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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Oxidative and antioxidative responses in submandibular and parotid glands of rats exposed to long-term extremely low frequency magnetic field

Uzun süreli oldukça düşük frekanslı manyetik alanın submandibular ve parotis bezlerinde oluşturduğu oksidatif ve antioksidatif yanıt

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ABSTRACT

Objective: Some epidemiologic and laboratory studies have suggested a possible associations between exposure to extremely low frequency magnetic field (ELF-MF) and cancer. However, it is not known underlying mechanisms of this interaction. The aim of the study was to investigate the possible oxidative damage induced by long-term ELF-MF exposure on submandibular and parotid glands of rats.

Methods: Rats in the experimental group were exposed to 100 and 500 μT ELF-MF (2 h/day, 7 days/week, for 10 months) corresponding to exposure levels that are considered safe for humans. The same experimental procedures were applied to the sham group, but the ELF 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 submandibular and parotid gland.

Results: Although some oxidative and antioxidative parameters of submandibular gland were altered by ELF-100 and ELF-500 exposure groups, these changes were not statistically significant ($p > 0.05$). However, a decrease observed in CAT levels of parotid gland in both the ELF-100 and ELF-500 exposure groups ($p<0.05$, $p<0.01$). No significant alterations was found in other endpoints related to parotid gland ($p>0.05$).

Conclusion: Our results showed that long-term ELF-MF exposure did not alter oxidative, antioxidative processes and lipid peroxidation in submandibular gland of rats. However, 100 μT and 500 μT ELF-MF exposure decreased CAT activity in parotid gland. *J Clin Exp Invest 2014; 5 (2): 219-225*

Key words: Extremely low-frequency magnetic field, oxidative stress, antioxidant enzyme, submandibular gland, parotid gland

ÖZET

Amaç: Bazı epidemiyolojik ve laboratuar çalışmalar oldukça düşük frekanslı manyetik alan ile kanser arasında ilişki olduğunu ileri sürmektedir. Ancak bu etkileşimin altında yatan mekanizma tam olarak bilinmemektedir. Çalışmamızın amacı ratlardaki submandibular ve parotis bezleri üzerindeki uzun süreli oldukça düşük frekanslı manyetik alanın oksidatif ve antioksidatif etkilerini araştırmaktır.

Yöntemler: Ratlar; çalışma düzeneği olarak günde 2 saat, yedi gün ve 10 ay boyunca insanlar için güvenli kabul edilen 100 ve 500 μT'lık manyetik alana maruz bırakıldı. Aynı prosedür şham konrol grubuna uygulandı, fakat manyetik alan jeneratörü kapalı tutuldu. Çalışmanın bittiği 10. ay'dan sonra katalaz, malondilaldehit, myeloperoksidaz, total antioksidan kapasite, total oksidan status ve oksidatif stres indeks parametreleri ölçüldü.

Bulgular: ELF-100 ve ELF-500 uygulama grubunda bulunan ratların submandibular dokusunun bazı oksidatif ve antioksidatif parametrelerinde değişiklik olmakla birlikte, bu değişiklikler istatistiksel olarak anlamlı değildi ($p>0.05$). Ancak hem ELF-100 ve hem de ELF-500 uygulama grubunda bulunan ratların parotis bezlerinin katalaz düzeyinde anlamlı azalma gözlendi ($p<0.05$, $p<0.01$). Parotisle ilgili diğer değişkenlerde anlamlı değişiklik gözlenmedi ($p>0.05$).

Sonuç: Sonuç olarak, uzun süreli oldukça düşük frekanslı manyetik alanın submandibular glandın oksidatif, anti oksidatif süreçleri ve lipit peroksidasyon düzeylerini değiştirmediği gözlendi. Ancak 100 ve 500 μT'lık uzun süreli manyetik alan uygulamasının parotis bezinin katalaz aktivitesinde azalmaya neden oldu.

