Can exposure to manganese and extremely low frequency magnetic fields affect some important elements in the rat teeth?

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Abstract. – ***Background:*** **Length and level of exposure to electromagnetic fields (EMFs) is increasing in association with the widespread use of electrical and electronic devices and technological progress. The undesirable effects of extremely low frequency magnetic fields (ELF-MFs) on health have attracted considerable interest.**

Materials and Methods: **Sixty-four four-month-old male Wistar rats divided into eight groups of eight rats each were used. Seven groups were exposed to varying dosages of manganese (Mn) and a 50 Hz magnetic field (MF) of approximately 1 mT, while the last group was set aside as the cage control group and not subjected to any procedure.**

This study was intended to investigate the interactions between the application of MF and Mn and the elements Ca, Zn, Mg, and P thought to be involved in caries, in rat teeth.

Results: **Levels of Ca, Mg, Zn, and P in the experimental group rats were different to those in the control group.**

Conclusions: **The results demonstrate that ELF-MF and Mn can have significant effects on levels of elements in rat teeth. Further experimental and epidemiological studies of ELF-MF and Mn are needed in order to evaluate their dental effects.**

Key Words:

Magnetic field, Manganese, Elements.

Introduction

Production, distribution, transmission and usage of electromagnetic fields (EMFs) produce an electric and magnetic field with a frequency of 50-60 Hertz (Hz) falling within an extremely low frequency (ELF) of the electromagnetic spectrum (between 3 and 3000 Hz)[1]. The effects of EMFs associated with widespread use of electronic devices parallel to technological progress in many spheres of life have attracted considerable interest from researchers. Various devices and equipment with a wide technical range of technical properties are sources of EMFs. With its many different properties, various forms of EMF are used in the treatment of disease in the health sector. However, some forms, such as extremely low frequency magnetic fields (ELF-MFs) are thought to be potentially harmful to human health[2].

Radiation from electronic devices and the environment has an ionizing or non-ionizing effect on the atoms that comprise a body appearing inside a substance. In terms of its ionizing effects, radiation can be divided into non-ionizing (electromagnetic radiation) and ionizing (neutron, proton, alpha, beta, X and gamma rays). Non-ionizing radiation consists electromagnetic waves, from ELF-MF to radio waves, infrared light, visible light and ultraviolet rays[3-7].

There has been an increase in *in-vivo*, *in-vitro*, clinical and epidemiological studies of the effects on human health of electromagnetic waves in recent years. These have reported that non-ionizing radiation affects the environment and human health depending on the intensity and frequency of the source[8,9].

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Major and trace elements play a significant role in human health. A deficiency or excess of these elements as a result of exposure to natural and human-made effects can lead to serious clinical consequences[10].

Trace elements can be seen in different intensities in dental enamel[11]. Manganese (Mn) is a trace element and a basic element essential for many physiological processes such as bone mineralization, protein and energy metabolism and free radical species production[12]. Mn involvement in calcified tissues (bone and teeth) may be related to exposure to environmental factors[13]. Mn absorption in humans takes place predominantly through such foods as spinach, tea, herbs, and plants. Foodstuffs containing high concentrations of Mn are cereals and rice, soy bean, eggs, hazelnut, olive oil, green beans and oyster[14].

Previous studies have reported that elements such as calcium (Ca), zinc (Zn), phosphorus (P) and magnesium (Mg) in the dental structure are associated with decay[15].

One in vitro study regarding the classification of elements in dental structure described five different groups[16].

Cariostatic elements: Fluoride and phosphorus.
Mildly cariostatic: Molybdenum, vanadium, silver, strontium, boron, lithium, gold.
Doubtful: Beryllium, cobalt, manganese, tin, zinc, bromine iodine.
Inert: Barium, aluminum, nickel, iron, palladium, titanium.
Cariogenic: Selenium, magnesium, cadmium, platinum, lead, silicon.

This study was intended to investigate the interactions between the application of MF and Mn and the elements Ca, Zn, Mg, and P present in rat teeth.

