Effect of a static magnetic field on bone healing in the dog: radiographic and histopathological studies

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Summary

Although the promotional effects on bone healing of pulsed electromagnetic fields (PEMF) have been well demonstrated, the effects of static magnetic fields (SMF) remained unclear. In this study, effects of a custom-made magnetic wrap on radiographic and histopathological aspects of bone healing using a canine unstable osteotomy gap model were investigated. After an osteotomy of the midshaft radius, bone healing was evaluated over an 8-week-period in the control dogs (n = 5) and dogs exposed to SMF (1000 gauss) (n = 5). Bone healing was evaluated by qualitative and quantitative evaluation of serial radiographs every 2 weeks. Histopathological study was also performed on osteotomized radii upon completion of the experimental period. Dogs exposed to SMF had more advanced radiographic healing of osteotomy sites. Cellular morphology scores of the SMF group was significantly greater as compared with the control group ($P<0.05$). These results suggested that using the osteotomy gap model, SMF enhanced the radiographic and histopathological aspects of bone healing in dogs. Dogs at risk for delayed healing of fractures may benefit from treatment with SMF.

Key words: Static magnetic field, Ostectomy, Radiography, Histopathology, Dog

Introduction

Biomagnetics is an interdisciplinary field where magnetism, biology and medicine overlap (Ueno *et al*., 2002). The use of electromagnetic fields in the healing arts dates back as far as the fifteenth century, although the magnetic effects of "lodestone" were first described by the shepherd Magnes circa 1000 BCE in the region known today as Turkey (Steyn *et al*., 2000). In the 18th century, Mesmer began treating hysteria and other disorders (today recognized to be psychosomatic in origin) with lodestones. He also claimed that non-magnetic substances such as paper, wool, silk and stones had similar healing properties. Mesmer was later declared a charlatan, and interest in magnetic field therapy subsequently waned. Recently, however, interest in magnetic field therapy has revived, and a variety of products are available for treatment of humans and horses (Steyn *et al*., 2000).

The mechanism of action whereby such effects might be attained remains elusive, but one hypothesis is that there is an increase in blood flow in areas influenced by the magnetic field (Steyn *et al*., 2000). Research into magnet therapy is divided into two distinct areas: pulsed bioelectric magnetic therapy (pulsed electromagnetic field (PEMF)) and fixed magnetic therapy (biomagnetic therapy) (Null, 1998). Pulsed elctromagnetic fields have been used to treat various conditions, such as inflammation, osteoarthritis, soft-tissue injuries, chronic pelvic pain, and tendonitis and have been reported to be useful for nerve regeneration, healing of osseous defects, bone grafts, and fractures and prevention of osteoporosis (Steyn *et al*., 2000). Probably 85-90% of the

scientific literature is on pulsed elctromagnetic therapy; the remainder is on therapy with fixed solid magnets (Null, 1998). Since it can not necessarily be assumed that a positive result from PEMF will automatically translate to a positive result from a static magnetic field (SMF), there needs to be more study in the area of fixed magnets. Therefore, the study reported here was conducted to evaluate the effect of an Iranian-brand SMF on bone healing in a radial osteotomized model in dog, determined by means of radiography and histopathology.

Materials and Methods

Animals

Ten apparently healthy intact male mongrel dogs were used in the experiment. The animals' mean age was 4.4 ± 0.4 years, and their mean body weight was 18.68 ± 2.32 kg. Dogs were randomly assigned to either control or experiment group (n = 5 dogs/group). The experimental procedures and animal care were approved by the Veterinary Clinical Sciences Committee at University of Urmia.

Magnetic wraps

Custom-made magnetic wraps composed of three square-shaped permanent magnetic plates (L, 1 inch; H, 1/8 inch) (Aerospace Complex, Malek-e-Ashtar University, Isfahan, Iran) were used to create the SMF, in this study. Wraps were made of commercial 15 cm elastic bandage rolls. Each magnetic plate was made of an alloy of strontium-ferrite ($SrFe_{12}O_{19}$). The magnetic power of each plate was 1000 gauss. The magnetic force between two facing magnet plates was attractive to each other, one polarity is north (N) and another one is south (S). Inactive plates, without magnetic power, were used in the control group. With the aid of a Hall probe (teslameter, 0.3-3000 millitesla (mT), Phywe, Germany) the regional magnetic density of each magnetic plate was scanned. The field intensity of the magnetic wraps in each animal was also examined weekly to ensure the steady magnetism during the experimental study.