Anahtar kelimeler: Oldukça düşük frekanslı manyetik alan, oksidatif stres, antioksidan enzim, submandibular bez, parotis bezi

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INTRODUCTION

Since the distribution of electricity and increasing use of electric and electronic appliances, which emit Extremely Low Frequency Magnetic Field (ELF-MF), the number of humans exposed to ELF electromagnetic fields has increased during the last century in the worldwide [1]. Therefore, questions have been raised ELF-EMF can produce adverse health outcomes since the late 1970s. Several epidemiologic and laboratory studies have suggested a possible associations between ELF-MF exposure and certain type of cancer, particularly leukemia and brain cancer [2,3]. Extremely Low Frequency Magnetic Field have also been classified as being possibly carcinogenic to humans in group 2B by the International Agency Research on Cancer (IARC) reports [4], based on data pooled from epidemiological studies by Ahlbom et al.[5] and Greenland et al.[6], that show the risk of childhood leukemia doubles with exposure to ELF-MF. There are lots of studies try to detect biological effects of ELF-MF in vivo and in vitro. However, the molecular mechanism, which ELF-MF can effect on living system, is still unclear.

One of the proposed interaction mechanisms between ELF-MF and cells is the radical pair mechanism which static and ELF magnetic fields can affect the chemistry of individual molecules [7-11]. This interaction mechanism involves a specific type of chemical reaction: the recombination of a pair of short-lived, reactive free radicals generated either from a single molecule or from two molecules by intermolecular electron or hydrogen atom transfer [12]. The effect of an applied magnetic field depends upon its interaction with the spin of unpaired electrons of the radicals. Importantly, this effect may constitute a mechanism for the biological effects of very weak fields [13,14]. Free radicals are a chemical species formed during many metabolic processes and thought to contribute to various disease states such as neurodegenerative disease [12]. Most cells contain a variety of radical scavengers such as glutathione peroxidase that provide antioxidant defense mechanisms. If these are depleted, for example from exposure to an agent that generates excess reactive oxygen species (ROS), tissue damage may occur [12].

Several studies were performed to test whether ELF magnetic field can modulate oxidative stress and antioxidant systems in tissues by measuring free radical levels and antioxidant enzyme activities, respectively [15,16]. According to their results, Falone et al. 17 suggest that the exposure to ELF-MFs may act as a risk factor for the occurrence of oxidative stress-based nervous system pathologies associated with ageing. Chu et al. [18] investigated whether extremely low frequency magnetic field (ELF-MF) induces lipid peroxidation and reactive oxygen species in mouse cerebellum. They indicate that ELF-MF may induce oxidative stress in mouse cerebellum [18]. Ciejka et al. [19] have shown that ELF-MF applied for 30 min/day for 10 days can affect free radical generation in the brain. They also suggested that the effect of ELF-MF irradiation on oxidative stress parameters depends on the time of animal exposure to magnetic field. Cui. et al. [20] found that ELF-MF exposure (1 mT, 50 Hz) induced serious oxidative stress in the hippocampus and striatum and impaired hippocampal-dependent spatial learning and striatum-dependent habit learning. There are several studies in relation to the effect of ELF-MF on ROS production and oxidative damage of brain tissue. However, no studies have been found to investigate oxidative damage induced by long-term ELF-MF exposure on salivary glands. Since the lack of studies, we don't know whether long-term ELF-MF exposure can effect on oxidative and antioxidative processes of submandibular and parotid glands in rats. The underlying mechanisms of oxidative and antioxidative events induced by ELF-MF in the salivary glands is still unclear. Therefore, in the present study, we investigated the possible oxidative damage induced by long-term ELF-MF exposure on submandibular and parotid glands of rats.

METHODS

Subjects and animal care

The experiments were performed on 30 male Sprague-Dawley rats obtained from Medical Science Application and Research Center of Dicle University. Experimental protocols were approved by the local ethics committee. The rats (4 months of age and weighing 339.10±36.95 g) were kept in 14/10 h light/dark environment at constant temperature of 22±3°C, 45±10% humidity and received standard laboratory food (TAVAS Inc, Adana, Turkey) and tap water ad libitum.

Rats were assigned into three groups (sham group, n: 10 and two experimental groups: n: 10 for each group). Rats in the experimental groups were randomly divided into two groups named as ELF 100 and ELF 500 exposure groups, respectively. The first experimental group rats were exposed to 100 μT while second experimental group exposed

to 500 μT ELF-MF for 2 h/day (7 days in a week) during 10 months in a Plexiglas cage. For the sham group, the similar experimental procedure was applied to the rats which are kept in a Plexiglas cage identical to that for the exposed group and the ELF-MF generator was turned off. This cage was maintained within a Helmholtz coil and inside a Faraday cage during the experiment

