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Effects of ELF-MF and Strontium Ranilate on Periodontium in Rats (Duble Blind)

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Abstract: In the present experimental study, it was investigated the periodontium of rats that exposed to Extremely Low Frequency-Magnetic Field (ELF-MF), overectomized bilaterally and treated with Strontium Ranelate (SR). The experiments were performed on 75 female Sprague-Dawley (4 months) rats. The rats were divided into five groups (n = 15). All rats were subjected to bilateral ovariectomy except those in I and III groups. Bilateral ovariectomy was performed before 4 days at the beginning of the experiments under ketamine anesthesia (100 mg kg^{-1}, intramuscularly) and II, IV and V groups animals were subjected to 1.5 mT ELF-MF exposure during 6 months, 4 h a day starting 5th day after the surgery. There were statistically significant differences among the all groups in the case of periodontal ligament, alveolar bone and gingiva ($p<0.05$). As a result of the study there were different levels of histopathologic changes occured between control and experimental changes in the case of all periodontal tissue examination. These results may hypothese that both ELF-MF and Strontium renalate have effects on periodontal tissues.

Key words: Extremely low frequency-magnetic field, strontium ranelate, periodontium, osteoporosis, histopathologic, Turkey

INTRODUCTION

Numerous sources of Electromagnetic Fields (EMF) exist in nature and in the occupational and residential environments. In nearly all instances, these fields pose no obvious threat to human health or safety and are generally discussed as an inevitable by-product of modern technology. Volta discovered the battery; numerous trials have been performed to use electrcal stimulation in medicine.

The discovery of piezoelecticity (Fukada and Yasuda, 1957) and bioelectric potentials (Friedenberg and Brighton, 1996) in bone raised the possibility that externally applied electric energy could modify the behavior of bone cells (Bassett *et al*., 1964; Bassett, 1982).

Bassett (1982) was the first to use a pair of Helmholtz coil to produce a magnetic field across a fracture site and enhance osteogenesis. After Yasuda induced osteogenesis by electrical stimulation in 1953; the use of electricity in medicine was accelerated (Kubota *et al*., 1995). Recently, electically induced osteogenesis has been studied intensely, both *in vivo* and *in vitro* (Kubota *et al*., 1995; Fredericks *et al*., 2000). Despite the clinical success, negative reports on the *in vitro* effects of electric stimulation on cellular proliferation, differentation and bone formation were reported (Norton, 1982; Iannacone *et al*., 1988).

Chang and Chang (2003) 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 prevention of osteoporosis resulting from ovariectomy and that PGE_2 might relate to these preventive effects Chang and Chang (2003). 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 (Zhang *et al*., 2006).

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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; Zhu *et al*., 2007; Reginster *et al*., 2007).

The periodontium consists of the investing and supporting tissues of the tooth (gingiva, Periodontal Ligament (PL), cementum, Alveolar Bone (AB)) (Carranza and Newman, 1996). The loss of ovarian function at menapause is assciated with loss of oral bone (Hildebolt *et al*., 2002). The impact of estrogen deficiency and osteopenia/osteoporosis on periodontal disease is unclear partially due to the lack of longitudinal studies evaluating clinical signs of gingival inflammation and periodontitis progression (Reinhardt *et al*., 1999).

In the present experimental study, it was investigated the periodontium 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 five groups (n = 15): cage-control (Cg-Cnt, I group), ovariectomy (OVX, II group), ELF-MF exposure (ELF-MF, III group), ELF-MF exposure with Strontium ranelate treatments and OVX application (ELF-MF+SR+OVX, IV group), ELF-MF exposure with OVX application (ELF-MF+OVX, V group). All rats were subjected to bilateral ovariectomy except those in Cg-Cnt and ELF-MF groups. Bilateral ovariectomy was performed before 4 days at the beginning of the experiments under ketamine anesthesia (100 mg kg^{-1}, intramuscularly) and ELF-MF, ELF-MF+SR+OVX and ELF-MF+OVX animals were subjected to 1.5 mT ELF-MF exposure during 6 months, 4 h a day starting 5th day after the surgery. ELF-MF+SR+OVX group was used to test whether the parameters of the present study in Strontium ranelate treated rats was affected by ELF-MF. The animals in this group received 308 mg kg^{-1} of Strontium ranelate (Protelos 2 g, 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 two 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 between coils was 47 cm. An AC current produced by an AC power supply (DAYM, 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

