Effects of Electromagnetic Pulse on Bone Metabolism of Mice *in vivo*[1]

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Objective To study the effects of electromagnetic pulse (EMP) on bone metabolism of mice *in vivo*. **Methods** Twenty-four male BALB/c mice were divided into a control group and 2 experimental groups (n=8). The whole-body of mice in experimental groups were exposed to 50 kV/m and 400kV/m EMP, 400 pulses daily for 7 consecutive days at 2 seconds intervals. Alkaline phosphotase (ALP) activity, serum calcium concentration and osteocalcin level and trabecular bone volume (BV/TV, %) were measured immediately after EMP exposure by biochemical，ELISA and morphological methods. **Results** The ALP activity, serum calcium concentration and osteocalcin level and BV/TV in experimental groups remained unchanged after EMP exposure. **Conclusion** Under our experimental conditions, EMP exposure cannot affect bone metabolism of mice *in vivo*.

Key words: Electromagnetic pulse; Alkaline phosphotase; Serum calcium; Osteocalcin; Trabecular bone volume

INTRODUCTION

The biological effect of electromagnetic field is more and more concerned by researchers. However, its influence on bone metabolism remains controversial. Some studies reported the effect of electromagnetic field on ALP activity[1-2], serum calcium concentration[3] and bone mass[4-6], while other studies showed no effect of electromagnetic field on these parameters[7-11]. Available data about electromagnetic field exposure are generally insufficient to show its role in bone metabolism. In the present study, EMP was used as a kind of pulsed electromagnetic field with an electric intensity up to 400kV/m, and the ALP activity, serum calcium concentration and osteocalcin level and BV/TV in BALB/c mice were measured after 7 days of EMP exposure to explore whether EMP with a high electric intensity has effects on bone metabolism of mice *in vivo*.

MATERIALS AND METHODS

Animals and EMP Exposure

Twenty-four male BALB/c mice (20±2 g) at the age of 8-10 weeks were bred at Experimental Animal Center of the Fourth Military Medical University (FMMU) under specific pathogen-free conditions, with free access to laboratory chaw and water. The mice were divided into a control group and 2 experimental groups (n=8). The whole-body of mice in 2 experimental groups was exposed to 50 kV/m and 400 kV/m EMP at 0.5 Hz, 400 pulses daily for 7 consecutive days.

ALP Activity and Calcium Concentration Assay

Eight blood samples were collected from each group after 7 days of EMP exposure. ALP activity and serum calcium concentration were measured using an autoanalyser (Cobas Integra 400 Plus, Roche, Switzerland).

Osteocalcin Level Measurement

Eight blood samples were collected from each group after 7 days of EMP exposure. Serum osteocalcin level in mice was measured using a commercially available ELISA kit (Biomedical Technologies, Stoughton, MA) according to its manufacturer's instructions.

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Trabecular Bone Volume Measurement

After the animals were killed by cervical dislocation, 8 femurs of mice in each group were immediately fixed in buffered 10% formalin for 72 h. The specimens were decalcified in 4% EDTA, dehydrated through a graded series of alcohol bath, embedded in paraffin and transversely cut into 4μm-thick sections. The sections were stained with hematoxylin and eosin (H & E). The trabecular bone volume (BV/TV, %) was measured at a magnification of 40×. All sections were read blindly by an experienced investigator. The length of new bone circumference was measured and converted into the area of new bone formation using a digital picture analysis system (Photoshop), and the histology between the groups was compared.

Statistical Analysis

The data were expressed as $\bar{x} \pm s$ and analyzed using analysis of variance (ANOVA). $P<0.05$ was considered statistically significant. Statistical analyses were performed using the SPSS 13.0 for Windows.

RESULTS

Effect of EMP on Serum ALP Activity in Mice

The serum ALP activity in mice was lower in 50 kV/m and 400 kV/m groups than in control group (Table 1), whereas the difference was not statistically significant.

TABLE 1

Serum ALP Activity in Mice after 7 Days of Exposure to EMP ($\bar{x} \pm s$)

Groups	ALP Activity（U/L）
Control	460.15±28.20
50 kV/m	448.35±21.15
400 kV/m	439.25±10.24

Note. $P>0.05$ *vs* control group.

Effect of EMP on Serum Calcium Concentration in Mice

The serum calcium concentration remained unchanged in mice after exposure to 50 kV/m and 400 kV/m of EM (Table 2).

TABLE 2

Serum Calcium Concentrations in Mice 7 Days after Exposure to EMP ($\bar{x} \pm s$)

Groups	Ca Concentration (mmol/L)
Control	1.240±0.0548
50 kV/m	1.283±0.3231
400 kV/m	1.280±0.3899

Note. $P>0.05$ *vs* control group.

Effect of EMP on Serum Osteocalcin Level in Mice

After 7 days of exposure to EMP, no significant difference in serum osteocalcin level (8.75±1.77 *vs* 8.45±1.67 and 9.30±1.38) was observed between the control and experimental groups (Fig. 1).

