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ELF Electromagnetic Field and Strontium Ranilate Influences on the Trace Element Content of Rat Teeth

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Abstract: Exposure to Extremely Low Frequency (ELF) Electromagnetic Field (EMF) emanating from the generation, distribution and utilization of electricity. The major debate in recent years has focused on the possibility that exposure to EMF may result some health consequences such as differentiation on bone constitute. In this study, the effect of ELF-EMFs and strontium ranelate on teeth constitute amount of mineral were investigated in rats. Seventy-five four month old adult female Sprague-Dawley rats were randomly divided into 5 different groups (n = 15). After all applications, some mineral levels such as: Ca, Mg and Zn in rat teeths were determined with Atomic Absorbtion Spectrophotometry (AAS) and the phosphorus content of teeth was determined by Ultraviolet Spectrophotometer (UVS). It was determined that the levels of Ca and P were not statistically different in comparison to Cg-Cnt and between groups ($p>0.05$). However, it was observed that the levels of Zn significantly alterations between some groups ($p<0.05$). The levels of Mg in ELF-EMF+OVX, ELF-EMF and OVX groups decreased significantly in comparison to Cg-Cnt group ($p<0.05$). As a result, it can be suggested that mineral amount of rat teeth can change after ovariectomy and ELF-EMF exposure, also strontium ranelate treatments can't increase mineral amount of teeth.

Key words: Magnetic field, strontium ranelate, teeth, trace elements, mineral

INTRODUCTION

Osteoporosis is an elevated fracture risk characterized by decreased bone mineral content in menopausal females. Due to the clinical importance of osteoporosis, there are being developed new treatment modalities each day. Strontium ranelate is a new drug that is shown to be effective in decreasing the risk of fractures in postmenopausal women. Investigations on strontium ranelate indicate that this drug is an antiosteoporotic medication possessing a physiological effect mechanism. Strontium ranelate synchronically improves bone formation and decreases bone resorption. This effect stabilizes bone cycle in favor of bone formation again (Akbulut *et al*., 2005; Ling-Ling *et al*., 2007; Reginster *et al*., 2007).

Strontium ranelate has been reported to have beneficial effects on bone. Strontium ranelate treatment of laying hens, which are susceptible to osteoporosis and bone fracture, improved the mechanical performance of whole bone, but had no effect on the estimated material properties of the bone tissue (Shahnazari *et al*., 2006).

Alternative other treatment regimens like EMF are proposed for osteoporosis. EMF had been used for the past 25 years to deal with different kinds of osteoporosis in both animal and clinical experiments. Experimental results suggested that EMF exposure stopped bone loss and exerted a preventive effect against bone loss of osteoporotic hindlegs (Chang and Chang, 2003). They also demonstrated that extremely low intensity, low frequency, single pulse electromagnetic fields significantly suppressed the trabecular bone loss and restored the trabecular bone structure in bilateral ovariectomized rats. They conclude that Pulsed Electromagnetic Field (PEMF) may be useful in the

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prevention of osteoporosis resulting from ovariectomy and that PGE_2 might relate to these preventive effects. The clinical results showed that bone mineral density of the treated radii increased significantly as compared to the contralateral control radii (Tabrah *et al*., 1990). Zhang *et al*. (2006) showed that many bone indexes are significantly elevated after rotary non-uniform Magnetic Field (RMF) exposure compared to the control Ovariectomized (OVX) group and confirmed mechanistic evidence that strong Magnetic Field (MF) exposure could effectively increase bone density and might be used to treat osteoporosis.

Some ions, such as Cu and Zn, have multiple functions as cofactors of enzyme activity and their variations have been suggested as part of the biological effects caused by EMF. Some authors suggested that these effects are caused by a direct interaction of the EMF and the cell membrane (Burchard *et al*., 1999). They also reported that exposure to electric and magnetic fields resulted in decreased concentrations of Mg in blood plasma and in increased concentrations of Ca and P and decreased concentrations of Fe and Mn in cerebrospinal fluid.

Trace elements play an important and complex role in the human and animal metabolism. The trace elements in teeth have been examined for a number of reasons, for example there are some studies of dental health where trace element concentrations have been correlated with the presence of dental caries. The mineral tissue of the tooth consists of hydroxyapatite crystals $Ca_{10}(PO_4)_6(OH)_2$ with incorporated trace elements, which can provide information of the habitat environment or dietary habits (Falla *et al*., 2005). Teeth are reported to be suitable indicators of trace element exposure for a wide range of effects (Brown *et al*., 2004). However, very little is known concerning the MF influence on electrolyte balance and concentration of some trace elements in teeth and there are no studies have quantified the changes of Ca, Mg, Zn and P in teeth of rat, which overectomized, treated to Strontium ranelate and exposed to ELF-EMF.

