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Effects of Extremely Low Frequency Magnetic Field on Pulp Tissue of Rats

[1]Sadullah Kaya, [3]M. Salih Celik, [3]Mehmet Zulküf Akdag, [1]Ozkan Adiguzel,
[2]Izzet Yavuz, [4]Yusuf Nergiz and [4]Ercan Ayaz
[1]Departmant of Operative Dentistry and Endodontics, [2]Departmant of Pediatric Dentistry,
Faculty of Dentistry, Dicle University, Diyarbakir, Turkey,
[3]Departmant of Biophysics, [4]Departmant of Histology,
Faculty of Medicine, Dicle University, Diyarbakir, Turkey

Abstract: This study aims to investigate the effect of Extremely Low Frequency Magnetic Field (ELF-MF) on dental pulp of ovariectomized rats. About 75 female Wistar-albino rats were randomly divided into 5 groups: cage-control, ovariectomy, ELF-MF exposure, ELF-MF exposure with strontium ranelate treatments and ovariectomy application, ELF-MF exposure with ovariectomy application. All groups except cage-control and ovariectomy groups were exposed to 1.5 mT ELF-MF exposure throughout 6 months, 4 h a day. After all applications, pulp tissues of rat teeth were investigated under light microscope. Between the groups, histological differences in collagen fibrils, fibroblasts, blood vessels and odontoblasts that create the pulp were found. We concluded that osteoporosis affected the odontoblasts and fibroblasts in rat dental pulp, ELF-MF exposure did not resolve the histopathological effect due to osteoporosis but enhanced this pathological effect, strontium ranelate was more efficient to eliminate the effects of osteoporosis than ELF-MF application.

Key words: Pulp, electromagnetic fields, strontium ranelate, ovariectomy, pathological effect, histopathological

INTRODUCTION

Osteoporosis is defined as a skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, predisposing to an increased risk of fracture (Vlasiadis *et al*., 2008). Osteoporotic fractures are associated with substantial morbidity and mortality risk (Friendlander, 2002; Melton, 2003). Some researchers have also established a significant association between postmenopausal osteoporosis and tooth loss (Krall *et al*., 1994; Taguchi *et al*., 1999; Kribbs, 1990; Krejc and Bissada, 2002; Jeffcoat *et al*., 2000). Dentists must investigate new treatment modalities to treat oral diseases due to osteoporosis.

Strontium ranelate is a new drug 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 mechanism of action. Strontium ranelate synchronically improves bone formation and decreases bone resorption. This effect stabilizes bone cycle in favor of bone formation once more (Akbulut *et al*., 2005; Zhu *et al*., 2007; Reginster *et al*., 2007). In addition to drug treatments for osteoporosis, some researchers have investigated alternative treatment regimes using Extremely Low Frequency Electromagnetic Field (ELF-EMF) and Pulsed Electromagnetic Field (PEMF) in both animal and clinical experiments.

In Chang and Chang (2003)'s recent bone histomorphometric study of ovariectomized rats revealed that considerable trabecular bone loss in the proximal tibiae was observed 1 month after bilateral ovariectomies.

They 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 (Chang and Chang, 2003). They conclude that PEMF may be useful in the prevention of osteoporosis resulting from ovariectomy (Chang and Chang, 2003). Several studies also found that ovariectomy could alter the bone turnover rate, i.e., bone formation rate and bone resorption rate which is induced by the disappearance of estrogen (Ikeda *et al*., 1996; Baldock *et al*., 1999; Tanizawa *et al*. 2000). Akpolat *et al*. (2009) determined that Bone Mineral Content (BMC) and

Corresponding Author: Sadullah Kaya, Departmant of Operative Dentistry and Endodontics, Faculty of Dentistry, Dicle University, Diyarbakir, Turkey

Bone Mineral Density (BMD) values decreased in overectomized rats and increased in rats exposed to ELF-MF.

The clinical results showed that bone mineral density of the treated radii increased significantly as compared to contralateral control radii (Tabrah *et al*., 1990). However, the same investigators continued another 8 year follow up study based on their previous clinical trial and revealed that no significant differences in bone mineral density of radii were found (Tabrah *et al*., 1998). 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.

A study conducted by Takayama *et al*. (1990) however had found that PEMF (square wave with frequency of 15 Hz, peak magnetic field strength of 15 G) did not show any protective effects against bone loss in estrogen deficient osteoporosis in rats in dicating that signal parameters of PEMF such as waveform, frequency, repetition rate in tensity of magnetic field or induced electric field affect PEMF efficacy.