Magnetic field generation and exposure of rat

The magnetic field strength used in present study is within the limits contained in occupational and public environment Magnetic Field (MF) exposure guideline standards and it exists in both the public and the occupational environments [21]. The MF was generated by using a pair of Helmholtz coils of 25 cm in diameter in a Faraday cage (130×65×80 cm) that earthed shielding against the electric component. This magnet was constructed by winding 225 turns of insulated soft copper wire with a diameter of 1.0 mm and coils were placed horizontally facing each other. An AC current (0.12 A for 100 μT and 0.50 A for 500 μT, which yielded 50 Hz MF) was produced by an AC power supply (DAYM, Ankara, Turkey) was passed through the device. The MF intensities were measured once a week as 100 μT and 500 μT in 15 different points of methacrylate cage by using a digital teslameter (Phywe, S/N; 209101074, Göttingen, Germany) to ensure homogeneity of the field during the course of the experiment. All field measurements were performed by persons who were unaware of the groups so that the subsequent analysis could be performed blind. The rats were kept freely moving in methacrylate cage inside the coils. After 10 months of MF exposure, the study was terminated. Immediately after the last exposure, rats were euthanized under ketamine anesthesia (100 mg/kg, intramuscularly). The submandibular and parotid glands were removed for CAT, MDA, TAC, TOS, OSI, and MPO measurements.

Tissue sampling and homogenization

Before biochemical assays, all submandibular and parotid tissues were weighed, then they were placed in empty glass tubes. Ten milliliters of 140 mM KCl solution/gram of tissue were added to each tubes and all tissue were then homogenized in a motor-driven homogenizer. The homogenates were centrifuged at 2,800×g for 10 min at 4°C and the supernatant obtained were used for the measurement of CAT, MDA, TAC TOS, OSI, MPO levels. Homogenized all tissues were placed in labeled vials and stored in deep-freezer at −80°C. Microprotein level was measured by the method of Lowry et al [22].

The determination of myeloperoxidase

The method described by Wei and Frenkel [23] was used for the tissue MPO activity assay [23], which is a lysosomal oxidative enzyme that is found in white blood cells, and data are expressed as units per gram protein.

Measurement of total antioxidative capacity

The TAC levels of all tissues were measured using a novel automated colorimetric measurement method developed by Erel [24,25].

Measurement of total oxidant status

TOS levels of all tissues were determined using a novel automated measurement method, developed by Erel [26]. In this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per gram protein (micromoles H_2O_2 equivalents per gram protein).

Oxidative stress index

The percent ratio of the TOS to the TAC gave the OSI, an indicator of the degree of oxidative stress (15). The OSI value was calculated as the formula: OSI=[(TOS; micromoles H_2O_2 equivalents per gram protein)/(TAC; micromoles Trolox equivalents per gram protein)]

Determination of catalase activity

CAT activity in supernatant of tissues was assayed by a method described by Goth 27. From the homogenate, 0.2 mL homogenate was incubated in 1.0 mL substrate (65 μmol per H_2O_2 in 60 mmol/L sodium-potassium phosphate buffer, pH 7.4) at 37°C for 60 s. The enzymatic reaction was stopped with 1.0 mL of 32.4 mmol/L ammonium molybdate ($(NH_4)6Mo_7O_{24}•4H_2O$), and the yellow complex of molybdate and H_2O_2 was measured at 405 nm against blank 3. Homogenate CAT activity is linear up to100 kU/L. If the CAT activity exceeded 100

kU/L, the homogenate was diluted with the phosphate buffer (two- to tenfold), and the assay was repeated. One unit of CAT decomposes 1 μmol of H_2O_2/L min under these conditions. Results were expressed as kilounits per gram protein which was calculated as follows:

CAT kU/g protein= A(sample)-A(blank 1)/A (blank 2)-A(blank 3).271

Determination of malondialdehyde levels

Lipid peroxidation of all tissues was evaluated by the fluorometric method based on the reaction between MDA and thiobarbituric acid[28]. Briefly, 50 μL of supernatant of tissues was added to 1 mL of 10 mmol/L diethylthiobarbituric acid reagent in a phosphate buffer (0.1 mol/L, pH 3). The mixture was mixed for 5 s and incubated for 60 min at 95°C. Samples were placed on ice for 5 min, and then 5 mL of butanol was added. The mixture was shaken for 1 min to extract the MDA adduct and then centrifuged at 1500×g for 10 min at 4°C. Fluorescence of the butanol extract was measured at an excitation wavelength of 539 and emission wavelength of 553. 1,1,3,3 Tetraethoxypropane was used as the Standard solution, and the values were presented as micromoles per gram protein.

