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Effects of Extremely Low Frequency Magnetic Fields on Periodontal Tissues and Teeth in Rats

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Abstract: The aim of this study is to research the effects of ELF magnetic fields on periodontal tissues and teeth in rats histopathologically. The experiments were performed on 27 male Sprague-Dawley rats, aged 4 months at the beginning of the study, weighing (342.4±38.89) g and fed with standard pelleted food. The rats were divided into three groups; two experimental and one control (sham). Experimental groups were exposed to 100 and 500 μT ELF magnetic fields during 10 months, 2 h a day, respectively. Third group was sham that were treated like experimental group except ELF magnetic fields exposure in methacrylate boxes. There was no statistical difference between the experimental groups among all tissues ($p>0.05$). Alveolar bone, pulp and gingiva there were statistically significant differences among the groups of sham and 1st experimental, sham and 2nd experimental ($p<0.05$). The statistical difference between the experimental and sham groups in the study makes us to think that ELF magnetic fields may have effects on periodontal tissues and teeth.

Key words: ELF magnetic field, periodontal tissues, teeth, rats, pulp, gingiva, Turkey

INTRODUCTION

Everyone is exposed to a complex mix of electromagnetic fields of different frequencies that permeate the environment. Exposures to many electromagnetic fields, frequencies are increasing significantly as technology advances unabated and new applications are found (http://www.who.int).

While the enormous benefits of using electricity in everyday life and health care are unquestioned during the past 20 years the general public has become increasingly concerned about potential adverse health effects of exposure to electric and magnetic fields at Extremely Low Frequencies (ELF). Such exposures arise mainly from the transmission and use of electrical energy at the power frequencies of 50/60 Hz. Sources and typical upper limits of ELF magnetic fields found in the community, home and workplace are given below.

There are established biological effects from acute exposure at high levels (well above 100 μT) that are explained by recognized biophysical mechanisms. External ELF magnetic fields induce electric fields and currents in the body which at very high field strengths cause nerve and muscle stimulation and changes in nerve cell excitability in the central nervous system (http://www.who.int). Much of the scientific research examining long-term risks from ELF magnetic field exposure has focused on childhood leukaemia (Washburn *et al.*, 1994) and cancers in workers (Savitz, 1995; Kheifets *et al.*, 1995; Miller *et al.*, 1996). A number of other adverse health effects have been studied for possible association with ELF magnetic field exposure. These include other childhood cancers, cancers in adults, depression, suicide, cardiovascular disorders, reproductive dysfunction, developmental disorders, immunological modifications, neurobehavioural effects and neurodegenerative disease.

However, the epidemiological evidence is weakened by methodological problems such as potential selection bias. In addition, there are no accepted biophysical

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mechanisms that would suggest that low-level exposures are involved in cancer development. Thus, if there were any effects from exposures to these low-level fields, it would have to be through a biological mechanism that is as yet unknown. Additionally, animal studies have been largely negative. Thus, on balance, the evidence related to childhood leukaemia is not strong enough to be considered causal (http://www.who.int).

The periodontium consists of the investing and supporting tissues of the tooth (gingiva, Periodontal Ligament (PL), cementum, Alveolar Bone (AB)). The gingiva is the part of the oral mucosa that covers the alveolar processes of jaws and surrounds the necks of the teeth. The periodontal ligament is the connective tissue that surrouns the root and connects it with the bone. It is continuous with the connective tissue of the gingiva and communicates with the marrow spaces through vascular channels in the bone. Cementum is the calcified mesenchymal tissue that forms the outer covering of the anatomic root. There are two mian types of root cementum: Acellular (primary) and cellular (secondary). The alveolar process is the portion of the maxilla and mandible that forms and supports the tooth sockets (alveoli). It forms when the tooth erupts to provide the osseous attachment to the forming periodontal ligament; it disappears gradually after the tooth is lost (Carranza and Newman, 1996).

The researchers have examined all the studies in which the ELF magnetic fields effects on health were invesigated. The researchers did not encounter any histopathologic study focused on the ELF magnetic fields effects on periodontal tissues or teeth. The aim of this study is to research the effects of ELF magnetic fields on periodontal tissues and teeth histopathologically.

