BIOMEDICAL AND ENVIRONMENTAL SCIENCES **22**, 157-160 (2009) www.besjournal.com

Influence of Electromagnetic Fields on Bone Fracture in Rats: Role of CAPE

EKREM CICEK[#,1], OSMAN GOKALP[‡], REMZI VAROL[Δ], AND GOKHAN CESUR[§]

[#]Department of Physics, Faculty of Arts and Science, Mehmet Akif Ersoy University, Burdur, Turkey; [‡]Department of Pharmacology, [Δ]Department of Mechanical Engineering, Faculty of Engineering, [§]Department of Physiology Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Objective To study the effects of radiation emitted by mobile phones on bone strength and caffeic acid phenethyl ester (CAPE) on the changes induced by radiation. **Methods** Forty-eight Sprague-Dawley rats were divided into five groups. Rats in the control group (first group) were left within the experimental setup for 30 min/day for 28 days without radiation exposure. Nine hundred MHz radiation group was broke down into 2 subgroups (group 1/2). Both subgroups were exposed to radiation for 28 days (30 min/day). The next group was also divided into 2 subgroups (group 3/4). Each was exposed to 1800 MHz of radiation for 28 days (30 min/day). The third and fifth groups were also treated with CAPE for 28 days. Treatment groups received ip caffeic acid phenethyl ester (10 μmol/kg per day) before radiation session. Bone fracture was analyzed. **Results** Breaking force, bending strength, and total fracture energy decreased in the irradiated groups but increased in the treatment groups. **Conclusion** Radiation and CAPE can significantly improve bone.

Key words: Mobile phone; Biomechanical study; Caffeic acid phenethyl ester

INTRODUCTION

An increased risk of bone fracture due to exposure to electromagnetic fields (EMF) has been reported in studies dealing with EMF-increased tumor incidence[1] and effect of EMF on reproduction and development[2-3].

EMF has certain biological effects on the behavior of bone cells, and increases the maturation bone trabecula, bone volume and formation[4-5].

Over the past two decades, there has been increasing interest in the biological effects and possible health outcomes of weak, high-frequency electric and magnetic fields. Some studies on magnetic fields and cancer, reproduction and neurobehavioral reactions showed that different system diseases are related to electromagnetic fields such as mobile phones[6].

Becker[7] is one of the first researchers to discuss the effect of electromagnetic fields on bone. Shamos, a physicist and Lavine, an orthopedist, have characterized the osseous stimuli produced by electromagnetic fields. It was reported that electromagnetic fields have a beneficial effect on the repair of bone fracture and may increase bone mass[8], although other authors dispute these claims[9].

Biomechanical testing is important for evaluation of bone strength, which is associated with susceptibility to bone fracture[10]. Three-point bending test has commonly been used in evaluation of bone strength, showing that bending breaking force, bending strength, and total fracture energy, are good indicators of the mechanical strength of cortical bone[11].

Caffeic acid phenethyl ester (CAPE) exhibits significant cytotoxicity in oral cancer cells, and has free radical scavenging and antioxidant properties that do not require interaction of CAPE with a receptor. It was reported that CAPE intake by peripheral tissues may be enhanced and can be used as a free radical scavenger[12].

The aim of this study was to investigate the effects of 900 MHz and 1800 MHz EMF on bone fracture.

[1]Correspondence should be addressed to Dr. Ekrem CICEK, Department of Science Education, Faculty of Education, Mehmet Akif Ersoy University, Bahcelievler, Burdur, 15100 Turkey. Tel: 90 505 6618737. Fax: 90 248 2346005. E-mail: ekrcicek@yahoo.com

Biographical note of the first author: Ekrem CICEK, male, born in 1973, Ph. D., majoring in biomedical and works in Mehmet Akif Ersoy University.

0895-3988/2009
CN 11-2816/Q

MATERIALS AND METHODS

Study Protocol

Forty-eight Sprague-Dawley male rats at the age of 7 weeks were used in the study. The animals were housed in a 12 h light/12 h dark cycle, at ambient temperature (22 ℃) and relative humidity (45%) throughout the study, and fed with standard pellet food. CAPE was purchased from Sigma Chemical Company (St Louis, MO, USA).

