Effects of low level pulsed radio frequency fields on induced osteoporosis in rat bone

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Effect of modulated pulsed electromagnetic fields (PEMFs; carrier frequency, 14 MHz. modulated at 16 Hz of amplitude 10 V peak to peak) on sciatic neurectomy induced osteoporosis in rat femur and tibia resulted in statistically significant increase in bone mineral density, and deceleration in bone resorption process and consequently further osteoporosis in rat bone. These results suggest that such an effective window of pulsed radio frequency fields may be used therapeutically for the treatment of osteoporosis.

Keywords: Bone mineral density, Pulsed electromagnetic field, Osteoporosis.

Bone formation and resorption are continuous processes occurs throughout life. As a living and constantly changing tissue normally there is a balance between the amount of old bone being removed and the amount of new bone replacing it\(^1\). When the resorption is more than the formation, the bone loss occurs resulting in osteoporosis. Osteoporosis, or porous bone, is a disabling disease characterized by low bone mass and structural deterioration of bone tissue. It reduces the density and strength of bones, leading to bone fragility and an increased susceptibility to fractures\(^2\). Osteoporosis is probably the most common metabolic disorder controlled by several systemic and local factors, which regulate the formation and activity of bone cells (osteoclasts and osteoblasts). People over 45 years of age experience bone fractures due to osteoporosis (senile or osteoblast mediated) in which fracture of proximal femur and inter trochantric fractures are common. Around 250 million women worldwide have osteoporosis now. By the year 2020, the number of women affected will double\(^3\). Clinical surveys have demonstrated that adult bone mass diminishes at a mean rate of 0.5% per year\(^4\), and it can reach a loss of 2% per year after menopause. As the probability of fracture is related to a person's effective bone mass, a modality that could prevent or retard loss of bone may provide a substantial reduction in the incidence of skeletal morbidity.

Several prophylactic measures to prevent loss of bone are available; these include estrogen therapy, calcitonin, parathyroid hormone, bisphosphonates, vitamin D, prostaglandin E\(_2\), supplemental dietary calcium and exercise. There are also treatment regimens that stimulate formation of bone, such as sodium fluoride and parathyroid hormone. Although these regimens have been effective in the treatment of osteoporosis, limitations, cautions, and dangers are inherent in their extended use. The clinical potential for increasing bone mass or simply preventing bone loss, by alternative non-invasive means is therefore substantial.

According to Brighton et al.\(^5\) a capacitatively coupled electrical signal, delivered through gel-coated electrodes, could largely reverse an established disuse osteoporosis due to neurectomy in the rat tibia. Bioelectric effects therefore appear to provide a link between mechanical stimuli and cellular behavior. The application of electricity, later on as a treatment for delayed unions and non-unions of bone has received attention\(^6\). Rubin et al.\(^7\) concluded that there is an effective window of pulsed electromagnetic fields which could control the bone mass. Mcleod and Rubin\(^8\) also demonstrated that a simple, low power sinusoidal field at 15 Hz is the most osteogenic of any induced field they studied. This is achieved by comparatively short daily exposure generated within a physiological intensity and frequency. These electrical fields can slow, inhibit or even reverse the osteoporotic processes that normally accompany disuse in animal model. Behari et al.\(^9\) and Behari\(^10\) showed that
pulsed radio frequency electrical field can accelerate bone fracture healing in rats. Rubin and McLeod\textsuperscript{11} reported that the mechanical stimuli at 15-30 Hz frequencies results in a bone formation rate than do stimuli at 1-10 Hz frequencies. Wang et al.\textsuperscript{12} suggested that direct current stimulation promoted the osteogenic processes in bone metabolism and expanded osteoblast-like cells able to be entering into massive skeletal defects to promote cell mediated regeneration of skeletal tissues.

As there is no established treatment of osteoporosis, the efforts are on for prevention of bone loss and fragility. The objective of the present study is to examine the extent bone afflicted with osteoporosis responds to the stimulation of a non-invasive method of pulsed electromagnetic field (PEMF).

**Materials and Methods**

Three months old 24 male Wistar rats weighing approximately 200 g each were divided into 3 groups (10, 10, and 4). Each rat of first group (10 rats) underwent only one-sided sciatic neurectomy on the first day of 70 days experiment. After the animal had been anaesthetized with phenobarbitone sodium (30 mg/kg of body wt. ip), one hind limb was shaved over the thigh and disinfected. An incision was made on the upper thigh just posterior to the femoral trochanteric region. The sciatic nerve was mobilized within the incision and about 0.5cm section was excised. The incised skin was closed with sterilized ethicon thread by using surgical needle. Sciatic neurectomy was done in rats of the 2\textsuperscript{nd} group (10 rats) in both legs by the same procedure (one leg “sham-exposed” and other as “exposed”) on the same day as the first group. Antibiotic (anthrocin 250; equivalent of 250 mg of erythromycin estolate IP) was given (as needed) through drinking water for early healing of wound and inhibition of any infection. Third group (4 rats) was left as “control” without any surgical procedures. Each rat was housed in 17×18×24 cm sized plastic cage and allowed free access to tap water and pelleted commercial diet.