MATERIALS AND METHODS

Subjects and animal care: The experiments were performed on 27 male Sprague-Dawley rats obtained from Medical Science Application and Research Center of Dicle University, aged 4 months at the beginning of the study, weighing (342.4±38.89) g and fed with standard pelleted food (TAVAS Inc. Adana, Turkey). The rats were divided into three groups; two experimental and one control (sham). The animals were kept in 14/10 h light/dark environment at constant temperature of 22±3°C, 45±10% humidity. All animal procedures were in agreement with the Principles of Laboratory Animal Care and the rules of Scientific and Ethics Committee of Dicle University Health Research Center.

Magnetic field generation and exposure of rat to magnetic field: The magnetic fields was generated in a device designed by us that had one pair of Helmholtz coils of 25 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 225 turns of insulated soft copper wire with a diameter of 1.0 mm. Coils were placed horizontally as facing one another. The distance between coils was 25 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 0.12 A for 100 μT and 0.50 A for 500 μT which resulted 50 Hz magnetic fields. The magnetic fields intensities were measured once per week as 100 and 500 μT in different 15 points of methacrylate cage by using digital teslameter (Phywe, 209101074, Gottingen, Germany) to ensure homogeneity of the field during the course of the experiment. Magnetic field measurements showed that at the conditions of the experiment, the magnetic field exposure system produced a stable flux density of 100, 500 μT 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 measured with a Bell 7030 Gauss/Teslameter (F.W. Bell, Inc., Orlando, FL).

Fig. 1: The experimental setup

The component parallel to the exposure field was 14 μT and the component perpendicular to the exposed field was 34 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 coils during the exposure. The 1st (n = 10) and 2nd (n = 10) experimental groups were exposed to 100 and 500 μT ELF magnetic fields during 10 months, 2 h a day, respectively. Third group (n = 7) was sham that were treated like experimental group except ELF magnetic fields exposure (corresponding to 1st and 2nd groups, respectively) in methacrylate boxes (17×17× 25 cm). The rats were free in methacrylate cage inside the coils. After 10 months of magnetic fields exposure, the study was terminated. Immediately after the last exposure, 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 evaluation was performed in Dicle University, Pathology Department. Tissues was fixed in 10% buffered formalin and fixed in Bouin's fixative. All tissues were processed in paraffin. Consecutive 4 mm thick sections were stained with haematoxylin and eosin and examined microscopically. Histological and pathological assessments were done with a light microscope.

Statiscal analysis: Exact test, Pearson Chi-square (χ^2) 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 and focal haemorragies areas were determined in periodontal ligament, alveolar bone, gingiva and pulpa among some individuals. 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 Table 1 and 2 and the differences among the groups are evaluated statistically. The vasodilatation rates and comparisions are shown in Table 1, focal haemorragies areas comparision in Table 2. There were no changes in cement, dentin and enamel tissues among all the groups because of this these tissues are not shown.

In Table 1, the tissues vasodilatation percentages are shown. Although, there was no statistical difference between the control and sham groups among all tissues ($p>0.05$) in the case of alveolar bone, pulp and gingiva there were statistically significant differences among the groups of sham and 1st experimental, sham and 2nd experimental ($p<0.05$).

In the case of alveolar bone: In the 1st experimental group in 2 individual mild (Fig. 2) and in 8 individual no vasodilatation was determined.

In the 2nd experimental group in 4 individual severe (Fig. 3) and 6 individual no changes were determined. In the sham group in 1 individual mild and in 6 individual no vasodilatation (Fig. 4) was determined.

In the case of pulp: In the 1st experimental group in 4 individual mild in 2 individual severe and in 4 individual no vasodilatation was determined. In 2nd experimental groups in 3 individuals severe and in 4 individuals, no vasodilatation was determined. In the sham group in 7 individual no vasodilatation was established.