Statistical analysis

Means values and standard deviations were calculated, and statistical significance of the differences between exposed samples and controls was evaluated. A computer program (SPSS 10.0, SPSS Inc., Chicago, IL, USA) were used for statistical analysis. Data were analyzed by Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks and post hoc multiple comparison tests using a Tukey's Honestly Significant Difference (HSD). All hypothesis tests used a criterion level of p=0.05.

RESULTS

The results of oxidative and antioxidative parameters in rats' submandibular and parotid glands, which were exposed to long-term 100 and 500 μT ELF-MF, were shown on table 1 and 2.

Although some oxidative and antioxidative parameters of submandibular gland were altered by ELF-100 and ELF-500 exposure, these changes were not statistically significant (p >0.05) (Table 1). However, the statistically significant decrease in CAT levels of parotid gland was found in both the ELF-100 and ELF-500 exposure groups (p<0.05, p<0.01) (Table 2). No significant alterations was found in the other endpoints related to parotid gland (p>0.05) (Table 2).

Table 1. The concentrations of submandibular gland myeloperoxidase (MPO), catalase (CAT), malondialdehyde (MDA), total antioxidant capacity (TAC), total oxidant status (TOS) and oxidative stress index (OSI) in rats in the exposed and sham groups

Parameters	Sham	ELF-100	ELF-500
CAT (kU/gr protein)	103.5±22.3	83.5±6.2	153.7±115.4
MPO (U/gr protein)	1.97±1.04	2.23±1.67	5.60±6.27
MDA (μmol/gr protein)	3.988±1.432	4.433±4.322	5.349±4.134
TOS (μmol H_2O_2 equiv/gr protein)	6.425±1.710	6.773±5.186	8.473±5.480
TAC (μmolTroloxEqv./gr protein)	6.685±1.871	7.561±1.796	9.480±6.292
OSI (AU)	1.058±0.459	1.063±1.048	1.194±1.050

No significant difference was found between the groups (p>0.05). The values represent mean ± SD, Arbitrary units (AU).

Table 2. The concentrations of parotid gland myeloperoxidase (MPO), catalase (CAT), malondialdehyde (MDA), total antioxidant capacity (TAC), total oxidant status (TOS) and oxidative stress index (OSI) in rats in the exposed and sham groups.

Parameters	Sham	ELF-100	ELF-500
CAT (kU/gr protein)	124.2±18.9	[a]100.7±9.8	[a*]100.0±12.9
MPO (U/gr protein)	6.747±5.272	5.167±2.669	6.948±3.385
MDA (μmol/gr protein)	3.427±1.166	2.771±0.506	3.281±1.625
TOS (μmolH_2O_2 equiv/gr protein)	6.591±2.135	5.292±0.755	5.854±3.576
TAC (μmolTroloxEqv./gr protein)	7.071±4.367	7.693±3.761	7.725±3.054
OSI (AU)	1.356±0.915	0.843±0.385	1.034±1.159

The statistically significant decrease in CAT levels of parotid gland was found both the ELF-100 exposure group and ELF-500 exposure group in comparison to sham group respectively (a=Statistically significant, compares sham group (p<0.05). a*=Statistically significant, compares sham group (p<0.01)). The values represent mean ± SD, Arbitrary units (AU).

DISCUSSION

In the present study, it was indicated that long term ELF-MF exposure, which is below exposure limits for public and occupational safety as determined by International Commission on Non Ionizing Radiation Protection (ICNIRP) [21], could not affect oxidative and antioxidative parameters on submandibular gland of rats. Whereas, 100 and 500 μT ELF exposure altered the catalase activity of parotid gland. On the other hand, no significant differences were found in relation to the other parameters, which was presented in this study, on parotid gland of rats.

Salivary glands are responsible, to a large extent, for maintaining soft and hard oral tissue homeostasis by the secretion of saliva. Salivary glands also play an important role in metabolism, digestion, oral and systemic health by regulating, and maintaining the integrity of the oral hard and soft tissues, and even in the humoral immunity by IgA secretion [29-31]. The biological mechanism of the impairment of salivary gland function has been studied, [32-33] but the details are not yet understood. ROS are involved in the impairment of salivary gland function due to aging, inflammation, drugs, and ionising irradiation [34-37]. However, we don't know whether long-term ELF-MF exposure can induce ROS on salivary glands such as parotid and submandibular gland. In order to explore this interaction mechanisms, we intend to do the present study.