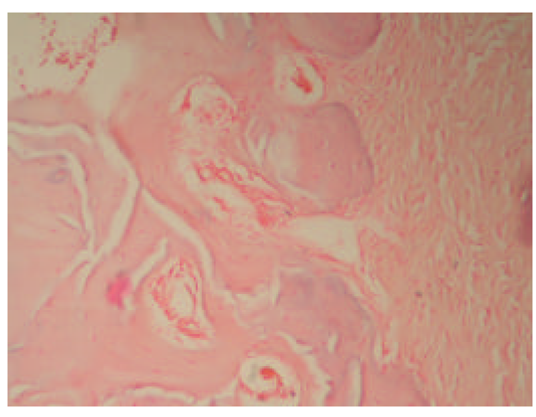

Fig. 1: An example of alveolar bone in group I (HE×200)

were observed between exposure and sham cages during the exposure. Animals in the groups III, IV and V were exposed to 1.5 mT ELF-MF exposure during 6 months, 4 h a day in methacrylate boxes (43×42×15 cm). II group were treated like V group except ELF-MF exposure in methacrylate boxes. For the I group, nothing applied to rats in this group and they completed their life cycle in the cage during the study period. The rats were free in methacrylate cage inside the coils. Immediately after the last exposure, total body images of the animals were obtained with a DEXA scanner having the Coefficient Variations (CV) 3% for BMC, 2.1% for BMD.

Accuracy: About 1% (based on hydroxyapatite phantom) (Hologic, Discovery QDR 4500A Series bone densitometer, Hologic Corp, USA) using small animal scan software available from Hologic. The scan resolution was 0.5 mm and scan speed was 60 mm sec^{-1}.

The blood of the animals was collected by cardiac puncture under ketamine anesthesia (100 mg kg^{-1}, intramuscularly) to kill rats and maxillary posterior teeth with the surrounding soft and hard tissues were extripated completely. Tissues was fixed in 10% buffered formalin.

Histo-pathological procedure: Histopathologic double blind evaluations were performed by pathologists of Dicle University, Faculty of Medicine, Department of Pathology and Canakkale 18 Mart University, Faculty of Medicine, Department of Pathology, who had no knowledge of each other. Samples were fixed in 10% buffered formalin and fixed in Bouin's fixative. All samples were processed in paraffin. Consecutive 4 micron thick sections were stained with haematoxylin and eosin and examined microscopically.

Statiscal analysis: Exact test, Pearson Chi-square test and Comporision of Proportion tests were used in analyses. SPSS-15.0 for Windows ve med calc version 9.4.2.0 stathistic software programs were used to make calculations and evaluations.

RESULTS

By the histopathologic evaluation abnormal changes like vasodilatation was determined in periodontal ligament, alveolar bone and gingiva among some individuals (Fig. 1-5). These degenerations were in different levels. Because of this, the vasodilatation scores were recorded as no, mild and severe. The abnormal change rates are shown as percentages in tables and the differences among the groups are evaluated statistically. The vasodilatation rates and comparisions are shown in Table 1. There was no changes cement tissue among all the groups because of this these tissues are not shown in Table 1.

There were statistically significant differences among the all groups in the case of periodontal ligament, alveolar bone and gingiva ($p<0.05$) (Table 2).

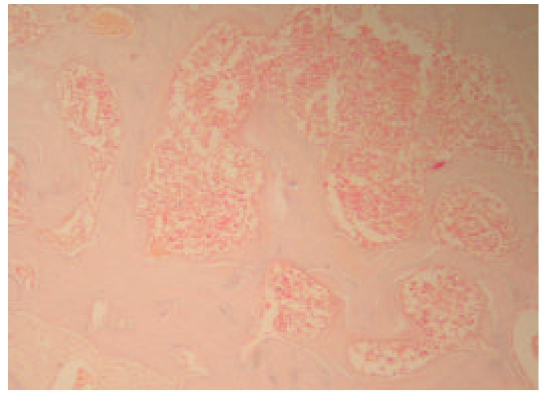

Fig. 2: An example of alveolar bone in group II (HE×100)

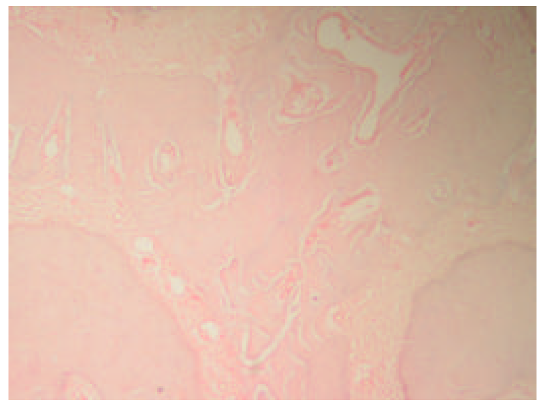

Fig. 3: An example of alveolar bone and periodontal ligament in group III (HE×100)

