other groups. Svanborg and her colleagues have identified HAMLET as a new perioral agent for colon cancer prevention and treatment [10]. EMF can enhance α-lactalbumin induced apoptosis. Ki-67 immunoreactivity of COLO-741 cell line was more than other groups. This data can provide that metastatic colon cancers have high proliferative rate. This high degree of proliferation in metastatic colon cancer cells can minimize exposure of EMF. Although no change in colonic cell line proliferation, the balance between proliferation and apoptosis in the direction of increased apoptosis may be appropriate for treatment in in vitro condition; this effect may be analyzed also in vivo applications.

Conflict of Interest

The authors declared that they had no conflicts of interest.

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MSD
MEDICAL SCIENCE & DISCOVERY
ISSN: 2148-6832
Publisher LuLu Press, USA

ISBN 978-1-312-33833-3
90000
9 781312 338333

International Journal of
Medical Science and Discovery
Open Access Scientific Journal
Jun 2014, Vol.1, No.1,

www.medscidiscovery.com