The role of reactive oxygen species (ROS) have been implicated in tissue injury. At the cellular level, lipids, proteins, carbohydrates, and nucleic acids may be damaged by reactions with ROS [38]. Moreover, the increase of the concentration of ROS may cause to functional and morphological disturbances in the cell through the oxidative stress leading to reversible or irreversible tissue injury, e.g., DNA damage [38]. It is suggested that MF can prolong the life of free radical species and change some enzyme activities [39]. In our study, we observed that long-term ELF-MF exposure could not alter oxidative and antioxidative parameters on submandibular glands. However, we found that long-term 100 and 500 μT ELF-MF exposure can decrease catalase activity, which was one of bioindicator in antioxidation, on parotid gland. We also determined different response in two different salivary glands after ELF-MF exposure in relation to oxidative and antioxidative parameters. It is possible that these different response may be due to metabolic differences between the two glands, while the metabolism of parotid glands is predominantly aerobic, the metabolism of submandibular glands is predominantly anaerobic [40]. In view of this differences in metabolism, probably the parotid glands are more sensitive the action of the generated ROS than the submandibular glands.

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen [41]. It is also very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Furthermore, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second [41]. Because of catalase enzyme activity is one of the important enzyme to show antioxidative activity, catalase activity has been used in biological studies to explore antioxidative changes after physicochemical exposures. There are some studies, which is investigated the effect of ELF-MF on catalase enzyme activity. For example, Kula et al. [42] stated that ELF magnetic field (0.018 T, for 20 days, 2 h a day) increased significantly catalase activity in the liver and kidney of rats. Regoli et al. [43] reported that catalase activity decreased at in the land snail Helix aspersa after 50 μT ELF-MF exposure intensity up to 2 months. Falone et al.[17] demonstrated that 50 Hz, 100 μT MF exposure (for 10 days) did not affect catalase levels in brain cortices of youg rats. However, they reported that this exposure procedure reduced on catalase activity in brain cortices of aged rats [17]. Amara et al. [44] also determined that the exposure of rats to static magnetic field (128mT, 1 h/day during 30 consecutive days) decreased the activities of glutathione peroxidase (GPx), catalase (CAT) and the superoxide dismutase (SOD) in liver and kidney. Martinez-Samano et al. [45] reported no significant differences in CAT activity of rat's liver, heart, kidney and plasma after 2h ELF-MF (60 Hz, 2.4 mT). Sahebjamei et al. [46] reported that the activity of the catalase (CAT) of suspension-cultured tobacco cells was decreased by MF, compared with those of the control cells. In our previous study, we determined that CAT activity decreased in brain of rats, which was exposed to long-term ELF-MF [47]. However, in a recently published our study, it was concluded that long-term ELF-MF exposure did not affect oxidative or antioxidative processes including catalase activity, lipid peroxidation,or reproductive components such as sperm count and morphology in testes tissue of rats [48]. Duan et al. [49] reported

that catalase activity decreased significantly after ELF-MF exposure (50 Hz, 8 mT, 28 days) in the hippocampus and serum of mice. In the present study, it was determined that long-term ELF-MF exposure could cause decrease in CAT activity of parotid gland. However, it was demonstrated no significant differences in regarding to oxidative and antioxidative processes in submandibular gland and parotid gland except CAT activity. These results are in agreement with some studies, which reported that ELF-MF exposure can cause decrease CAT activity [17,43, 44, 46, 47,49]. However, some studies demonstrated that CAT activity increased or not altered after ELF-MF exposure, and oxidative/antioxidative processes were affected by ELF-MF. A possible reason for the inconsistencies between present study and other studies may be due to different experimental conditions. Exposure parameters such as frequency, waveform, intensity, duration and timing of MF exposure have generally not been controlled in the experiments and may be a cause for the inconsistent results [50].

CONCLUSIONS

We showed that long-term ELF-MF exposure did not affect oxidative, antioxidative processes and lipid peroxidation in submandibular gland of rats. However, 100 μT and 500 μT ELF-MF exposure could decrease only CAT activity and not alter other endpoints. Additionally, we can state that submandibular and parotid glands reacted differently to 100 and 500 microTesla ELF-MF.

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