Surgical procedure

Dogs were premedicated with atropine (Darou pakhsh, Tehran, Iran) (0.04 mg/kg, IM) and acepromazine (Hoogsrraten, Belgium) (0.1 mg/kg, intramuscularly), anaesthetized with sodium thiopental (Biochemie GmbH, Vienna, Austria) (10 mg/kg, 2.5%, intravenously) and maintained with halothane in oxygen in a semiclosed circle system. For creation of midshaft osteotomy right radius was chosen in each dog. The selected limb was prepared for aseptic surgery and then draped. The radius was approached through a craniomedial incision. To protect the ulna from damage during osteotomy, a Kelly haemostat was passed between the radius and ulna. A Gigli wire was used to create an osteotomy, leaving a 2-mm gap between the bone ends. The area was lavaged. Closure was routine. No internal or external fixation devices were applied.

All dogs received sodium ampicillin (Zakaria Pharmecutical Co., Tabriz, Iran) (25 mg/kg, intravenously, every 6 hrs) and gentamicin sulfate (Darou pakhsh, Tehran, Iran) (5 mg/kg, intravenously, every 24 hrs) for 5 consecutive post-operative days. Diazepam (Chemi Darou, Iran) (0.2 mg/kg, intravenously) was administered every 6 hrs after surgery for 24 hrs and as needed thereafter to control pain and discomfort.

Two days post-operative, the active magnetic wraps were placed over the operated regions (right mid-antebrachium) in dogs of the experiment group. They were fixed with non-compressive dressings and oriented in a way to assure the North Pole facing the limb. The operated regions in the control dogs were covered by inactive magnetic wraps, as well. The operated regions in each experimental group were exposed to SMF continuously throughout the entire experimental period (8 weeks), except during the short period of radiography (generally within 15-20 min).

Radiographic studies

The operated antebrachii were radiographed in lateral and anterioposterior views under sedation with acepromazine (0.1 mg/kg, IM) before and after osteotomy and 2, 4, 6 and 8 weeks after osteotomy. Qualitative and quantitative features of fracture healing were then evaluated. Qualitative assessment of fracture healing

included the relative amount of periosteal reaction and bony callus, overall callus quality, and degree of bridging callus at the osteotomy sites. Progression of fracture healing was quantified using a radiographic bone callus index based on Millis *et al*. (1998). A caliper was used to measure the maximal length and width of bony callus at four points on the lateral radiographs: (1) the cranial, (2) caudal aspects of the proximal radius, (3) the cranial and (4) caudal aspects of the distal radius. The radiographic bone callus index was determined by summing the eight measurements.

Bone callus index values were analysed using a repeated measures analysis of variance (ANOVA) with time and treatment as factors.

Histopathological studies

During week 8 of the study, dogs were euthanized by intravenous administration of sodium thiopental solution. Immediately after euthanasia, operated radii were removed, cleaned of soft tissue, and samples were cut from osteotomized regions and placed in 10% neutral buffered formalin for subsequent histopathological studies. Specimens were decalcified in 5% formic acid solution and embedded in paraffin. Five-micrometer paraffin sections were stained with haematoxylin and eosin. The bone defect was evaluated for each osteotomized radius under light microscope. The characteristics of the regenerated tissue were scored according to Ulutas *et al*. (2005), as follows:

Cellular morphology: 100% fibrous tissue: 0 point; fibrous tissue + mesenchyme (less): 1 point; fibrous tissue + mesenchyme (more): 2 points; mesenchyme + bone tissue (less): 3 points; mesenchyme + bone tissue (more): 4 points and 100% bone: 5 points

The points of both groups were calculated using the scale above. Total scores and mean values were calculated for each group. Results were statistically compared using Kruskal-Wallis test.

Results

Radiography

Radiographs indicated that dogs in the experiment group had more progressive signs of healing than control dogs in the operated radii. In the experiment dogs, periosteal reaction was seen by week 4 and then became more prominent at 6 and 8 weeks (Fig. 1). Evidence of callus formation was appeared at the osteotomy sites, and progressive healing was apparent throughout the study period. Whilst in the control dogs, the osteotomy site showed relatively little periosteal reaction at week 4 (Fig. 2). Moreover, little bony callus formation was evident at week 8, and bony resorption was visible at the osteotomy sites. Widening of the osteotomy gap was occurred consequently.