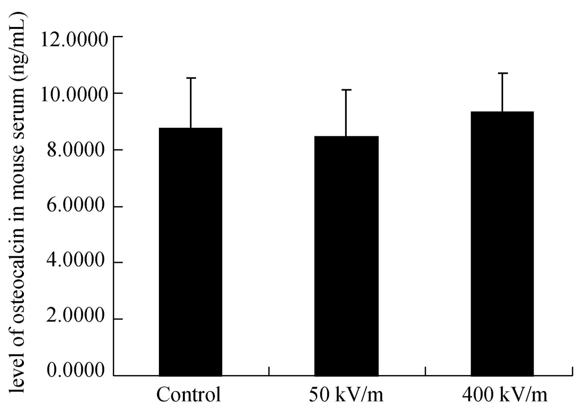

FIG. 1. Serum level of osteocalcin in mice after exposure to EMP. Data are expressed as $\bar{x} \pm s$, $P>0.05$ *vs* control group.

Effect of EMP on Trabecular Bone Volume in Mice

The morphological features of trabecular bone stained with H&E remained unchanged after 7 days of exposure to EMP in either 50 kV/m group or 400 kV/m group compared with control group (Fig. 2A). A similar value of relative bone volume (BV/TV, %) was 32.73±3.53 in 50 kV/m group, 31.58±2.72 in 400 kV/m group and 31.84±3.15 in control group, respectively (Fig. 2B).

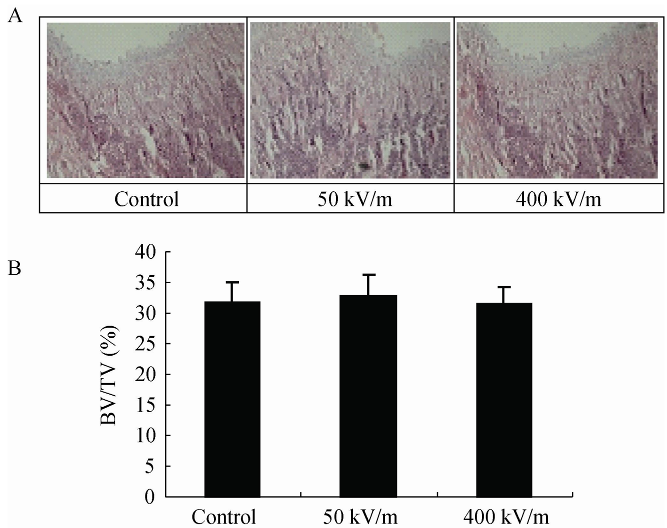

FIG. 2. Effects of EMP on trabecular bone volume in mice. A: Morphological changes of trabecular bone in mice after exposure to EMP with eosinophilic trabecular bone stained with H&E (40×); B: Percentage variations of trabecular bone volume (BV/TV, %) in mice after exposure to EMP. Data are expressed as $\bar{x} \pm s$. $P>0.05$ *vs* control group.

DISCUSSION

Bone metabolism reflects the activities of two osteons. Osteoclastic resorption shifts Ca, phosphate, and matrix constituents from bone to interstitial fluid and plasma[12], osteoblasts are deposited in matrix that is mineralized by Ca and phosphate shifting from interstitial fluid into bone. Parameters, such as serum ALP, calcium, osteocalcin and bone mass, are associated with the process of bone metabolism.

Serum ALP is one of the bone metabolism-related parameters. It has been reported that serum ALP activity does not undergo significant changes after exposure to electromagnetic field[9-11]. In the present study, the serum ALP activity was not significantly changed after 7 days of exposure to 50 kV/m and 400 kV/m EMP. Pashovkina and Akoev reported that the activity of serum ALP in guinea pigs is increased during *in vitro* exposure to electromagnetic field[2]. While Hashish *et al.*[1] showed that serum ALP activity is significantly lower in groups exposed to electromagnetic field.

In this study, neither 50 kV/m EMP nor 400 kV/m EMP changed the serum calcium concentration in mice, which is consistent with reported data[8, 10]. However, Spadaro and Bergstrom reported that serum calcium is decreased in rats after exposed to pulsed electromagnetic field[3].

Osteocalcin is secreted after the onset of mineralization, binds to bone minerals and indicates transition to mineral formation[13]. Few experimental data are available on serum osteocalcin. Stanosz *et al.* [14] found that serum osteocalcin concentration is insignificantly increased in women with osteoporosis after exposed to electromagnetic field. In our experiment, EMP did not affect the serum osteocalcin level.

It is still controversial about the effects of electromagnetic field on bone mass. Akca *et al.*[7] have obtained similar values of relative bone volume in rats exposed or not exposed to pulsed electromagnetic field. On the contrary, Fini *et al.*[5] reported that BV/TV is increased in guinea pigs after stimulation with pulsed electromagnetic field. Chang and Chang[4] found that pulsed electromagnetic field exposure increases bone volume percentage in rats. It has been shown that BV/TV is decreased after electromagnetic field exposure[6]. We failed to show any changes of relative bone volume in mice after 7 days of EMP exposure.

The difference between our and other results may be due to the different exposure condition, lapse of time as well as the age and strain of animals. The effect of the highest electric intensity of EMP was evaluated in this study with no statistical changes observed in the selected parameters related to bone metabolism, indicating that ALP activity, serum calcium concentration and osteocalcin level and BV/TV are not sensitive to EMP even with a high electric intensity.

In conclusion, EMP we used does not exert significant effects on bone metabolism in mice.

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