After 30 days of sciatic neurectomy, rats of first group were scanned \textit{in vivo} by DEXA (Dual Energy X-ray Absorptiometry). Then all rats of the first group were sacrificed and femur and tibia were resected for further analysis. After 30 days of denervation, all rats of the 2\textsuperscript{nd} group received the exposure of pulsed radio frequency signal. For the exposure, we used a bone stimulator with following specification\textsuperscript{16}.

- **Carrier frequency**: 14.0 MHz
- **Modulating frequency**: 16.0 Hz
- **Amplitude**: 10 V (peak to peak)
- **Output waveform**: Square
- **Electrode diameter**: 1 cm
- **Average electric field between electrodes**: 7.8 Volt/m (10)

Calibration was done with the help of Iwatsu Oscilloscope SS -5711 C, Japan.

Output of bone stimulator was given to each rat separately by a pair of electrodes (Fig. 1) in only one leg daily for 2 hr. Current density at the point of application was 80 \mu A/cm\textsuperscript{2}. Other leg was tied with same type of electrodes without any connection to stimulator (Sham-exposed). Rats were lightly anaesthetized, before giving the exposure, so that they could not disturb the experimental process. \textit{In vivo} DEXA scan of rats of second group was done after a 30 days of exposure. After the \textit{in vivo} DEXA scan of rats of third group, all rats of 2\textsuperscript{nd} and 3\textsuperscript{rd} group were sacrificed and femur and tibia were resected for further analysis.

**DEXA scanning** — All DEXA scans were performed on Hologic QDR-4500A scanner (Hologic, Waltham, MA) by using software for small animal at Nuclear Medicine Department, Indraprastha Apollo Hospital, New Delhi, India. Before scanning rats were lightly anaesthetized with phenobarbitone sodium (ip) to make them stable and immovable. Then they were fixed on hard paper (150 gsm) in supine position with the help of surgical tape. After the scanning, we carefully selected the regions of interest (ROI, i.e. tibia and femur) on the monitor of the instrument and obtained the bone mineral content and bone mineral density of the areas under investigation.

**Scanning electron microscopy** — Resected femur and tibia of five rats of 1\textsuperscript{st} and 2\textsuperscript{nd} group were made free of all soft tissues. Transverse sections of femur

![](image)

**Fig. 1** — Schematic diagram of exposure set up.
and tibia of each leg ('normal' and 'denervated' of 1st group, 'exposed' and 'sham-exposed' of 2nd group) of 0.5 cm thickness were made by fine saw edged knife very carefully as osteoporotic bone was fragile and generally could break during cutting. They were separately put into fixative (glutaraldehyde; 2ml-25%, generally could brake during cutting. They were very carefully as osteoporotic bone was fragile and glutaraldehyde solution, 3 ml-37% formaldehyde solution, 1.58g-dehydrated calcium acetate, aqua ad 100 ml) for minimum fixation time (8-24 hr for anatomical details of bone) at room temperature. Then these samples were washed carefully with phosphate buffer solution and dehydrated till the critical point shrinking. With silver paint these samples were conductive for taking image. Same procedure was repeated with femur and tibia of two rats of 3rd group. SEM photography was performed on 'low vacuum SEM' LEO 435 VP (Cambridge, England).

Bone ash — Resected femur and tibia of rest of the rats of all groups were made free from soft tissues and their wet weights were determined by electronic balance. Femur and tibia were next placed in individual porcelain crucibles and dried for twenty-four hours in oven at 100°C. After dry weights were determined, femur and tibia were placed in a muffle furnace for 2 hr at 800°C and ash weights were determined.

Results
The results of DEXA measurements in the region of interest of femur and tibia are summarized in Table 1. A significant decrease in the BMD was observed in the femur and tibia of denervated leg after one month of denervation, which was further lowered in ROI of sham-exposed legs, whereas, BMD increased in the femur and tibia of the legs exposed to PEMF. Although, it was not up to the normal level but it was certainly more than the osteoporotic and sham-exposed legs. P-value in one month sham-exposed versus one-month exposed rat bone was less than 0.05 but versus denervated it was more than 0.05 (Table 1). Results of percentage of bone ash content also confirms the results of DEXA.