By week 4, the callus index at the operated sites was significantly greater in the experiment dogs in compare to the control dogs. These differences were even more noticeable at 6 and 8 weeks after osteotomy (Fig. 3).

Histopathology

At week 8, the defects in radii of experiment dogs were completely filled with mesenchymal connective tissue, hyaline cartilage, and newly formed bone. The new bone did reach and integrated with both edges of the defect (Figs. 4a and b), whereas in the control dogs, the new bone was scarce at the bony defect regions. Thus, the spontaneous bony repair of control bone defects was limited to the immediate edges of the osseous gap. Cellular morphology scores of the experiment dogs based on Ulutas *et al*. (2005) and its comparison with the control group were summarized in Table 1. There were statistically significant differences between the experiment and control groups ($P<0.05$).

Table 1: Quantitative assessment of the cellular morphology of bony repair tissue in the studied groups (scored according to Ulutas *et al*., 2005)

Dogs	Groups	
	Control	Experiment
1	2	3
2	2	4
3	2	3
4	1	2
5	1	2
Mean	1.6[a]	2.8

[a] $p = 0.0019$

The external callus and repair tissue volume was not identical in the control and experimental radii. The quantity of callus formed by the application of the SMF, at the end of the experimental period, appeared visually to be more than that formed in the control group.

Fig. 1: Lateral radiographs of the antebrachium of an experiment dog after osteotomy. (A) 4, (B) 6 and (C) 8 weeks after osteotomy. Note to the periosteal reaction and bony callus, which were pronounced at 6 and 8-week radiographs

Discussion

The radial osteotomy gap model we used in this study is a hypertrophic non-union model (Millis *et al*., 1998; Inoue *et al*., 2002). While stability and rigidity of the bone defect fixation has been found to be a critical factor in achieving consistent osteotomy gap healing (Inoue *et al*., 2002), in this model the ulna acts as an internal support but micromovement at the osteotomy gap prevented any bone union. The results obtained in the control dogs revealed that this model could reliably produce non-union or greatly delayed fracture healing in dogs.

The osteotomy gap size in this study was selected based on that employed by Muller *et al*. (1968), Larsson *et al*. (2001) and Inoue *et al*. (2002).

In the present study we showed that in dogs, the SMF associated with application of the 1000 gauss custom-made wraps for 8

weeks promoted bone healing in osteotomized radii. Although the radii of dogs exposed to SMF did not completely reach bony union during the 8-week study period, there was progressive fracture healing and some areas had bridging callus by the end of the study period, whereas

Fig. 2: Lateral radiographs of the antebrachium of a control dog after osteotomy. (A) 4, (B) 6 and (C) 8 weeks after osteotomy. Little periosteal reaction and bony callus formation were noted in compare with the experiment group

Fig. 3: Mean bone callus index of the experiment and control dogs

bones of the control dogs displayed little healing and had resorption of bone from the osteotomy site, indicative of a developing non-union. Previous studies of Bruce *et al.* (1987) and Darendeliler *et al.* (1997) also demonstrated positive effects of SMF on bone healing.

The radiographic callus index provided a quantitative measure of bone healing and

Fig. 4: The histopathological views of the control (a) and experiment (b) groups at 8th week (H&E, ×10). Newly synthesized bony spicules (BS) are observed, indicating differentiation of the mesenchymal connective tissue (MT). Note to the integration of the new bony spicules with both defect edges [surrounding bone (SB)] in the experiment group (white arrows). Incomplete integration is obvious in the control group. Note to the tiny fissure between SB and MT in the control group

further substantiated the greater degree of bone healing in treated dogs. The amount and progression of callus production suggest that the experiment dogs likely would bridge the osteotomy sites in time, although we can not certain of this without studies of longer duration.

Strazza (1996) showed a reduction of 40-50% in the healing time of simple fractures by incorporating magnets into the bandage. He also observed that in magnetic therapy of over fifty different animal fracture cases, no cases of non-union developed.

The effect of SMF and PEMF on bone healing in guinea pigs was investigated. The study concluded that both static and pulsed electromagnetic fields seemed to accelerate the rate of bone repair when compared to the control group (Darendeliler *et al*., 1997).

