Brazilian Journal of Medical and Biological Research (2006) 39: 1501-1505
ISSN 0100-879X Short Communication

Electrical field stimulation improves bone mineral density in ovariectomized rats

A.P.R. Lirani-Galvão[1],
C.T. Bergamaschi[2],
O.L. Silva[3]
and M. Lazaretti-Castro[1]

[1]Divisão de Endocrinologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil
[2]Divisão de Ciências da Saúde, Universidade Federal de São Paulo, Campus Baixada Santista, Santos, SP, Brasil
[3]Departamento de Bioengenharia, Universidade de São Paulo, São Carlos, SP, Brasil

Correspondence
A.P.R. Lirani-Galvão
Rua Gonçalo da Cunha, 130/74
04140-040 São Paulo, SP
Brasil
Fax: +55-11-5579-6636
E-mail: analirani@fcr.epm.br

A.P.R. Lirani-Galvão has a fellowship from CNPq.

Publication supported by FAPESP.

Received November 29, 2005
Accepted July 21, 2006

Abstract

Osteoporosis and its consequent fractures are a great social and medical problem mainly occurring in post-menopausal women. Effective forms of prevention and treatment of osteoporosis associated with lower costs and the least side effects are needed. Electrical fields are able to stimulate osteogenesis in fractures, but little is known about their action on osteoporotic tissue. The aim of the present study was to determine by bone densitometry the effects of electrical stimulation on ovariectomized female Wistar rats. Thirty rats (220 ± 10 g) were divided into three groups: sham surgery (SHAM), bilateral ovariectomy (OVX) and bilateral ovariectomy + electrical stimulation (OVX + ES). The OVX + ES group was submitted to a 20-min session of a low-intensity pulsed electrical field (1.5 MHz, 30 mW/cm^2) starting on the 7th day after surgery, five times a week (total = 55 sessions). Global, spine and limb bone mineral density were measured by dual-energy X-ray absorptiometry (DXA Hologic 4500A) before surgery and at the end of protocol (84 days after surgery). Electrical stimulation improved ($P < 0.05$) global (0.1522 ± 0.002), spine (0.1502 ± 0.003), and limb (0.1294 ± 0.003 g/cm^2) bone mineral density compared to OVX group (0.1447 ± 0.001, 0.1393 ± 0.002, and 0.1212 ± 0.001, respectively). The OVX + ES group also showed significantly higher global bone mineral content (9.547 ± 0.114 g) when compared to both SHAM (8.693 ± 0.165 g) and OVX (8.522 ± 0.207 g) groups ($P < 0.05$). We have demonstrated that electrical fields stimulate osteogenesis in ovariectomized female rats. Their efficacy in osteoporosis remains to be demonstrated.

Key words
• Rats
• Osteoporosis
• Ovariectomy
• Electrical stimulation
• Bone mineral density
• Osteogenesis

Osteoporosis is characterized by low bone mineral density (BMD) and deterioration of bone microarchitecture that increases bone susceptibility to fracture (1). Postmenopausal and senile osteoporosis are the most common primary forms of bone loss seen in clinical practice. In the development of osteoporosis, there is often a long latent period before the appearance of the main clinical manifestation, i.e., pathologic fractures (2).

Women are more affected than men because of the decrease in sexual steroids after menopause. Osteoporosis evaluated by dual-energy X-ray absorptiometry (DXA) accord-

ing to WHO criteria was found in 33.2% of females and 16.1% of males in an elderly population from the city of São Paulo, Brazil (3). In white women over 50 years old, the risk of osteoporotic fracture is nearly 40% over their remaining lifetime (4) and has a huge impact on their life quality and mortality.

In general, all available pharmacological treatments for osteoporosis decrease the relative risk of a new fracture by about 50 to 65% (1). Nevertheless, besides the high costs of these treatments, adverse events such as dyspeptic conditions (nausea, vomiting, abdominal pain) (5) and metabolic and thromboembolic disturbances are not infrequently observed in patients receiving any of the available drugs for the treatment of osteoporosis, limiting the compliance with this kind of treatment.

Considering its high incidence, alternative treatments with best cost benefit rates for postmenopausal osteoporosis have been investigated. Since bone is a piezoelectric material - i.e., when deformed it can transform mechanical energy into electrical energy - some physical agents have been suggested. Electrical stimulation for bone repair has been used for three decades and was approved by the Food and Drug Administration (FDA) for pseudarthrosis and bone nonunion (6). Mechanical or electrical stimuli of relative low amplitude and high frequency can influence bone formation and resorption *in vitro* and *in vivo*, suggesting that these modalities can be used clinically to inhibit or reverse osteopenia (7).