In the present experimental study, it was investigated the alterations of some mineral contents in teeth of rats that exposed to ELF-EMF, overectomized bilaterally and treated with Strontium ranelate.

MATERIALS AND METHODS

Animal care and preperations for experimental animals: The experiments were performed on 75 female Sprague-Dawley rats with initial weights of 157-226 g obtained from Medical Science Application and Research Center of Dicle University, aged 4 months at the beginning of the study. All rats were allowed free access to water and standard pelleted food diet (TAVAS Inc. Adana, Turkey) during the experimental period. The rats were divided into 5 groups (n = 15): Cage-Control (Cg-Cnt), Ovariectomy (OVX), ELF-EMF Exposure (ELF-EMF), ELF-EMF exposure with Strontium ranelate treatments and OVX application (ELF-EMF+Str.Ran+OVX), ELF-MF exposure with OVX application (ELF-EMF+OVX). All rats were subjected to bilateral ovariectomy except those in Cg-Cnt and ELF-EMF groups. Bilateral ovariectomy was performed before 4 days at the beginning of the experiments under ketamine anesthesia (100 mg kg^{-1}, intramuscularly) and ELF-EMF, ELF-EMF+Str.Ran+OVX and ELF-EMF+OVX animals were subjected to 1.5 mT ELF-EMF exposure during 6 months, 4 h a day starting fifth day after the surgery. ELF-EMF+Str.Ran+OVX group was used to test whether the parameters of the present study in Strontium ranelate treated rats was affected by ELF-EMF. The animals in this group received 308 mg kg^{-1} of Strontium ranelate (Protelos 2g, Les Laboratories Servier-France) a day orally. The animals were kept in 14/10 h light/dark environment at constant temperature of 22 ±3°C, 45 ± 10% humidity. This protocol was approved by the local ethics committee.

Magnetic field generation and exposure of rat to magnetic field: The MF was generated in a device designed by us that had 2 pair of Helmholtz coils of 70 cm in diameter in a Faraday cage (130×65×80 cm) that earthed shielding against the electric component (Fig. 1). This magnet was constructed by winding 125 turns of insulated soft copper wire with a diameter of 1.5 mm. Coils were placed vertically as facing one another. The distance

Fig. 1: The experimental setup

between coils was 47 cm. An AC current produced by an AC power supply (Adakom, Turkey) was passed through the device. The current in the wires of the energized exposure solenoid was 40 A for 1.5 mT, which resulted 50 Hz MF. The MF intensities were measured once per week as 1.5 mT in different 15 points of methacrylate cage with a Bell 7030 Gauss/Teslameter (F.W. Bell, Inc., Orlando, FL) to ensure homogeneity of the field during the course of the experiment by a person who is not involved in the animal experiment. Magnetic field measurements showed that, at the conditions of the experiment, the magnetic field exposure system produced a stable flux density of 1.5 mT and stable frequency of 50 Hz with neglible harmonics and no transients. The 50 Hz stray fields in the sham-exposure system were 0.1 μT. The static earth magnetic field was also measured with a Bell 7030 Gauss/Teslameter (F.W. Bell, Inc., Orlando, FL).

The component parallel to the exposure field was 16 μT and the component perpendicular to the exposed field was 37 μT. All field measurements were performed by persons not involved in the animal experiments. Observers were not aware of which group of rats was ELF Magnetic Field-or sham-exposed, i.e. the whole study was done blind. No temperature differences were observed between exposure and sham cages during the exposure. ELF-MF, ELF-MF + Str.Ran + OVX and ELF-MF + OVX animals were exposed to 1.5 mT ELF-MF exposure during 6 months, 4 h a day in methacrylate boxes (43×42×15 cm). OVX group were treated like ELF-MF + OVX group except ELF-MF exposure in methacrylate boxes. For the cage control, nothing applied to rats in this group and they completed their life cycle in the cage during the study period. The rats were free to move in a methacrylate cage inside the coils. Immediately after the last exposure, teeth of the animals were collected under ketamine anesthesia (100 mg kg^{-1}, intramuscularly) in sterile saline to measure the levels of mineral amount.