For each experiment, animals were randomly assigned to one of the five groups:

I. Control group in which rats receiving no treatment and radiation exposure before testing (*n*=10);

II. GSM 900-MHz treatment group in which rats were exposed to GSM 900-MHz, 2 W EMF (30 min/day) for 28 days (*n*=8);

III. CAPE treatment group in which rats received 0.569 mg CAPE (10 μmol/kg·bw/day ip) before exposed to a GSM 900-MHz, 2 W EMF (30 min/day) for 28 days (*n*=10);

IV. GSM 1800-MHz treatment group in which rats were exposed to GSM 1800-MHz, 1.5 W EMF (30 min/day) for 28 days (*n*=10);

V. CAPE (0.569 mg) treatment group in which rats were exposed to GSM 1800-MHz, 1.5 W EMF (30 min/day) for 28 days (*n*=10).

The experiment time was 28 days. At the end of experiment, the animals were anesthetized with sevofluorane and decapitated. The left femur was removed and the ultimate strength of a femoral shaft was measured.

Experimental Setup and Radio Frequency Irradiation

The 900 and 1800 MHz continuous wave electromagnetic energy generator (the peak specific absorption rate (SAR) was 2 W/kg, average power density 1±0.4 mW/cm^2) from the Electromagnetic Compatibility Laboratory of Suleyman Demirel University was used in the study. The power density was measured using an EMF meter (Holaday Industry Inc., Adapazari, Turkey). The exposure system consists of a round plastic tube cage (diameter: 5.5 cm, length: 12 cm) and a dipole antenna (Fig. 1). The whole body of the rat was positioned above the dipole antenna, and the tube was ventilated to decrease the stress on the rats. Rats were exposed to 900 MHz or 1800 MHz EMF (30 min/day), 5 days a week for 4 weeks.

Biomechanical Measurement

Soft tissues were removed from the left femur for mechanical tests. Prepared femurs were kept at -20 ℃ for tests. Before mechanical test, the femoral lengths and outer mid-shaft widths in both directions (medial-lateral, anterior-posterior) were measured using a digital vernier caliper.

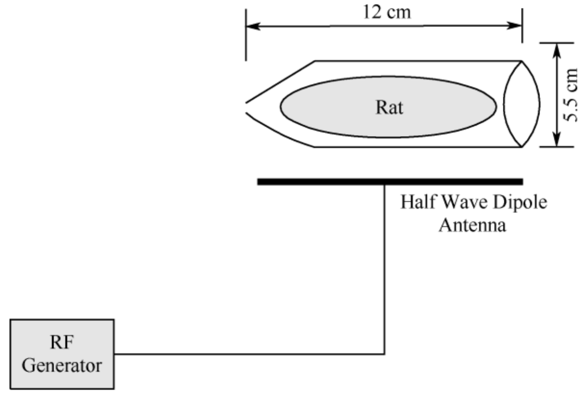

FIG. 1. A sketch map of the exposure system.

Mechanical tests were performed at room temperature. Mechanical properties of the femurs were determined by three-point bending test using a mechanical testing device (Tecquipment, England). During mechanical testing each femur was placed on two supports that are 13-15 mm apart. Each support was arranged to stabilize the tested bone. Force was applied at the mid-point on the anterior surface and lower supports touched the posterior surface. The three-point bending test was performed to test the posterior surface in tension and the anterior surface in compression. The load was kept on the mid-point of the tested femur until the occurrence of bone fracture. After mechanical testing, femur cross sections at the fracture site were used for all measurements. The external dimension of each bone was measured using a digital calliper. The mechanical properties of each femur were determined following equation (1):

$$f \ \frac{F_f .L.c}{4.I} \tag{1}$$

Where f is the stress on the outer mid-shaft surface of the tested femur in bending, F_f is the fracture load, L is the support length (13-15 mm) in three-point bending, c is the distance to outer surface from centroid of the femoral cross section, I is the second moment of area at mid-shaft of the tested femur. The cross section area of femur was measured after mechanical testing. Cross sections of fractured femurs at the fracture site were prepared to measure cross-sectional properties, such as area and moment of inertia. Cross-sectional moment of inertia was measured using Renishaw cyclone Sp 620 (Ireland) and calculated by Solid Works-2004 software. Total fracture energy values were calculated using experimental data. The load and maximal deflection values for each bone fracture were determined, the total fracture energy was calculated using area under the load-deflection curve with the last least square method (LSM)[10-11].