The objective of the present study was to determine by bone densitometry the effects of long-term electrical stimulation in ovariectomized rats.

The study was approved by the Ethics Committee for Animal Research, School of Medicine of the Federal University of São Paulo, São Paulo, SP, Brazil, under protocol number 1381/04. Thirty female Wistar rats (220 ± 10 g) were operated according to the ethical principles for animal experimentation of the International Council for Laboratory Animal Science (8). They were subjected to 20 mg/kg xilazine anesthesia (Vetbrands®, Jacareí, SP, Brazil), and 40 mg/kg ketamine, *ip* (Vetbrands®) for bilateral ovariectomy (OVX, N = 20) or sham operation (SHAM, N = 10). The surgical experimental model of ovariectomy described in the osteoporosis study by Giardino et al. (9) was used in the present investigation since ovariectomized rats have been used as an accepted model of postmenopausal osteoporosis. The other 10 rats (SHAM group) were submitted to the same procedure but the ovaries were not removed.

Animals were kept in cages under appropriate light and temperature conditions (alternate cycles of 12 h and temperature approximately 25ºC), with free access to water and food.

Rats were divided into three groups (N = 10 per group): OVX and SHAM rats did not receive any treatment. OVX + ES rats (OVX rats submitted to electrical field stimulation) received a low-intensity pulsed electrical field treatment (1.5 MHz, 1:4 duty cycle, 30 mW/cm^2) in 55 sessions of 20 min, five times a week for 11 weeks, starting on the 7th day after surgery (DAS) in order to avoid pain due to early manipulation.

The electrical field stimulation equipment was constructed in the electronics laboratory of Bioengineering Department (University of São Paulo, São Carlos, SP, Brazil). Metal electrodes (25 x 35 cm) were positioned on the upper and lower parts of the cage in order to submit the entire body of the rats to this low-intensity electrical field through capacitated coupling, which does not cause any discomfort to the animals. The electronic unit and the dosage parameters were the same as those of low-intensity pulsed ultrasound, which is approved by American FDA to accelerate bone healing (6).

All animals were submitted to bone den-

sitometry (DXA - Hologic, model 4500 A, Waltham, MA, USA) using the specific software for small animals to evaluate global, spine and lower limb BMD (g/cm^2), global bone mineral content (BMC, g) and body composition. The exam was performed in a blind fashion on the 7th DAS and on the day before sacrifice (84 DAS).

Groups were compared by one-way analysis of variance (ANOVA). The Tukey-Kramer post test was applied to determine significant differences among the 3 groups, with the level of significance set at 5% in all analyses. The GraphPad Prism, version 3.03 (GraphPad Inc., San Diego, CA, USA) software was used. Data are reported as means ± SEM.

On the 7th DAS (basal), there was no difference between groups regarding weight, global BMD or global BMC. At the end of the 84-day protocol (Table 1), BMD and BMC were lower in the OVX group compared to the SHAM group, demonstrating the efficiency of ovariectomy in our model. Final global BMD was significantly lower in the OVX than the OVX + ES and SHAM groups, as shown in Figure 1. Percent gains of global BMD (Δ%) were significantly higher in the SHAM and OVX + ES groups than in the OVX group. Similarly, the spine and lower limb BMD of SHAM and OVX + ES were higher than that of the OVX group. At the end of treatment, the group that received electrical stimulation (OVX + ES) presented a significantly higher BMC than the OVX and SHAM groups. The same occurred when BMC + lean mass was compared among groups, with statistical relevance for OVX + ES when compared to OVX and SHAM. All data are summarized in Table 1.

Ovariectomy-induced osteoporosis in female rats is a well-accepted and established model for the study of postmenopausal osteoporosis (9). The efficacy of ovariectomy in our experiment could be demonstrated by BMD values, which did not increase in the OVX group as much as in the SHAM group. Using this model, our results demonstrated that the exposure of ovariectomized rats to electrical fields for 20 min a day, five times a week maintained the same bone mass as that detected in the sham-operated rats. This fact was consistent in all bone regions evaluated (global, spine and lower limb).

The beneficial effects of low-intensity electrical stimulation on bone have been demonstrated in other models, such as male rats with disuse-induced osteoporosis after limb immobilization (10) or after sciatic neurectomy (11). Pulsed electromagnetic fields (PEMF) can significantly suppress trabecular bone loss and restore trabecular bone structure in bilaterally ovariectomized rats evaluated by histomorphometry (12). However, the present study is the first to demonstrate the efficacy of electric stimulation to

Table 1. Dual-energy X-ray absorptiometry (DXA) parameters obtained at the end of the experiment.