The teeth were placed in a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 500°C in 1 h. The samples were ashed for about 4 h until a white or grey ash residue was obtained. The residue was dissolved in 3 mL of the mixture of the 65% nitric acid (HNO_3), 30% hydrogen peroxide (H_2O_2) (3:1), when necessary, was heated slowly to dissolve the residue. The solution was transferred to a 10 mL volumetric flask and made up to the volume. The Calcium (Ca), Magnesium (Mg) and Zinc (Zn) content of teeth was determined by Atomic Absorption Spectroscopy (AAS) and the phosphorus content of teeth was determined by ultraviolet spectrophotometer (UV).

Statistical analysis: A computer program (SPSS 11.5, SPSS Inc., Chicago, IL, USA) was 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 Dunnet's procedure. All hypothesis tests used a criterion level of $\alpha = 0.05$.

RESULTS

It was determined that the levels of Ca and P were different but that is not as a statistically different in comparison to Cg-Cnt and between groups (Fig. 2 and 3) ($p>0.05$).

It was observed that the levels of Zn and Mg were statistically significant differentiation in rats' teeth of all groups in comparison to Cg-Cnt group (Fig. 4 and 5) ($p<0.05$).

The levels of Zn in all groups were increased significantly in comparison to Cg-Cnt group (Fig. 3) ($p<0.05$).

The levels of Mg in all groups were decreased significantly in comparison to Cg-Cnt group (Fig. 5) ($p<0.05$).

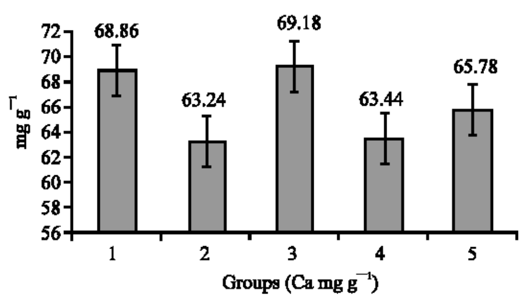

Fig. 2: Comparison of the Ca levels of the groups (1: ELF-EMF+OVX, 2:ELF-EMF+Str.Ran+OVX, 3: ELF-EMF, 4:OVX, 5:Cg-Cnt)

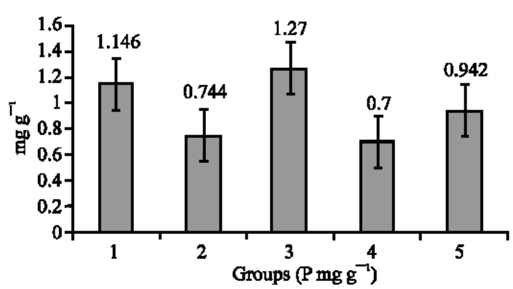

Fig. 3: Comparison of the P levels of the groups (1: ELF-EMF+OVX, 2:ELF-EMF+Str.Ran+OVX, 3: ELF-EMF, 4:OVX, 5:Cg-Cnt)

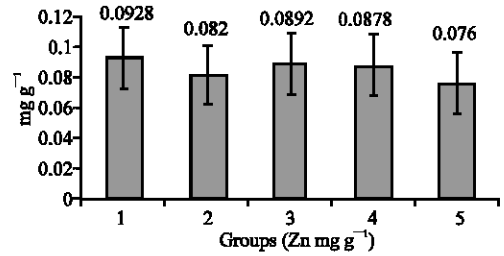

Fig. 4: Comparison of the Zn levels of the groups (1: ELF-EMF+OVX, 2:ELF-EMF+Str.Ran+OVX, 3: ELF-EMF, 4:OVX, 5:Cg-Cnt)

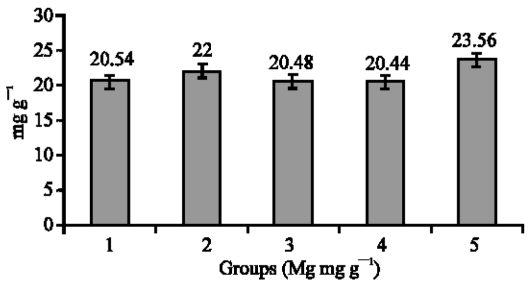

Fig. 5: Comparison of the Mg levels of the groups (1: ELF-EMF+OVX, 2:ELF-EMF+Str.Ran+OVX, 3: ELF-EMF, 4:OVX, 5:Cg-Cnt)

DISCUSSION

In the present study, no significant difference was found between Cg-Cnt and other groups in relation to Ca and P levels of rat teeth. The increase of Zn levels in all groups were found significant compared to cage control group. However, it was determined a significant decrease on Mg levels in all groups compare to Cg-Cnt group. The direct comparison of results from this research with previous studies is complex because of no body have been interested with this issue. So source of the tooth, sample population, tooth type, sample preparation and analytical methodology gave some difficulties. However, some useful comparisons can be made with control group. This was not detrimental to this research, as the primary aim was to compare the trace element content of teeth from experimental and nonexperimental.