Materials and Methods

Sixty-four four-month-old male Wistar albino rats were divided into eight groups of eight animals each. The first three groups were administered 60 mg/kg, 15 mg/kg and 3.75 mg/kg dosages of Mn plus 4 h a day of a 50 Hz MF of approximately 1 mT obtained by applying an alternative current (AC) obtained from power sources were applied to two pairs of Helmholtz coils placed opposite one another on the vertical axis inside flexiglass cages. Rats in group 4 were administered only 4 h of MF per day. Rats in groups 5, 6 and 7 were administered Mn in dosages of 60 mg/kg, 15 mg/kg and 3.75 mg/kg per day. Rats in the eighth, cage control group, were not subjected to any procedure. All animal procedures were performed in agreement with the Principles of Laboratory Animal Care and the rules of the Scientific and Ethics Committee of the Dicle University Health Research Center.

The administration of Mn and MF continued for 45 days. A Faraday cage was used to prevent any MF interactions from the outside, while flexiglass cages were used to permit unobstructed access to the MF groups (Figure 1).

All measurements were performed by individuals uninvolved in the animal experiments. Observers were blinded to which groups constituted the ELF-MF and Mn or control groups, and the study was performed entirely blinded. Rats' teeth were extracted by observers using ketamine anesthesia (intramuscular 100 mg/kg). Teeth were then kept in sterile saline solution until analysis and measurement of changes in Ca, Mg, Zn and P using chemical techniques.

Measurements of all elements were performed as follows. First, 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 ap-

Figure 1. MF exposure in Faraday and flexiglass cages.

Table I. Group mean rank values obtained from the Kruskal-Wallis test.

Groups	Calcium (Ca) mean rank	Zinc (Zn) mean rank	Magnesium (Mg) mean rank	Phosphorus (P) mean rank
1- Mn 60 mg/kg + MF	10.44	52.69	37.81	46.25
2- Mn 15 mg/kg + MF	11.94	28.00	24.25	22.63
3- Mn 3.75 mg/kg + MF	24.13	32.88	56.38	17.81
4- MF	26.19	29.31	44.25	41.06
5- Mn 60 mg/kg	36.44	14.00	27.31	33.81
6- Mn 15 mg/kg	47.06	35.88	30.75	34.13
7- Mn 3.75 mg/kg	46.69	44.44	33.38	7.56
8- Cage control	57.13	22.81	5.88	56.75

proximately 4 h until a white or grey ash residue was obtained. The residue was dissolved in 3 ml of a mixture of the 65% nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) (3:1), and when necessary was heated slowly to dissolve the residue. The solution was transferred to a 10 ml volumetric flask and made up to volume. The Ca, Mg, and Zn content of teeth was determined using atomic absorption spectroscopy and the P content using ultraviolet spectrophotometry.

Statistical Analysis and Results

Kruskal-Wallis one-way analysis of variance, a non-parametric test, was used to compare the groups since the data in all groups did not exhibit normal distribution. A statistical difference was determined between the groups on the basis of the Kruskal-Wallis analysis of variance results ($p < 0.001$).

Group rank values obtained from the Kruskal-Wallis test are shown in Table I.

Measurement results for elements obtained from the groups by mean rank values are shown in Figure 2.

The Mann Whitney U test with Bonferroni correction, a post hoc multiple comparison test, was used in multiple intragroup comparisons to determine the origin of significant differences among the groups.

Comparison of mean rank, median and p value results obtained from the groups is shown in Table II.

Discussion

Previous studies have reported that high or low frequency MFs fields have different effects on biological tissues. Low frequency magnetic fields have been reported to have harmful effects on deep tissues in organisms, while high frequency magnetic fields have a limited effect on superficial tissues[17]. A correlation has been determined between ELF-MF and human health in several recent studies[18-20]. But all the effects of ELF-MF on human health have not been fully explained in the emerging findings. There are reports in the litera-

