

# Sensory motor behavior, histological, biochemical evidences indicate reversal of osteoporosis in spinal cord injury model.

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## Abstract

The present study was carried out to evaluate the effect of extremely low frequency and intensity magnetic field in the development of SCI induced sublesional osteoporosis. Adult Male Wistar rats (n=30) were equally divided into SCI, SCI + MF, Sham exposed groups. Complete transection of spinal cord (T11 vertebra) was surgically performed after laminectomy in ketamine anesthetized rats. Sensory-motor functions were repeatedly assessed during the 8-weeks study period in support of magnitude of injury and the recovery if at all by ELF-MF. Femur and tibia bones were removed at the end of study for evaluation of BMD, BMC (Ca<sup>++</sup>, P, C contents), histological (SEM), radiological (DEXA) and biochemical (bone Osteocalcin, alkaline phosphatase, Collagen I; spinal cord 5HT, adrenalin, glutamate) evidences. MF rats received 17.9 $\mu$ T, 2h/d post SCI for 8 weeks, while sham group of rats were similarly treated except for switch 'ON' was not pressed. The OC, Collagen I and ALP in femur significantly decreased (459.74 $\pm$ 52.99  $\mu$ g/g, 213.33 $\pm$ 22.73 mg/g, 93.10 $\pm$ 11.57 I.U/g) in SCI versus control rat group (790.14 $\pm$ 76.71  $\mu$ g/g, 296.59 $\pm$ 34.8 mg/g, 134.67 $\pm$ 8.84 I.U/g) respectively which was significantly attenuated in SCI+MF rat group. Moreover, sensory motor parameters including BBB score, motor responses to noxious and non-noxious thermal/electrical stimuli and bladder control recovery was more and enhanced in MF exposed rats. Besides, the mortality rate and self injurious behavior were markedly reduced in MF exposed rats. The histological, radiological and the neurochemical profile of spinal cord at the injury site and surrounding it support a faster recovery in MF exposed rats. SCI caused loss of BMC, BMD and altered biochemical properties of sub-lesional bones indicative of significant osteoporosis in our rats which was attenuated by low intensity MF (17.96  $\mu$ T and 50 Hz) for 2h/d x 8 weeks. The bone histological, radiological and the neurochemical profile of spinal cord and bone supports the recovery of sensory, motor and bone deficits by MF.

## 1. Introduction

The most rapid and severe forms of osteoporosis following SCI, which is detected by micro CT as early as 10 days in only distal metaphysis of femur while, at 3 wks in metaphysis, epiphysis and diaphysis of both femur and tibia in lower thoracic SCI rats. A decrease in dry and ash weight are reported as early as 3 wks while the wet weight at 6 wks post SCI in rats. Moreover, deterioration in bone mass and micro-architecture has been detected at 3 wks, while increase in water content at 6 months (1).

Biochemical indicators of bone loss support SCI induced osteoporosis which is contributed by osteoclast dysfunction and suppressed osteoblast activity. The former is indicated by Osteocalcin (OC), alkaline phosphatase (ALP) and procollagen; while later by serum carboxyterminal telopeptide of type I collagen and urinary hydroxyproline, pyridinoline, collagen type I telopeptide etc. The ALP activity remains unchanged upto 3 wks, 6 wks and 6 months after SCI. Intensive exercise regimens or, standing may partially prevent bone loss in femoral shaft but not at the proximal hip, while functional electrical stimulation has been reported to be almost ineffective even at 3 to 12 months post SCI. Moreover, the usually advocated pharmacological agents (etidronate, alendronate) have also been reported to be ineffective in SCI patients because of probably an exorbitant bone consequent to osteoclastic activity at all the resorption pits.

Pulsed electro-magnetic field (PEMF) therapy has been beneficial in promoting peripheral *nerve regeneration* and *osteogenesis* (2). EMF can stimulate osteoblasts, induces differentiation of cartilage cells and enhances alkaline phosphatase (ALP) activity in rat osteoblastic cells, although the mechanism is not clear (3, 4). However, there are no reports of bone loss prevention by PEMF following SCI either in human

or animal model. In the present study we have adopted the complete spinal cord transection model to determine the efficacy of repeated (2 h/d for 8 wks) magnetic field (MF, 50 Hz, 17.96  $\mu$ T) whole body exposure for 8 wks on osteoporosis.

## 2. Materials and Methods

**Animals:** Equal number (n=8) of adult male wistar rats (bw 230-250 g) were divided into study groups Control, SCI and SCI+MF.

**Exposure to magnetic field:** modified Helmholtz coil was utilized for exposure to provide 17.96  $\mu$ T and 50 Hz in the central area of the axis, where rats were kept (5).