Images of various parts of transverse section of femur and tibia show the clear differences in normal, osteoporotic and exposed bone. Compactness of cancellous part of bone in transverse section indicates the mineral deposition in normal and exposed bone with respect to osteoporotic bone (Fig. 2a-c). Moreover in normal tibia, bone marrow is attached to the cortex (Fig. 3a) and cortical thickness was more than that of the osteoporotic tibia in which marrow was also detached from the cortex (Fig. 3b). But after the PEMF exposure, new growth in the endosteal part of the cortex was clearly observed (Fig. 3c). Denervation induced the osteoclastic activities in bone and made it porous (Fig. 4b) with respect to normal (Fig. 4a). After exposure of low-level PEMF for 30 days, the amount of porosity started to reduce and pores were being filled with minerals (Fig. 4c).

Discussion
The study of the bioelectric effects in bone had its modern origin in 1957, when Fukada and Yasuda presented their experimental and theoretical work demonstrating that bone possessed piezoelectric properties. Their work indicated that when external forces were applied to bone it generated electrical potential. The bone contains mechanosensing cells (osteocytes) distributed throughout the bone matrix, monitor mechanical strain and activate corrective biological processes. These osteocytes produce a electrical signal proportional to mechanical loading either by sensing strain on bone surfaces through stretch-activated ion channels or electrical potentials or flow of interstitial fluid or some other phenomenon. Cell to cell communication of electrical signals and small molecules through gap junctions has been

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<th>Table 1 — Bone mineral density (BMD) and bone ash (% in relation to dry wt) of femur and tibia at one month of exposure to PEMF</th>
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<td>[Values are mean ± SE]</td>
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P < 0.05 sham exposed vs. exposed; >= 0.05 sham exposed vs. one month denervated.
demonstrated in osteoblasts. Similar gap junctions in osteocytes participate in such communication with osteoblasts, and bone lining cells as well. Mechanical loading is converted to an electrical signal that can be transmitted intracellularly to the bone lining cells, creating intracellular or transmembrane potential changes in the osteoblasts. According to above-mentioned works it is established that mechanical stress generates electrical signals, which induces bone remodeling. In osteoporotic bone we

Fig. 2—T. S. of cancellous part of femur bone showing compactness, (a) normal, (b) osteoporotic and (c) exposed.

Fig. 3—T. S. of tibia showing (a) normal, (b) osteoporotic, and (c) exposed bone. In exposed bone (c) new growth observed in endosteal part of the cortex.
couldn't induce mechanically stress-generated potential as bone was fragile. Hence we induced osteogenic potential in bone through non-invasive capacitor plates. It is yet to be settled that which form of electrical energy (AC, DC, Pulsed) is most efficient stimulator of osteogenesis. In an attempt to quantitate these a series of experiments have been carried out to study the high frequency responses.\(^\text{22}\) Stimulation caused by the PEMFs is likely to communicate this signal to osteocytes and may well as perturb the fluid flow in bone. Experimental and clinical research studies have shown positive effects of PEMFs on endochondral bone formation and on osteogenesis. It is shown that extremely low frequency EMF exposure can alter Ca\(^{2+}\) transport, probably through Ca\(^{2+}\) channel without having any such effect on non-activated cells.\(^\text{23}\) The temporary application of a 60 Hz sinusoidal E-fields causes some dynamic changes in cell membrane components and/or within the vicinity of cellular membrane, reflecting in reduced or induced Ca\(^{2+}\) influx respectively through ATP (cyclic AMP) or histamine induced ion channels. Even though the observed effects have been temporary, it is possible that chronic exposure of low intensity EMFs could have long lasting effects on cell physiology, through changes of Ca\(^{2+}\) distribution within the cells.\(^\text{24}\) Increased BMD and increased percentage of bone ash of exposed bone in comparison to sham-exposed bone (Table 1) suggest that PEMF exposure reduces the process of induced osteoporosis and retains the mineralization inside bone by the same mechanism.

Bone strength depends on the mechanical quality and the spatial distribution of the mineralized matrix. Thus, the effects of treatments for bone-weakening diseases should not be evaluated according to the changes elicited in the bone mass, but in the stiffness of bone material and/or in the architectural design of the bone as an organ.\(^\text{25}\) Although, it is clear that mineralization occurs in the osteoporotic bone and bone mass and bone mineral density (BMD) increase after the PEMFs exposure, it is not as much compact as the original structure. But it certainly gives the support to the osteoporotic bone by filling its pores and reduces the risk of fractures. Increase in duration of PEMFs exposure in hours as well as in days may provide compactness and strength to bone. As the beginning of tissue regeneration in endosteal part of tibia makes the cortical bone thick and must have positive effect on the bone strength.

Acknowledgement

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Fig. 4—T. S. of femur bone showing porosity, (a) normal, (b) osteoporotic, and (c) exposed. Porosity in osteoporotic bone (b) is more than exposed (c) and normal (a) bone.
References