Statistical Analysis

Data were analyzed using the statistical package SPSS for Windows (Ref. 9.05, SPSS Inc., Chigago, IL). Results were expressed as ($\bar{x} \pm s$). P<0.05 was considered statistically significant. Comparison between groups was made by one-way ANOVA and Tukey HSD test.

RESULTS

The results of the biomechanical study are shown in Table 1.

The breaking force, bending strength, and total fracture energy were lower in the irradiated groups than in the control group (P<0.05) and significantly higher in the CAPE treatment group than in the irradiated and control groups (P<0.05). The bending strength was significantly higher in the CAPE treatment group than in the 900 MHz irradiated group (P<0.05) and lower than in the 1800 MHz irradiated group (P>0.05).

TABLE 1

Mechanical Parameters of Rat Femora ($\bar{x} \pm s$)

	Control Group, I (*n*=10)	900 MHz Irradiated Group, II (*n*=8)	1800 MHz Irradiated Group, IV (*n*=10)	900 MHz Irradiated and CAPE Treated Group, III (*n*=10)	1800 MHz Irradiated and CAPE Treated Group, V (*n*=10)
Breaking Force (N)	135±12.43	105±7.07[a]	90±5[a]	116.66±10.15[b]	105±8.05[b]
Bending Strength (MPa)	164.5±14.30	117.27±11.88[a]	138.86±9.42[a]	151.58±13.14[b]	126±11[c]
Total Fracture Energy	177.7±7.1	91.99±8.7[a]	78.6±7[a]	127.76±9.7[b]	116.25±8.1[b]

Note. [a]P<0.05 *vs* control group, [b]P<0.05 *vs* irradiated group, [c]P>0.05 *vs* irradiated group.

DISCUSSION

There is accumulating evidence that exposure to radio frequency fields from mobile telephones or their base station affects people's health. The bone is a potential route for the absorption of hazardous materials encountered in environment [13].

EMF has some biological effects on the behavior of bone cells. For example, it increases maturation of the bone trabecula, bone volume and formation. Recent studies showed that EMF can stimulate osteoblasts. However, the mechanism by which EMF stimulates osteoblasts is not clear. The cell membrane has been speculated to be a target for EMF, suggesting that EMF can induce ligand receptor interaction and stimulate ion transport channels[14]. Extremely low-frequency magnetic field (ELFMF) increases DNA synthesis, transcription, and inter cellular calcium, by inducing the differentiation of cartilage cells and enhancing alkaline phosphatase (ALP) activity in rat osteoblastic cells[15].

However, there are contradictory and insufficient reports on this subject. Giavaresi *et al.*[16] applied 72-Hz, 7-mT pulsed EMF (PEMF) to ovariectomized rats, 1 h a day, for 16 weeks, but they did not observe any significant difference in the mineral density, gross and trabecular volume, or the vertical growth of the bone. McElhaney *et al.*[17] observed that 30-Hz sinusoidal fields could lead to bone loss in osteoporosis patients. The cell membrane is the target of many toxic factors including EMF. It has been showed that EMF causes different pathological cahnges in various tissues[4].

In our study, the total fracture energy, bending strength and breaking force of the femur were decreased in 900 MHz and 1800 MHz-irradiated groups compared to the control group.

CAPE, an active component of propolis, has many biological and pharmacological activities, including antioxidant, anti-inflammation, antiviral action, and anticancer effect[18-19]. In our experiment, CAPE improved the total fracture energy, bending strength, and breaking force of the femur.

In conclusion, both high-frequency EMF and CAPE have effects on bones. Bone metabolism in humans and rats may be different. Since the growth of mobile telecommunications is rapid, there will be about one billion mobile phone users before 2005. Therefore, mobile telephones will affect the health of almost all individuals in the world if they have adverse effects[20-21]. Further studies are needed to determine the effect of high-frequency EMF on bone strength.