The most plausible mechanism for SMF is the enhanced blood flow to the site of surgery, which is pooling oxygen and nutrients thereby speeding the overall healing process (Man *et al*., 1997). It has been suggested that SMF have a stimulatory effect on regional blood flow to the extremities and that this enhances healing of musculoskeletal injuries (Steyn *et al*., 2000). Kobluk *et al*. (1994) reported that a SMF significantly increased blood flow and metabolic activity in the metacarpus of horses. The magnet improves circulation, allowing blood vessels to dilate and bring a greater volume of blood flow to the injured area. This helps to bring in natural healers and to remove the toxic byproducts of inflammation, bradykinens and prostaglandins (Null, 1998).

When a magnet placed over flowing blood in which ionic charges such as Na^+ and Cl^- do exist, some force will be exerted on the ions. Furthermore, the separation of ionic charges will produce an electromotive force (EMF), which produces a very small amount of heat (Ramey, 1997).

It has been demonstrated that magnetic field increased the production of collagen in the in vitro grown rabbit marrow fibroblasts, defined as determined osteogenic precursors, and of glycosaminoglycans from cultures of chondrocytes and articular cartilage, possibly via an intracellular reduction of cyclic adenosine monophosphate (AMP). The increased formation of matriceal components may explain the accelerated fracture healing rate when subjected to magnetic field (Bruce *et al*., 1987). Bassett *et al*. (1981) speculated that magnetic field may perturb and/or modify the cellular membranes thereby allowing ionic movement from the extracellular environment into the osteogenic cells thereby promoting osteogenesis.

From the aforementioned evidences, it is tempting to conclude that magnetic fields may hasten the maturation of tissues thereby increasing the strength of a healing callus.

Calluses subjected to SMF either have thicker and/or stronger (more mature) trabeculae (Bruce *et al*., 1987).

In a recent study, it was observed that osteoblasts oriented parallel to the magnetic fields, but a mixture of osteoblasts and collagen oriented perpendicular to the magnetic fields (Ueno *et al*., 2002). Ueno *et al*. (2002) investigated the effects of SMF of 8 tesla (T) on bone formation in both in vivo and in vitro systems. After 60 hrs of exposure to the magnetic field, cultured mouse osteoblastic MC3T3-E1 cells were transformed to rod-like shapes and were oriented in the direction parallel to the magnetic field. They concluded that although the magnetic field exposure did not affect cell proliferation, it up-regulated cell differentiation and matrix synthesis.

Supronowicz *et al*. (1999) investigated the effects of SMF on select functions (especially adhesion) of bone cells. They observed that the number of adherent osteoblasts increased under magnetic stimulation. The use of magnetic fields in fracture healing increases the adherence of calcium ions to the blood clot formed at the site of the break. This allows for the proper formation of the callus that is necessary for fracture to heal properly (Messonnier, 2001).

The success of magnet therapy on bone healing is also attributed, in part, to its facilitating the migration of calcium ions and osteoblasts to heal broken bones in less than the usual time (Null, 1998). Madronero (1990) showed that bone healing was promoted by means of the influence of the magnetic field on the crystal formation of calcium salts.

Effects of SMF on bone formation in rat osteoblast cultures were studied by Yamamoto *et al*. (2003). During a 20-day culture, the values of the total area and the number and average size of bone nodules showed high levels in the presence of SMF. In the matrix development and mineralization stages, the calcium content in the matrix and two markers of osteoblastic phenotype (alkaline phosphatase and osteocalcin) also showed a significant increase. Accordingly, these findings showed that SMF stimulates bone formation by promoting osteoblastic differentiation and/or activation (Yamamoto *et al*., 2003).

Magnets lessen the stickiness of platelets; hence the amount of bleeding in the wounds may potentially increase (Null, 1998). Hence, in the present study, the magnetic wraps were applied 2 days post-operative to lessen the likelihood of post-operative bleeding.

The results of this study are encouraging and revealed that SMF produced by Iran-made magnetic plates (Aerospace Complex, Malek-e-Ashtar University, Iran) may have clinical application in the treatment of fractures in canine patients at risk of developing delayed or non-union fractures. Whilst it is still too early to predict the eventual role that SMF may play in the future treatment of bone defects and fractures, this report certainly suggests that the subject should be taken seriously and that more controlled, clinical investigation is warranted.

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