Table 1: The comparision of the groups in the aspect of vasodilatation among the evaluated tissues

	Periodontal ligament			Alveaolar bone			Gingiva			Pulp		
Groups	No	Mild	Severe	No	Mild	Severe	No	Mild	Severe	No	Mild	Severe
I (%)	70.0	20.0	10.0	80.0	20.0	0	90.0	10.0	0	40.0	40.0	20.0
n = 10	n = 7	n = 2	n = 1	n = 8	n = 2	-	n = 9	n = 1	-	n = 4	n = 4	n = 2
II (%)	60.0	0	40.0	60.0	0	40.0	60.0	0	40.0	70.0	0	30.0
n = 10	n = 6	-	n = 4	n = 6	-	n = 4	n = 7	-	n = 4	n = 7	-	n = 3
III (%)	85.7	14.3	0	85.7	14.3	0	85.7	14.3	0	100	0	0
n = 7	n = 6	n = 1	-	n = 6	n = 1	-	n = 6	n = 1	-	n = 7	-	-

Periodontal ligament: χ^2 = 9.088, p = 0.050; Alveaolar bone: χ^2 = 9.925, p = 0.019*; Gingiva: χ^2 = 9.520, p = 0.035*; Pulp: χ^2 = 8.849, p = 0.030*; *p<0.05

Table 2: The comparision of the groups in the aspect of focal bleedings in the evaluated tissues

	Periodontal ligament			Alveaolar bone			Gingiva			Pulp		
Groups	No	Mild	Severe	No	Mild	Severe	No	Mild	Severe	No	Mild	Severe
I (%)	100	0	0	100	0	0	100	0	0	100	0	0
n = 10	n = 10	-	-	n = 10	-	-	n = 10	-	-	n = 10	-	-
II (%)	90.0	0	10.0	90.0	0	10.0	90.0	0	10.0	80.0	10.0	10.0
n = 10	n = 9	-	n = 1	n = 9	-	n = 1	n = 9	-	n = 1	n = 8	n = 1	n = 1
III (%)	100	0	0	100	0	0	100	0	0	100	0	0
n = 7	n = 7	-	-	n = 7	-	-	-	-	-	n = 7	-	-

Periodontal ligament: χ^2 = 1.723, p = 1.000; Alveaolar bone: χ^2 = 1.723, p = 1.000; Gingiva: χ^2 = 1.723, p = 1.000; Pulp: χ^2 = 3.584, p = 0.715

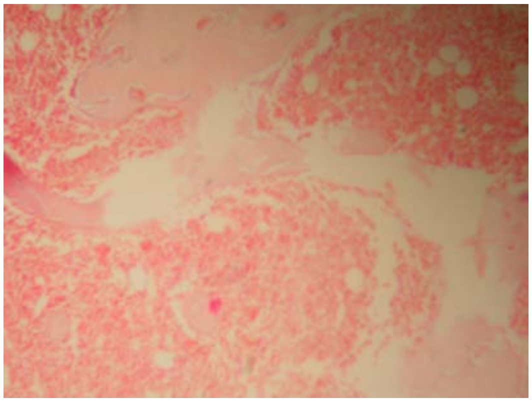

Fig. 2: An example of alveolar bone in 1st experimental exposure group (HE×100)

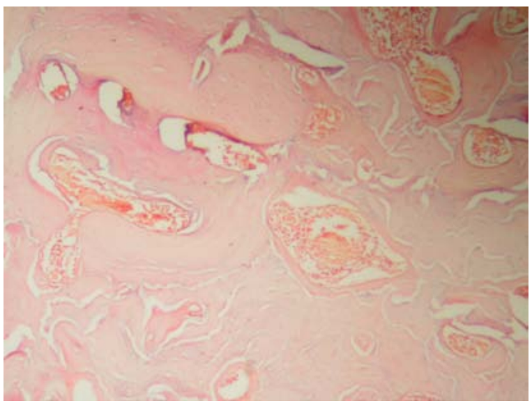

Fig. 3: An example of alveolar bone in 2nd experimental exposure group (HE×100)