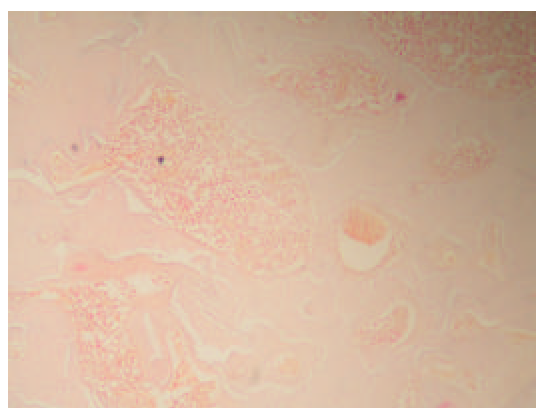

Fig. 4: An example of alveolar bone in group IV (HE×100)

Table 1: The vasodilatation rates and comparisions

	Periodontal ligament			Alveaolar bone			Gingiva		
Groups	No (%)	Mild (%)	Severe (%)	No (%)	Mild (%)	Severe (%)	No (%)	Mild (%)	Severe (%)
I group (n = 15)	46.7 (n = 7)	53.3 (n = 8)	0	60.0 (n = 9)	33.3 (n = 5)	6.7 (n = 1)	100 (n = 15)	0	0
II group (n = 15)	26.7 (n = 4)	26.7 (n = 4)	46.7 (n = 7)	26.7 (n = 4)	33.3 (n = 5)	40.0 (n = 6)	66.7 (n = 10)	33.3 (n = 5)	0
III group (n = 15)	20.0 (n = 3)	26.7 (n = 4)	53.3 (n = 8)	20.0 (n = 3)	26.7 (n = 4)	53.3 (n = 8)	26.7 (n = 4)	20.0 (n = 3)	53.3 (n = 8)
IV group (n = 15)	26.7 (n = 4)	66.7 (n = 10)	6.7 (n = 1)	26.7 (n = 4)	60.0 (n = 9)	14.3 (n = 2)	26.7 (n = 4)	66.7 (n = 10)	6.7 (n = 1)
V group (n = 15)	20.0 (n = 3)	60.0 (n = 9)	20.0 (n = 3)	26.7 (n = 4)	53.3 (n = 8)	20.0 (n = 3)	33.3 (n = 5)	53.3 (n = 8)	13.4 (n = 2)
	χ^2 = 24.670			χ^2 = 25.329			χ^2 = 42.764		
	p = 0.005*			p = 0.004*			p = 0.000*		

*p<0.05

Table 2: The comparision of the groups in the aspect of vasodilatation among the evaluated tissues

	Periodontal ligament		Alveaolar bone		Gingiva	
Groups	χ^2	p	χ^2	p	χ^2	p
I-II	6.716	0.0096*	NS	-	NS	-
I-III	8.344	0.0039*	NS	-	14.342	0.0002*
I-IV	NS	-	10.159	0.0014*	12.160	0.0005*
I-V	NS	-	8.344	0.0039*	8.344	0.0039*
II-III	NS	-	NS	-	8.344	0.0039*
II-IV	4.258	0.00391*	3.902	0.0482*	NS	-
II-V	NS	-	NS	-	NS	-
III-IV	5.695	0.01700*	7.284	0.0070*	4.805	0.0269*
III-V	NS	-	4.725	0.0297*	5.695	0.0170*
IV-V	NS	-	NS	-	NS	-

*p<0.05; NS: No Significant

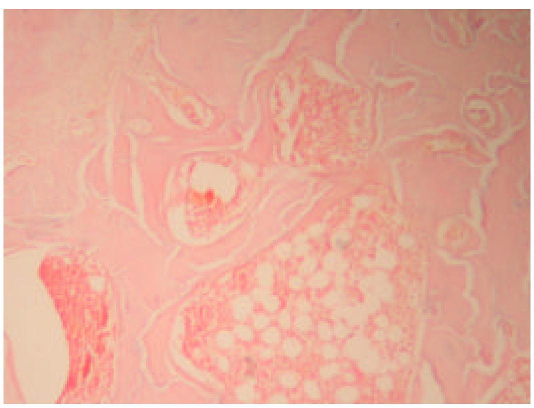

Fig. 5: An example of alveolar bone in group V (HE×200)

Periodontal ligament: The lowest vasodilatation levels were observed in I group (46.7%) and the highest in III group (53.3%). It was determined that the differences between the groups of I-II, I-III, II-IV and III-IV were statitically significant (p<0.05).