Although, much is known regarding the biological effects of PEMF on osteoporosis both in human and experimental animal models and there are also some reports in relation to the interaction between osteoporosis and tooth lose, no data were found about the effect of ELF-MF, strontium ranelate on odontoblast and fibroblast cells of bilateral ovariectomized rats' teeth. Therefore, it was investigated the effect of ELF-MF, strontium ranelate on odontoblast and fibroblast cells of bilateral ovariectomized rats' teeth.

MATERIALS AND METHODS

Animal care and preparations 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):

Group 1 = Cage-Control (Cg-Cnt)
Group 2 = Ovariectomy (OVX)
Group 3 = Extremely Low Frequency Magnetic Field (ELF-MF)
Group 4 = ELF-MF exposure with OVX application (ELF-MF+OVX)
Group 5 = ELF-MF exposure with strontium ranelate treatments and OVX application (ELF-MF +Str.Ran+OVX)

All rats were subjected to bilateral ovariectomy except those in Cg-Cnt and ELF-MF groups. Bilateral ovariectomy was performed 4 days before the beginning of the experiments under ketamine anesthesia (100 mg kg^{-1} in tramuscularly) and 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 starting from the 5th day after the surgery. The animals in ELF-MF+Str.Ran+OVX group received 308 mg kg^{-1} of strontium ranelate (Protelos 2 g, Les Laboratories Servier-France) per day orally. The animals were kept in 14/10 h light/dark environment at a constant temperature of 22±3°C and 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. 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 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 a week as 1.5 mT in different 15 points of methacrylate cage with a Bell 7030 Gauss/Teslameter (F.W. Bell in c., Orlando, FL) to ensure homogeneity of the field during the course of the experiment by a person who was 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 negligible harmonics and no transients. All field measurements were performed by the persons not involved in the animal experiments. Observers were not aware of which group of rats was ELF-MF or other groups, 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) and fixed in 10% formalin to observe the histological alterations.

Tissue preparation for light microscopy: Each group consisted of 15 rats and 2 specimens were obtained from each of the rats (30 specimens per group). After decalcification with 5% nitric acid, specimens were rinsed in 0.1 M phosphate-buffered saline (pH 7.4). They were then dehydrated in alcohol and embedded in paraffin. Serial sections were cut horizontally at a thickness of 5 μm.

Subsequently, they were dewaxed, rehydrated and rinsed in phosphate-buffered saline. Sections were stained with Hematoxylin and Eosin (H/E) and Periodic Acid Schiff (PAS) and investigated by light microscopy (BH2, Olympus, Tokyo, Japan). The sections were evaluated by different examiners in a blinded fashion (Examiners were not aware of which group of sections was Cg-Cnt, OVX, ELF-MF, ELF-MF+OVX and ELF-MF+Str.Ran+OVX). The histological findings of the samples were interpreted and compared.

RESULTS AND DISCUSSION

Normal structures of collagen fibrils, fibroblasts and odontoblasts which form the pulp of the teeth of the rats in cage control group were observed (Fig. 1). Collagen structures were not observed in the central pulp, irregularity in the shapes of the fibroblasts and odontoblasts was seen in molar teeth from ovariectomy group (Fig. 2). Oedema interstitial hemorrhagia, collagen loss and decomposition of odontoblast arrangement in tooth pulp of ELF-MF group were remarkable (Fig. 3). Extensive loss of collagen tissue along with thrombosis and irregularity in odontoblasts were observed in the sections of molar teeth of ovariectomy+ELF-MF group (Fig. 4). No pathology except aspiration of odontoblasts into dentin tubules was observed in molar tooth pulp of the rats from ovariectomy+ELF-MF+strontium ranelate group (Fig. 5). Interest on the Effects of Electromagnetic Fields (EMFs) has greatly increased in recent years,

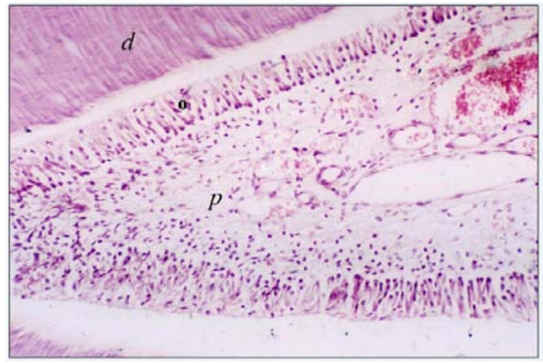

Fig. 1: Histological view of an incisor tooth from control group. Normal structures of collagen fibrils, fibroblasts, blood vessels and odontoblasts that form the pulp. d:dentin; o:odontoblast; p:pulpa; (PAS, original magnification ×80)