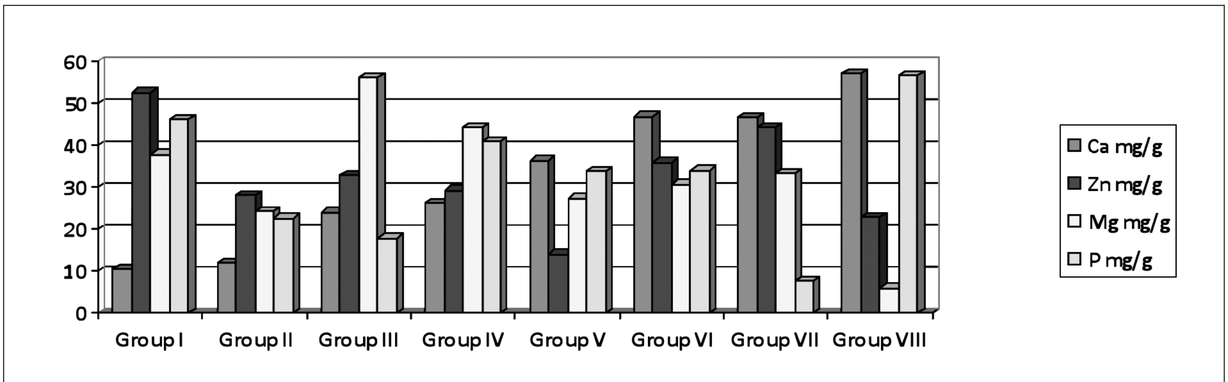

Figure 2. Element measurement results by mean rank values.

Table II. Comparison of mean rank, median and *p* value results obtained from the groups.

Groups (Ca)	Mean rank	Median values	Groups compared	Comparison results	*p* values
1	10.44	57.85	1-8	Difference	< 0.05
2	11.94	58.15	2-8	Difference	< 0.05
3	24.13	64.85	3-8	Difference	< 0.05
4	26.19	65.40	4-8	Difference	< 0.05
5	36.44	68.40	5-8	Difference	< 0.05
6	47.06	71.95	6-8	Difference	< 0.05
7	46.69	72.70	7-8	No difference	> 0.05
8	57.13	75.15			
Groups (Ca)	**Mean rank**	**Median values**	**Groups compared**	**Comparison results**	***p* values**
1	52.69	0.096	1-8	Difference	< 0.05
2	28.00	0.087	2-8	No difference	> 0.05
3	32.88	0.088	3-8	No difference	> 0.05
4	29.31	0.088	4-8	No difference	> 0.05
5	14.00	0.085	5-8	No difference	> 0.05
6	35.88	0.094	6-8	No difference	> 0.05
7	44.44	0.094	7-8	No difference	> 0.05
8	22.81	0.086			
Groups (Ca)	**Mean rank**	**Median values**	**Groups compared**	**Comparison results**	***p* values**
1	37.81	24.20	1-8	Difference	< 0.05
2	24.25	23.45	2-8	Difference	< 0.05
3	56.38	25.80	3-8	Difference	< 0.05
4	44.25	24.80	4-8	Difference	< 0.05
5	27.31	23.50	5-8	Difference	< 0.05
6	30.75	23.80	6-8	Difference	< 0.05
7	33.38	23.65	7-8	No difference	> 0.05
8	5.88	19.80			
Groups (Ca)	**Mean rank**	**Median values**	**Groups compared**	**Comparison results**	***p* values**
1	46.25	0.785	1-8	Difference	< 0.05
2	22.63	0.575	2-8	Difference	< 0.05
3	17.81	0.545	3-8	Difference	< 0.05
4	41.06	0.735	4-8	Difference	< 0.05
5	33.81	0.715	5-8	Difference	< 0.05
6	34.13	0.690	6-8	Difference	< 0.05
7	7.56	0.455	7-8	Difference	< 0.05
8	56.75	1.285			

ture that ELF-MF activates bone tissue mineralization and growth, inhibits osteoclastic activity, contributes to bone fracture healing and assists with wound healing by affecting granulation and fibrous tissue formation. In addition to studies reporting that a risky working environment and living near ELF-MFs do not pose a danger; research reporting that ELF-MF affects the immune system, establishes carcinogenic effects such as leukemia, gives rise to neurological disorders, triggers endocrine system disorders, activates degenerative coronary diseases and causes injury in blood vessels and pulmonary emphysema has also been published[3-7,14,21-26].