**Spinal cord injury:** Under katamine anesthesia (60mg/kg, bw, ip) complete transection of spinal cord (T10-12) was surgically performed. Post surgery care of bladder was taken.

**Determination of volumetric bone mineral content (BMC) and density (BMD):** The volume of fresh bones were determined by archimedes principle, wet weight was determined, bone was freeze dried, water % was calculated, fine powder of bone was obtained and bone ash was prepared in a muffle furnace. BMD was determined.

**Determination of element content:** Total calcium content was determined by the atomic absorption method, while phosphorus content by Vanado-Molybdo-phosphoric acid colorimetric method in UV-VIS spectrophotometer (470 nm). Total carbon content was determined by a carbon analyzer. Bone powder was taken in a sterile pretreated at 800°C ceramic boat and kept inside the analyzer (1200°C).

**Collagen I:** It was determined in rat bone by the method described by Galicka et al.

**Osteocalcin:** Bone powder was decalcified in ethylenediamine tetra-acetic acid, (10% w/v, pH 7.2), extracted by stirring at 4°C for 48 h. Concentration of osteocalcin in the supernatant was measured using a rat sandwich enzyme-linked immunosorbent assay kit.

**Alkaline Phosphatase Activity (ALP):** It was determined by plasma ALP kits.

**Rat sacrifice:** The rats were decapitated under deep anesthesia.

**Study plan:** The rats received exposure to magnetic fields each day for 2h/d from post SCI day 1 (10:00 - 12:00 h for 7d/wk x 8). The control and spinal cord injured groups of rats were similarly treated except for setting the switch of the power supply in “off” position. Tibia and femur were removed from both the sides and stored at -20°C.

**Statistical Analysis:** Data were analyzed by one-way ANOVA, and post hoc analysis was performed with Bonferroni test. P values less than 0.05 were accepted as significant.

## 3. Results

**Bone water content, BMC and BMD:** Water content of the femur and tibia were significantly (P<0.001) higher in SCI rats, while BMC and BMD were lower. There was no change in the bones of SCI+MF rats as compared to control rats.

**Total calcium, phosphorus and carbon contents:** In femur, calcium, phosphorus and carbon contents decreased post SCI, unlike SCI+MF rats. However, in tibia no difference was noted in SCI+MF exposed rats, except for carbon content.

**Collagen I:** SCI decreased collagen I concentration in bones, which was abolished in SCI+MF rats.

**Osteocalcin:** The OC was 790.14 $\pm$ 76.71, 850.37 $\pm$ 54.49 in femur, tibia, respectively in control, while it decreased (P<0.001) in SCI, which was abolished in SCI+MF rats.

**Alkaline phosphatase activity:** ALP activity after SCI decreased (P<0.001) in the femur (134.67 $\pm$ 8.84 vs. 93.10 $\pm$ 11.57, P<0.001) and tibia vs. control rats.

Histological and radiological evidences will be presented.

## 4. Conclusion

The present study was proposed to investigate the option of impediment of bone loss by extremely low frequency magnetic field in spinalized rats. Spinalization in our rats produced a significant decrease in

bone mineral (BMC, BMD and Ca, P, C) contents and biochemical (collagen I, osteocalcin and ALP) parameters of both the femur and tibia compared to the control rats. On the contrary, in our magnetic field (17.96  $\mu$ T and 50 Hz) exposed (2 h/day for 8 wks) spinalized rats there was no alteration in BMC, BMD or element contents of femur and except for C content in tibia. Moreover, bone collagen I, osteocalcin and ALP of femur and tibia of magnetic field treated spinalized rats, was found elevated as compared to SCI rats. Therefore, our results indicate that chronic exposure to MF has a potential to prevent bone loss induced by spinal cord injury.

## 5. References

1. Jiang SD, Jiang LS, Dai LY (2007) Changes in bone mass, bone structure, bone biomechanical properties, and bone metabolism after spinal cord injury: a 6-month longitudinal study in growing rats. *Calcif Tissue Int* 80(3):167-175.
2. Crowe MJ, Sun ZP, Battocletti JH, Macias MY, Pintar FA and Maiman DJ (2003) Exposure to pulsed magnetic fields enhances motor recovery in cats after spinal cord injury. *Spine* 28(24):2660-2666.
3. Tsai MT, Chang WH, Chang K, Hou RJ, Wu TW (2007) Pulsed electromagnetic fields affect osteoblast proliferation and differentiation in bone tissue engineering. *Bioelectromagnetics* 28(7):519-528.
4. Behari J (eds) (2009) *Biophysical Bone Behaviour: Principles and Applications*. Wiley, John & Sons, New Jersey, USA. pp 175-239
5. Kirschvink (1992) Uniform magnetic field and double wrapped coil system. *Bioelectromagnetics* 13:401-414.