REFERENCES

1. Savitz A D, Loomis P (1995). Magnetic Fields exposure inrelation to leukemia and brain cancer mortality among

Electric utility workers. *Am J Epidemiol* **141**(2), 123-134.
2. Barnothy J M (1964). Development of young mice. In: Barnothy MF (ed). Biological effects of magnetic fields. New York, Plenum Press pp. 93-99.
3. Coelho A M, Roger W R Jr, Smith H D (1995). Effects of current exposure to 60 Hz electric and magnetic fields on thesocial behavior of baboons. *Bioelectromagnetics Supplement* **3**, 71-92.
4. Rothman K J, Loughlin J E, Funch D P, *et al.* (1996). Overall mortality of cellular telephone customers. *Epidemiology* **7**, 303-305.
5. Tabrah F L, Ross P, Hoffmeier M, *et al*. (1998). Clinical report on long-term bone density after short-term EMF application. *Bioelectromagnetics* **19**, 75-78.
6. Knave B (2001). Electromagnetic fields and health outcomes. *Annals of the Academy of Medicine, Singapore* **30**(5), 489-493.
7. Becker R O (1978). Electrical osteogenesis-Pro end Co. *Calcif Tissue Res* **26**, 93-97.
8. Bassett C A L, Pawluk R J, Pilla A A (1974). Augmentation of bone repair by inductively coupled electromagnetic fields. *Science* **184**, 575-577.
9. Archer C, Ratcliffe N (1981). The effects of pulsed magnetic fields on bone and cartilage *in vitro*. *Trans Bioelec Rep Rowth Soc* **1**, 1.
10. Jamsa T, Tuukkanen J, Jalovaara P (1998). Femoral neck strength of mouse in two loading configurations: method evaluation and fracture characteristics. *Journal of Biomechnics* **31**, 723-729.
11. Jamsa T, Jalovaara P, Peng Z, *et al*. (1998). Comparison of Three-Point Bending Test and Peripheral Quantitative Computed Tomography Analysis in the evaluation of the Strength of Mouse Femur and Tibia. *Bone* **3**(2), 155-161.
12. Reiter J R (1998). Melatonin in the context of the reported bioeffects of environmental electromagnetic fields. *Bioelectrochemistry and Bioenergetics* **47**, 135-142.
13. Heikkinen P, Kosma V M, Alhonen L, *et al.* (2003). Effects of mobile phone radiation on UV-induced skin tumourigenesis in ornithine decarboxylase transgenic and non-transgenic mice. *Int J Radiat Biol* **79**(4), 221-233.
14. Aday W R, Lawrence A F (1984). Nonlinear Electrodynamics in Biological Systems. New York: Plenum.
15. Riggs B L, Melton L J (1986). III Involutional osteoporosis. *N Engl J Med* **314**, 1676-1686.
16. Giavaresi G, Fini M, Gnudi S, *et al.* (1999). Effect of pulsed electromagnetic fields on ovariectomized rats. *Electro-Magnetobiol* **18**, 119-131.
17. McElhaney J H, Stalnaker R, Bullard R (1968). Electric fields and bone loss of disuse. *J Biomech* **1**, 47-52.
18. Orban Z, Mitsiades N, Burke T R Jr, *et al.* (2000). Caffeic acid phenethyl ester induces leukocyte apoptosis, modulates nuclear factor-kappa B and suppresses acute inflammation. *Neuroimmunomodul* **7**, 99-105.
19. Fitzpatrick L R, Wang J, Le T (2001). Caffeic acid phenethyl ester, an inhibitor of nuclear factor-κB, attenuates bacterial peptidoglycan polysaccharide-induced colitis in rats. *J Pharmacol Exp Ther* **299**, 915-920.
20. Repacholi M H (2001). Health risks from the use of mobile phones. *Toxicol Lett* **120**(1-3), 323-331.
21. Yildiz M, Cicek E, Cerci S, *et al*. (2006). Influence of electromagnetic fields and protective effect of CAPE on bone mineral density in rats. *Arch Med Res* **37**(7), 818-821.

(*Received March* 19, 2007 *Accepted October* 25, 2007)