DXA parameters	SHAM	OVX	OVX + ES
Δ%BMD	8.432 ± 1.062*	3.305 ± 1.108	9.701 ± 1.483*
Final global BMD (g/cm^2)	0.1495 ± 0.001*	0.1447 ± 0.001	0.1522 ± 0.002*
Final spine BMD (g/cm^2)	0.1511 ± 0.003*	0.1393 ± 0.002	0.1502 ± 0.003*
Final lower limb BMD (g/cm^2)	0.1333 ± 0.002*	0.1212 ± 0.001	0.1294 ± 0.003*
Final global BMC (g)	8.693 ± 0.165	8.522 ± 0.207	9.547 ± 0.114**
Final BMC + lean mass (g)	206.820 ± 11.090	189.940 ± 6.010	237.960 ± 4.580*

Data are reported as mean ± SEM values of the DXA parameters obtained for the three groups at the end of the experiment after 55 sessions for 11 weeks. OVX = ovariectomy; OVX + ES = ovariectomy plus electrical stimulation; BMD = bone mineral density; Δ%BMD = percent BMD gain after the protocol; BMC = bone mineral content. *P < 0.05 *vs* OVX; **P < 0.05 *vs* OVX and SHAM (one-way ANOVA).

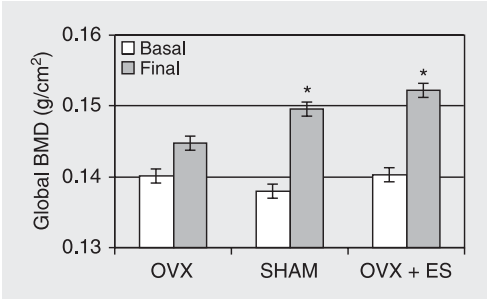

Figure 1. Global bone mineral density before and after electrical field stimulation. The ovariectomized rats received 5 sessions of irradiation per week for 11 weeks. BMD = bone mineral density; OVX = ovariectomy; OVX + ES = ovariectomy plus electrical stimulation. *P < 0.05 *vs* final OVX (one-way ANOVA).

prevent bone loss in all bone regions after ovariectomy in rats.

In human beings, electric and electromagnetic stimulation have been safely used for physical rehabilitation for a long time, but there are only few studies in the literature aiming at the prevention or treatment of osteoporosis using electrical stimulation. In a blind and randomized clinical trial, 40 post-menopausal osteoporotic patients were exposed at the spine and pelvis level to PEMF or placebo for 1 h/day, three times a week for 3 months. In the treated group there was a significant increase in serum osteocalcin and C-terminal peptide of pro-collagen type I levels, both markers of bone formation. These results suggest that PEMF can stimulate osteogenesis in a 3-month treatment by augmenting osteoblastic activity in postmenopausal osteoporotic women. The authors, however, could not demonstrate any effect on BMD, but it is known that three months is a short period of time to observe any alteration in bone mass (13). Tabrah et al. (14) determined the effect of a PEMF on bone density of the radii of osteoporosis-prone women for a period of 12 weeks. BMD of the treated radii measured by single-photon densitometry increased significantly in the area during the exposure period. Their data suggest that properly applied PEMF, if scaled for whole-body use, may have clinical application in the prevention and treatment of osteoporosis.

The cellular mechanism of electrical stimulation is not totally clear, but osteoblasts in culture are sensitive to electrical stimulation, resulting in an enhancement of the biomineralization process (15). Biochemical pathways are activated when electrical stimulation is applied to bone cells. Signal transduction of the capacitated coupling, the same as used here, has been shown to translocate Ca^{2+} ions through cell-membrane voltage-gated calcium channels, inducing an increase in cytosolic Ca^{2+} (16). Capacitated coupled electric fields accelerate cell proliferation and differentiation in osteoblast-like cells *in vitro* and enhance cell synthesis, leading to matrix formation and maturation (17). PEMF have been recently demonstrated to regulate osteoclastogenesis, bone resorption, osteoprotegerin, receptor activator of NFκB-ligand and macrophage colony-stimulating factor concentrations in a murine marrow culture system (18).

Martini et al. (19) studied the effects of an electrical field applied to osteoblast-like human cells in culture. After 24 h of treatment they noticed a significant increase in osteocalcin and nitric oxide (NO) production. In another study, osteoblasts of the MC3T3-E1 lineage with or without an NO synthase inhibitor (L-NMMA) were exposed to PEMF stimulation for 15 days. PEMF significantly increased NO synthesis, cellular proliferation and osteoblast differentiation. These effects were associated with an increase in NO synthesis since the addition of L-NMMA inhibited all the stimulatory effects of PEMF (20). Thus, the most recent hypothesis about the effects of electrical fields on bone cells has considered NO production as an essential pathway.