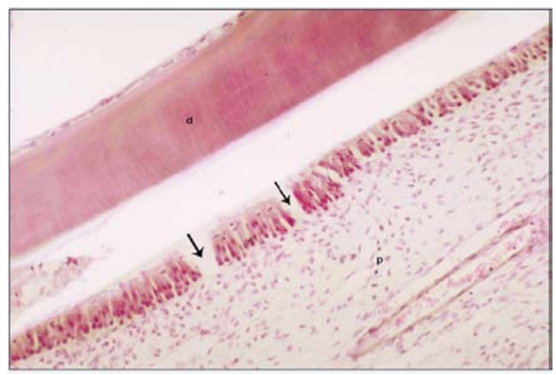

Fig. 2: A cross-section of a molar tooth from ovariectomy group. Decomposition and irregularity in patches in the odontoblast layer. p:pulp; ↓:decomposition points; (PAS, original magnification ×80)

especially in terms of their possible effects, their potential therapeutic and diagnostic applications (Albanese *et al*., 2009). Despite the large number of studies regarding the effects of ELF-MF on biological systems, there are very limited studies explaining the effects of long-term EMF exposure on human teeth tissues in a complete manner (Kaya *et al*., 2008a; Yavuz *et al*., 2008; Tumen *et al*., 2009; Adiguzel *et al*., 2008; Kaya *et al*., 2008b). In addition, risk of long-term ELF-MF exposure for the adverse health outcomes is a central issue in the study of possible ELF-MF health researches (Akdag *et al*., 2009). Therefore in the present study, it was investigated the effects of long-term ELF-MF exposure, osteoporosis and strontium ranelate on rat teeth pulp.The ovariectomized rat is considered an appropriate model for investigating about human postmenopausal osteoporosis because of many similarities in their pathophysiological mechanisms

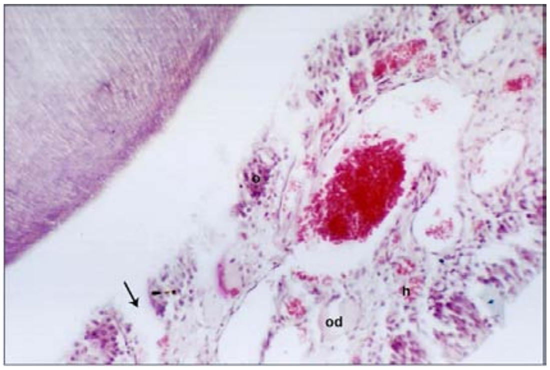

Fig. 3: Histological view of a molar tooth from extremely low frequency magnetic field group. Decomposition in odontoblast layer, oedema and interstitial hemorrhagia are seen o:odontoblast; od:oedema; h:hemorrhagia; ↓:decomposition. (HE, original magnification ×80)

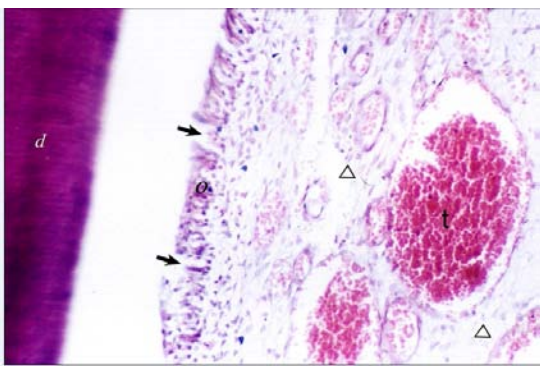

Fig. 4: Histological view of a molar tooth from ovariectomy+electromagnetic field group. Decomposition and irregularity in odontoblast layer along with collagen loss and thrombosis are seen. o:odontoblast;Δ:collagen loss; t:thrombosis; ↓:decomposition points; d: dentin. (HE, original magnification ×80)

(Kalu 1991; Wronski and Yen, 1991; Comelekoglu *et al*., 2007). Bone Mineral Density (BMD) and content measurements are generaly used for detecting osteoporosis and Dual-Energy X-ray Absorbsiometry (DEXA) is a noninvasive method that provides good precision for BMD and BMC assessments (Comelekoglu *et al*., 2007; Dequeker and Mundy, 1998). In other part of the present study, Akpolat *et al*. (2009) found that Bone Mineral Content (BMC) and bone mineral density values were decreased significantly in OVX groups' rats as comparing to cage-control group. Although there are studies on that postmenopausal osteoporosis causes to tooth loss, no study exists