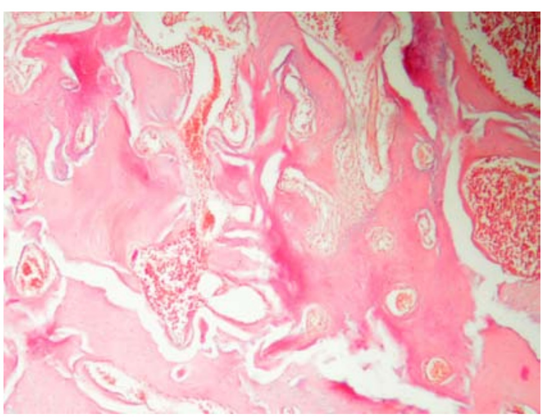

Fig. 4: An example of alveolar bone in sham group (HE×100)

In the case of gingiva: In the 1st experimental group in 1 individual mild and in 9 individual no vasodilatation was determined. In 2nd experimental groups in 4 individuals severe and in 6 individuals no vasodilatation was determined. In the sham group in 1 individual in mild and 6 individual no vasodilatation was established. In the aspect of focal haemorragies, although there were differences in the number of positive individual numbers there was no statistical differences among the groups ($p>0.05$).

DISCUSSION

Numerous sources of electromagnetic fields 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. In fact, the ability of the ELF magnetic fields to produce effects on living systems is still a matter of debate and contradictory results are available in the literature. However, public awareness of the ubiquitous nature of these fields and the growing controversy over their potential effects on living systems have stimulated the research community to define more precisely the physical properties of these fields and to delineate the thresholds for their possible effects on human health and environment (Tenforde and Kaune, 1987; Knave, 2001).

This research is performed on rats because the aim was to investigate the teeth and surrounding soft and hard tissues histopathologically. By this way, the researchers could be able to evaluate the structural and cellular changes in periodontium and teeth.

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.

Several organizations have established guidelines for occupational exposures to power frequency ELF magnetic fields. At 50 Hz magnetic fields exposure limits are 500 μT for occupational and 100 μT for public by the International Commission on Non-ionizing Radiation Protection (ICNIRP,1998). Therefore, 100 and 500 μT magnetic field strength were choosen to investigate the effects of ELF magnetic fields exposure on periodontal tissues and teeth in rats.

There is limited study in dental sciences which one of them is reporting that ELF magnetic fields treatment not only appears to increase bone formation as previously reported in the literature but acting on osteoclast activity also seems to improve bone quality during orthodontic treatment (Zaffe *et al*., 1998). The researchers could not find any articles related to ELF magnetic fields on periodontal tissues discuss directly. However, researchers can discuss the results of this study with some articles indirectly. There are some studies in which the effect of ELF magnetic field on cytosolic and intracelluler calcium levels and bone tissues were investigated (McCreary *et al*., 2002). 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 wound respond to an electromagnetically induced pulse (Glassman *et al*., 1986), these data giving to us opinion which is ELF magnetic fields could useful for dental treatment and on the pulp tissue repair that could be a subject of furter researchs. In the study, vasodilatation in alveolar bone in both groups and focal haemorrhagies in some individuals of 2nd group were determined. These findings may be the results of the effcts of ELF magnetic fields on bone.

Although, there was no statistical difference between the the experimental groups, the 2nd experimental group's findings were determened more severe than the 1st group. This condition may be explained as the increased tissue response by the higher ELF magnetic field intensity in the 2nd group.

There are many studies which indicates that ELF magnetic fields has got effects on different biologic structures (Liboff *et al*., 1984; Cossarizza *et al*., 1989; Goodman *et al*., 1983; Hiraoka *et al*., 1992; Cossarizza *et al*., 1993; Nagai and Ota, 1994; Fitzsimmons *et al*., 1995). The statistical difference between the experimental and sham groups in the study, makes us to think that ELF magnetic fields may have effects on periodontal tissues and teeth.

CONCLUSION

In this study, it is aimed to determine if ELF magnetic fields has effect on periodontal tissues and teeth. *In vivo* studies should focus on the potential for possible synergistic, genotoxic, immunological and carcinogenic effects associated with prolonged exposure of ELF magnetic fields. It is now clear that ELF magnetic fields can produce statistically highly significant biological and dental responses. In the light of these data, the study is important because it is the 1st study performed on periodontal tissues histologically.

The researchers hope that the study will guide the investigations on ELF magnetic fields effects in the aspects of dentistry. To evaluate the effects of ELF magnetic fieldson dental tissues there is need to further comprehensive biologic, histologic and epidemiologic studies.