Alveolar bone: The lowest vasodilatation levels were observed in I group (46.7%) and the highest in III group (53.3%). It was determined that the differences between the groups of I-IV, I-V, II-IV, III-IV and III-V were statitically significant (p<0.05).

Gingiva: The lowest vasodilatation levels were observed in I group (0%) and the highest in III group (53.3%). It was determined that the differences between the groups of I-III, I-IV, II-V, II-III, III-IV and III-V were statitically significant (p<0.05).

DISCUSSION

MF had been used for the past 25 years to deal with different kinds of osteoporosis in both animal and clinical experiments (Chang and Chang, 2003). Experimental results suggested that MF exposure stopped bone loss and exerted a preventive effect against bone loss of osteoporotic hindlegs (Kubota *et al*., 1995).

SR has been reported to have beneficial effects on bone. SR 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).

Systemic bone loss has been proposed as a risk factor for periodontal disease however, the relationship between these two diseases is still not clear. In recent studies it was suggested that osteoporosis formation in postmenopausal individuals is a risk indicator for periodontal disease (Tezal *et al*., 2000).

By the light of all these data we investigated the effects of ELF-MF and SR on the periodontium of ovariectomised rats histopathologically double blind.

This research is performed on rats because the aim was to investigate the periodontal tissues histopathologically. By this way we could be able to evaluate the structural and cellular changes in periodontium. Long-term animal exposure studies are difficult to accomplish and expensive. Ideally, constant environmental conditions should be maintained throught the experimental period, the handling of test animals should be rigidly controlled and standart operating procedures should be developed and followed. AIso in the study the same standart were maintained.

There are very limited number of studies conducted influential on clinical and laboratory. One of them which is ELF-MF 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).

Several investgators have subsequently studied the effects of electrical currents on osteogenesis in animals usin g different electrical modalities such as direct current (Friedenberg and Kohanim, 1968), pulsed voltage sources, (Levy and Rubin, 1972) constant-current sources (Friedenberg *et al*., 1970) and alternating currents (Iida *et al*., 1956).

Clinical studies in humans have reported enhanced healing and bone reorganization of nonunion fractures and pseudoarthroses by electrical stimulation following long-term unsuccessful conventional treatment (Ozbek *et al*., 2001; WHO, 1996; Tvinnereim *et al*., 1999; Tencate, 1998; Zaffe *et al*., 1998). Although, a major part of these results are derived from uncontrolled studies or case reports, there seems to be evidence of a positive effect of electrical impulses (Cetin and Malas, 2005; Al-Mahroos and Al-Saleh, 1997).

Vera *et al*. (1999) investigated the long term effects of 50 Hz MF on bone density in their study on 120 mices (Males 12 weeks and females 14 weeks). They observed slight alterations in some parameters like total density, total and trabecular area that showed quantitative alterations making sexual dimorphism decrease or disappear. In other parameters such as total mass or cortical and trabecular mass, the experimental MF made sexual differences increase. These slight variations were possibly induced by alterations in the experimental MF in rhythm of sex and adrenal hormone secretion. In the study control groups were also constituted of female animals to prevent the hormonal differences effects on study results. In the study, slight histopathologic periodontal changes were observed in group I (control group) and most severe in group III (non ovariectomised and only ELF-MF exposure group). This can be hypothesed that ELF-MF has more effects on young and non osteoporotic individuals.

By the aspect of the comparision of the experimental groups; there was no statistical difference observed in the case of all periodontal tissues of the groups only ELF-MF and ELF-MF together with Strontium ranelate. On the other hand the severity of vasodilatation was the lowest of the group SR (IV) among all the groups. This result may indicate that SR reduces the effects of ELF-MF or can be said that SR retards the development of osteoporosis.

As a result of the study there were different levels of histopathologic changes occured between control and experimental changes in the case of all periodontal tissue examination. These results may hypothese that both ELF-MF and Strontium renalate have effects on periodontal tissues.

CONCLUSION

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. ELF-MF and SR are also the two of these modals. Although, the relation between peridontal diseases and osteoporosis has not been clearly identified, there are studies which establish that there are relations between them and we tried to find the answer of the question are ELF-MF and SR effective on periodontal tissues which are beginned to be used in the treatment of the postmenopausal individuals. Due to be the first study in this topic, we could not compare the results with others. But the differences between control and experimental groups indicate that there is need to further studies on this matter. Clearifying of this may prove to include different treatment modals like ELF-MF and SR to periodontal treatment.

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