There are very limited number of studies conducted influential on clinical and laboratory. One of them which is ELFMF stimulation doesn't promote gains in clinical attachment or alveolar bone level to the extend that it can be adjunct to conventional periodontal therapy (Steffensen *et al*., 1988) another study was about direct electric current which indicated that direct electric current is a potent biological means to initiate and/or accelerate periodontal tissue and alveolar bone turnover by affecting cellular enzymatic phosphorylation activity (Davidovitch *et al*., 1980).

The body of an adult normally contains about 1200 g of calcium. At least 99% of this is present in the skeleton, where calcium minerals, provide the hard structure of the bones and teeth. According to the results of Ca and P concentrations, It was suggested that ELF-EMF can not affect the levels of Ca and P in rat teeth. We also suggest that Ca and P concentrations in rat teeth can not change after OVX application and Strontium ranelate treatments.

Magnesium is one of the most abundant elements in the earth's crust. It is widespread in food particularly whole grain and green vegetables. The previous studies indicate that Mg is a cofactor in many enzyme system and play an important role in regular development of enamel and dentin, mineralization and binding of Ca and P (Ozbek *et al*., 2001). In this study, the decreasing of magnesium concentrations are agreement with the results of Burchard *et al*. (1999) which reported that exposure to electric and magnetic fields resulted in decreased concentrations of Mg in blood plasma (Burchard *et al*., 1999).

Zinc is an essential element for humans and is homeostatically regulated in the body (WHO). It has an important role in protein synthesis and is also a co-factor for many enzymes regulating cell growth and hormone levels, including regulation of gene transcription and growth factor metabolism. Zinc plays an important role in the formation and metabolism of mineralized tissues. Animal studies suggest that the concentration of zinc in teeth may reflect absorption of the metal (Tvinnereim *et al*., 1999). We suggested that ELF-EMF exposure, OVX application and Strontium ranelate treatments can increase Zn levels of rat teeth. The increasing of teeth can be due to by affecting Zn metabolism pathways in teeth or induce enzyme systems that affect Zn metabolism.

Enamel is the hardest and most highly mineralized substance of the body. 96% of enamel consists of mineral, with water and organic material comprising the rest. Dentin is made up of 70% inorganic materials, 20% organic materials and 10% water by weight; 90% of the organic material is collagen type 1 and the remaining 10% ground substance which includes dentin-specific proteins (Ten Cate, 1998). One possible explanation according to us why the trace elements of enamel and dentin were affected by ELF treatment is that while ELF magnetic field can altere chemical bonds of trace elements.

The fact that some environmental factors like magnetic fields may have some harmful effects continues to arouse more interest especially last 2 decades. Some literature has been reported that environmental effects such as ELMF can affect health status in accordance with the altered physiological conditions (Steffensen *et al*., 1998; Cetin and Malas, 2005; Al-Mahroos and Al-Saleh, 1997; Frank *et al*., 1998; Knave, 2001; Lyle *et al*., 1991). For this reason, further studies are needed to reveal the effects of environmental factors on health status and oral tissues more clearly.

CONCLUSION

This study has highlighted that the crowns of the experimental and non experimental rat teeth did contain significantly different concentrations of certain elements.

This study concludes that the concentrations of the major and trace elements Ca and P do not differ significantly, in rat teeth, on the contrary the trace element Mg and Zn do differ.

It is therefore concluded, that there is positive evidence in rat teeth, for the hypothesis that EMF is associated with don't differ Ca and P, reduced Mg and increased Zn amount. However, this does not exclude the possibility that EMF does play an important aetiological role in mineralization.

These data which were got from this research will help to the further epidemiological research which will be related with ELF-EMF and human.

The possibility of these differentiation being deficient in the elements warrants further investigation. The implications of excess Zn and its link with caries prevelans would also be an important area for further research.

We believe our results, which have been obtained from study on animals, should be further investigated by histologic, endocrinologic and epidemiologic studies.

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