REFERENCES

Carranza, F.A. and M.G. Newman, 1996. Clinical Periodontology. 8th Edn., WB Saunders Company, Philadelphia, PA., USA., pp: 12-51.

Cossarizza, A., D. Monti, F. Bersani, R. Paganelli and G. Montagnani *et al*., 1989. Extremely low frequency pulsed electromagnetic fields increase interleukin-2 (IL-2) utilization and IL-2 receptor expression in mitogen-stimulated human lymphocytes from old subjects. FEBS Lett., 248: 141-144.

Cossarizza, A., S. Angioni, F. Petraglia, A.R. Genazzani and D. Monti *et al*., 1993. Exposure to low frequency pulsed electromagnetic fields increases interleukin-1 and interleukin-6 production by human peripheral blood mononuclear cells. Exp. Cell Res., 204: 385-387.

Fitzsimmons, R.J., J.T. Ryaby, S. Mohan, F.P. Magee and D.J. Baylink, 1995. Combined magnetic fields increase insulin-like growth factor-II in TE-85 human osteosarcoma bone cell cultures. Endocrinology, 136: 3100-3106.

Glassman, L.S., M.H. McGrath and C.A. Bassett, 1986. Effect of external pulsing electromagnetic fields on the healing of soft tissue. Ann. Plastic Surg., 16: 287-295.

Goodman, R., C.A. Bassett and A.S. Henderson, 1983. Pulsing electromagnetic fields induce cellular transcription. Science, 220: 1283-1285.

Hiraoka, M., J. Miyakoshi, Y.P. Li, B. Shung, H. Takebe and M. Abe, 1992. Induction of c-fos gene expression by exposure to a static magnetic field in HeLaS3 cells. Cancer Res., 52: 6522-6524.

ICNIRP, 1998. Guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields (up to 300 GHz). Health Phys., 74: 494-522.

Kheifets, L.I., A.A. Afifi, P.A. Buffler and Z.W. Zhang, 1995. Occupational electric and magnetic field exposure and brain cancer: A meta-analysis. J. Occup. Environ. Med., 37: 1327-1341.

Knave, B., 2001. Electromagnetic fields and health outcomes. Ann. Acad. Med. Singapore, 30: 489-493.

Liboff, A.R., T. Jr. Williams, D.M. Strong and R. Jr. Wistar, 1984. Time-varying magnetic fields: Effect on DNA synthesis. Science, 223: 818-820.

McCreary, C.R., A.W. Thomas and F.S. Prato, 2002. Factors confounding cytosolic calcium measurements in Jurkat E6.1 cells during exposure to ELF magnetic fields. Bioelectromagnetics, 23: 315-328.

Miller, A.B., T. To, D.A. Agnew, C. Wall and L.M. Green, 1996. Leukemia following occupational exposure to 60 Hz electric and magnetic fields among Ontario electirc utility workers. Am. J. Epidemiol., 144: 150-160.

Nagai, M. and M. Ota, 1994. Pulsating electromagnetic field stimulats-es Mrna expression of bone morphogenic protein-2 and -4. J. Dent. Res., 73: 1601-1605.

Savitz, D.A., 1995. Overview of occupational exposure to electric and magnetic fields and cancer: Advancements in exposure assessment. Environ. Health Perspect., 103: 69-74.

Tenforde, T.S. and W.T. Kaune, 1987. Interaction of extremely low frequency electric and magnetic fields with humans. Health Phys., 53: 585-606.

Washburn, E.P., M.J. Orza, J.A. Berlin, W.J. Nicholson, A.C. Todd, H. Frumkin and T.C. Chalmers, 1994. Residential proximity to electricity transmission and distribution equipment and risk of childhood leukemia, childhood lymphoma and childhood nervous system tumors: Systematic review, evaluation and meta-analysis. Cancer Causes Control, 5: 299-309.

Zaffe, D., D. Fraticelli, M.F. Sfondrini, D. Caielli and A.R. Botticelli, 1998. Rabbit bone behavior after orthodontic and pulsed low-frequency electromagnetic field treatments. Electromagn. Biol. Med., 17: 87-98.