Our data showed a consistently positive effect of electrical field stimulation on BMD and BMC, preventing ovariectomy-induced osteoporosis in female rats. The stimulation of female rats with an electrical field for 11 weeks was able to prevent the bone loss induced by ovariectomy. Our results encourage us to continue in this research field, studying the mechanisms of electrical field stimulation in bone metabolism for possible future application to the prevention and treatment of different forms of osteoporosis.

References

1. Zizic TM. Pharmacologic prevention of osteoporotic fractures. *Am Fam Physician* 2004; 70: 1293-1300.
2. Glaser DL, Kaplan FS. Osteoporosis. Definition and clinical presentation. *Spine* 1997; 22: 12S-16S.
3. Camargo MB, Cendoroglo MS, Ramos LR, de Oliveira Latorre MR, Saraiva GL, Lage A, et al. Bone mineral density and osteoporosis among a predominantly Caucasian elderly population in the city of São Paulo, Brazil. *Osteoporos Int* 2005; 16: 1451-1460.
4. Melton LJ III, Beck TJ, Amin S, Khosla S, Achenbach SJ, Oberg AL, et al. Contributions of bone density and structure to fracture risk assessment in men and women. *Osteoporos Int* 2005; 16: 460-467.
5. Biswas PN, Wilton LV, Shakir SA. Pharmacovigilance study of alendronate in England. *Osteoporos Int* 2003; 14: 507-514.
6. Lind M, Bunger C. Factors stimulating bone formation. *Eur Spine J* 2001; 10 (Suppl 2): S102-S109.
7. Rubin CT, McLeod KJ. Inhibition of osteopenia by biophysical intervention. In: Marcus R, Feldman D, Kelsey J (Editors), *Osteoporosis*. San Francisco: Academic Press; 1996.
8. COBEA. Ethical principles on animal experimentation. *Colégio Brasileiro de Experimentação Animal*. http://www.sbpqo.org.br/info/info6.html; 1991.
9. Giardino R, Fini M, Giavaresi G, Mongiorgi R, Gnudi S, Zati A. Experimental surgical model in osteoporosis study. *Boll Soc Ital Biol Sper* 1993; 69: 453-460.
10. Martin RB, Gutman W. The effect of electric fields on osteoporosis of disuse. *Calcif Tissue Res* 1978; 25: 23-27.
11. Brighton CT, Pollack SR. Uspto patent full-text and image database. US Patent No. 4,467,808. Access at http://patft.uspto.gov/ January 2005.
12. Chang K, Chang WH. Pulsed electromagnetic fields prevent osteoporosis in an ovariectomized female rat model: a prostaglandin E2-associated process. *Bioelectromagnetics* 2003; 24: 189-198.
13. Giordano N, Battisti E, Geraci S, Fortunato M, Santacroce C, Rigato M, et al. Effect of electromagnetic fields on bone mineral density and biochemical markers of bone turnover in osteoporosis: a single-blind, randomized pilot study. *Curr Ther Res Clin Exp* 2001; 62: 187-193.
14. Tabrah F, Hoffmeier M, Gilbert F Jr, Batkin S, Bassett CA. Bone density changes in osteoporosis-prone women exposed to pulsed electromagnetic fields (PEMFs). *J Bone Miner Res* 1990; 5: 437-442.
15. Wiesmann H, Hartig M, Stratmann U, Meyer U, Joos U. Electrical stimulation influences mineral formation of osteoblast-like cells *in vitro*. *Biochim Biophys Acta* 2001; 1538: 28-37.
16. Brighton CT, Wang W, Seldes R, Zhang G, Pollack SR. Signal transduction in electrically stimulated bone cells. *J Bone Joint Surg Am* 2001; 83-A: 1514-1523.
17. Hartig M, Joos U, Wiesmann HP. Capacitively coupled electric fields accelerate proliferation of osteoblast-like primary cells and increase bone extracellular matrix formation *in vitro*. *Eur Biophys J* 2000; 29: 499-506.
18. Chang K, Chang WH, Huang S, Huang S, Shih C. Pulsed electromagnetic fields stimulation affects osteoclast formation by modulation of osteoprotegerin, RANK ligand and macrophage colony-stimulating factor. *J Orthop Res* 2005; 23: 1308-1314.
19. Martini L, Giavaresi G, Fini M, Torricelli P, de Pretto M, Schaden W, et al. Effect of extracorporeal shock wave therapy on osteoblastlike cells. *Clin Orthop Relat Res* 2003; 269-280.
20. Diniz P, Soejima K, Ito G. Nitric oxide mediates the effects of pulsed electromagnetic field stimulation on the osteoblast proliferation and differentiation. *Nitric Oxide* 2002; 7: 18-23.