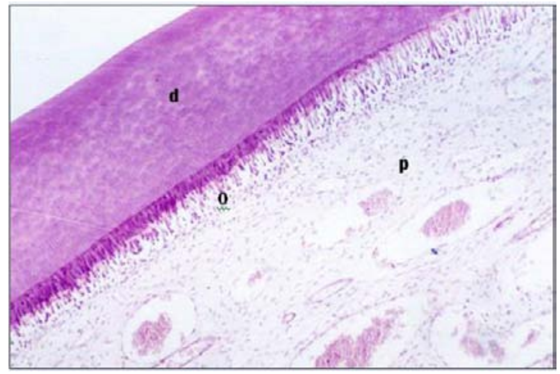

Fig. 5: Across-section of a molar tooth from ovariectomy+electromagnetic field+strontium ranelate group. A partial improvement in the odontoblast cell layer and in the pulp is remarkable. d:dentin; o:odontoblast; p:pulp. (HE, original magnification ×80)

about the effect of osteoporosis on tooth tissue in cellular level and about the extent to which strontium ranelate restores the pathology resulted from the osteoporosis by altered micro-architectural structure of pulp. Moreover, there are no data on how much the application of ELF magnetic field influences the oral pathology caused by osteoporosis. This present experimental study according to the observers opinion defined the effect of osteoporosis on dental pulp tissue in histological level. Furthermore, it was tried to identify whether the effect of ELF magnetic field determined in bone tissue by experimental and clinical studies occurs also in dental tissue.

Collagen structure of molar teeth from ovariectomy group was not observed in central pulp and an irregularity in shape of fibroblasts was seen. These data did not show that osteoporosis was a mechanism affecting dental pulp (Fig. 2). The comparison of the changes in dental pulps of ovariectomy+ELF-MF group with that of ovariectomy group showed that ELF-MF exposure could enhance the changes due to ovariectomy because ELF-MF application caused to pathologies such as collagen loss and thrombosis in rat dental pulp. The data that we achieved in this study suggest that ELF-MF application cannot be used to eliminate the changes in rat dental pulps resulted from ovariectomy; conversely, it leads to further pathologies such as inflammatory events in the pulp.

Moreover, the pathologies such as oedema in terstitial hemorrhagia, collagen loss and decomposition of odontoblast arrangement observed in the dental pulp of the rats exposed only to ELF-MF application show that ELF-MF exposure in the intensity that we used in the study is not accurate for the dental tissue of normal rats.

Kaya *et al.* (2008a) found abnormal histopathologic changes such as vasodilatation and focal bleeding areas in pulp of the rats that exposed to the 900 MHz RF radiation. The results of the present study are in accordance with the results determined by Kaya *et al.* (2008b).

In the study, there was no pathology except the aspiration of odontoblasts into dentin tubules in molar teeth pulps of the rats from ovariectomy+ELF-MF+strontium ranelate group (Fig. 5). With these data, it can be said that strontium ranelate can prevent the severe inflammatory reactions caused by ovariectomy plus ELF-MF together. A previous research suggested that ovariectomy+ELF-MF exposure and strontium ranelate application treatments could increase Zn levels in rat teeth (Tumen *et al.*, 2009). According to us, the increase in Zn levels can affect Zn metabolism pathways in pulp tissue in ducing enzyme systems that affect pulp metabolism.

Human teeth may be exposed to a wide range of EMF that may put the vital function of the pulp at risk. Yavuz *et al.* (2008) reported that EMF can have significant effects on teeth mineral content but there are no studies about the effect of EMF on the teeth pulp structure. Yavuz *et al.* (2008) and Glassman *et al.* (1986) suggested that since fibroblast behavior in bone healing can be altered electrically, it is plausible to hypothesize that fibroblast proliferation and function in soft tissue healing also would respond to an electromagnetically induced pulse, these data gave us the opinion of that EMF could be useful for dental treatment and the repair of pulp tissue, that is conflict opinion according to the results. In the present study, rats were used because of their easier availability; however because of the differences in body size, geometry and physiological responses, the extrapolation of these results into human is not straightforward and any such comparison should be made with great caution.

CONCLUSION

In conclusion, it was determined that odontoblast and fibroblast of dental pulp can affect in rats, subjected to bilateral ovariectomy; ELF-MF exposure can induce this effect. We concluded that strontium ranelate can more efficient agent to decrease effects, due to ovariectomy applications and ELF-MF exposure that we used in the study. However, we believe that the results which were obtained from the animal studies should be investigated further by histologic, endocrinologic and epidemiologic studies. Further studies on these topics will enhance the understandings regarding to the effects of ELF-MF on the pulp cells activities.

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