Apart from a few studies concerning the effect of ELF-MF on oral tissues and dental/tooth element content, the research and information available in the literature is inadequate[27-34].

This study determined significant changes in the levels of such trace elements as Ca, Zn, Mg and P in tooth structure as a result of exposure to ELF-MF and Mn for 45 days.

In the light of our findings, different dosages of Mn and the exposure to MF can alter the levels of the elements Ca, Mg, Zn and P in rat teeth.

Only a limited number of studies of the effects of ELF-MF and Mn on dental tissues and mineral structures have been published. However a correlation has been determined between ELF-MF and trace elements in some studies on rat blood, bone and spinal fluid. Sert et al[35] reported no change in Ca, Mg and lithium concentrations in rats exposed to ELF-MF. Akda et al[36], however, reported a significant difference in Cu, Zn and Fe concentrations in the sera of rats exposed to ELF-MF.

We investigated the effects on Ca, Mg, Zn and P levels in the teeth of eight different rat groups. Different levels of Mn and MF were administered to the first three groups. MF alone was applied to the fourth group, Mn alone in different dosages was administered to the next three groups and no procedure was performed on the eighth, cage control group.

A decrease in Ca and P levels in teeth was observed when MF and/or Mn were applied, while a significant increase was determined in Zn and Mg levels.

Intragroup comparisons were performed on the basis of the cage control group. Ca levels decreased in all the groups administered varying dosages of Mn and/or MF. We determined a statistically significant difference for Ca at comparisons between the groups, apart from groups 7-8 ($p > 0.05$) ($p < 0.05$).

An adult human body contains approximately 1200 g Ca, some 99% of which is in the skeletal system to form bones and hard dental structures. Calcium absorption can be impaired by Vitamin D deficiency or for other reasons[37]. Our study determined that exposure to ELF-MF and/or Mn reduced the level of Ca in rat teeth.

Zinc, one of the trace elements, is necessary for growth, development and biological functions. Various studies have reported that zinc can remain stable for a long time in the oral environment and it inhibits bacteria that cause dental caries and periodontal problems[38-41]. Zn has also been reported to reduce caries formation[38]. Exposure to ELF-MF and/or Mn increased the level of Zn in rat teeth in this study. Statistically no significant difference was determined for Zn at intergroup comparison, with the exception of groups 1-8 ($p < 0.05$) ($p > 0.05$).

Mg is an important inorganic element in dental enamel. It has been reported that the tissue removed in acid attacks arising in early enamel caries and in acid solubility is rich in Mg, because of Mg being closely related to dental caries. It has also been reported in various studies that because of caries increasing property of Mg it also significantly inhibits enamel mineralization[42-46]. This study determined a rise in Mg levels in the teeth of rats after exposure to ELF-MF and/or Mn. Statistically no significant difference was observed at intergroup comparisons for Mg in all groups apart from groups 7-8 ($p > 0.05$) ($p < 0.05$).

P is the chemical element present in the greatest quantities in the human body after Ca. P compounds (phosphordiester bonds) are of great importance for DNA structures in all living organisms. The human body also needs P for bone and teeth formation, cell growth and repair, energy consumption, heart muscle contraction, nerve and muscle movements and renal functions[47]. P has also been reported to have a preventive effect against dental caries[16]. Exposure to ELF-MF and Mn reduced the level of P in rat teeth in our study. Statistical significance was determined for P among all groups ($p < 0.05$).

In conclusion, we observed changes in the amounts of such elements as Ca, Mg, Zn and P in rat teeth in association with exposure to ELF-MF and/or Mn.

Conclusions

The data from this study show that exposure to ELF-MF and/or Mn reduces the Ca and P mineral content in rat teeth, while it increases the mineral content of Zn and Mg. On the basis of these results, we conclude that exposure to ELF-MF and/or Mn increases caries and reduces resistance to decay. In conclusion, the correlation between ELF-MF and Mn and Ca, P, Zn and Mg and caries is significant, but further histological, chemical, experimental and epidemiological studies are now needed.

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