Electromagnetic Fields

A Novel Prophylaxis for Steroid-induced Osteonecrosis

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Abstract Establishing a means to prevent osteonecrosis after corticosteroid administration is an important theme. We asked whether pulsed electromagnetic field stimulation, a noninvasive treatment, could prevent osteonecrosis. Ninety rabbits were divided into four treatment groups: (1) exposure of 10 hours per day to electromagnetic stimulation for 1 week, followed by injection of methylprednisolone (20 mg/kg), and exposure of 10 hours per day to electromagnetism for a further 4 weeks ($n = 40$); (2) methylprednisolone injection only ($n = 40$); (3) no treatment ($n = 5$); and (4) exposure of 10 hours per day to electromagnetism for 5 weeks ($n = 5$). After 5 weeks, we harvested and histologically examined femurs bilaterally. The frequency of osteonecrosis was lower in the steroid-electromagnetism group (15/40) than in the steroid-only group (26/40). No necrotic lesions were found in the two control groups. We observed no clear effects of electromagnetism on the number, location, extent, and repair of

necrotic lesions and intramedullary fat cell size in affected rabbits. Pulsed electromagnetic field stimulation reportedly augments angiogenesis factors and dilates blood vessels; these effects may lower the frequency of osteonecrosis. Exposure to pulsed electromagnetic field stimulation before corticosteroid administration could be an effective means to reduce the risk of osteonecrosis.

Introduction

Osteonecrosis (ON) has been recognized as a complication of high-dose corticosteroid administration. If the necrotic area is large, spontaneous healing is unlikely, and surgery is usually necessary if the necrotic area collapses. Arthroplasty effectively improves quality of life for patients, but the costs and risks of surgery are not insignificant; furthermore, an acceptably durable prosthesis is not yet available for the relatively young population in which this complication typically occurs [4, 5, 20, 21]. Therefore, ON must be prevented in patients who require high-dose corticosteroid therapy, but there is no established prophylactic measure.

Ischemia in the bone has long been believed one of the causes of ON after corticosteroid administration [7, 8, 28]. The pathogenesis of the ischemic necrosis remains elusive but is thought to involve hypercoagulable conditions [8], vasoconstriction [7], and disorders of lipid metabolism [28] after corticosteroid administration. Therefore, prevention of bone ischemia might also prevent steroid-induced ON. We considered pulsed electromagnetic field stimulation could be a preventive therapy because it promotes angiogenesis [11, 19, 27] and dilates blood vessels [23]. We speculated it might also influence the enlargement of intramedullary fat cells since that is reportedly a potential mechanism in bone necrosis [14, 15, 28].

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We first asked whether electromagnetism would reduce the frequency of ON. We then asked whether electromagnetism reduces the severity (number of lesions) of ON. Finally, we asked whether electromagnetism influenced the enlargement of intramedullary fat cells.

Materials and Methods

We divided 90 male Japanese white rabbits (Kitayama Labes Co, Ltd, Nagano, Japan) (body weight, 3.2–4.3 kg; age, 28–32 weeks) into four groups: (1) a steroid-electromagnetism group ($n = 40$), which received 1 week of exposure to pulsed electromagnetic field stimulation (10 hours/day) over the gluteofemoral area, followed by methylprednisolone (20 mg/kg body weight) intramuscularly into the gluteus medius muscle, and 4 subsequent weeks of exposure to electromagnetism (10 hours/day; total exposure period, 5 weeks); (2) a steroid-only group ($n = 40$), which received an injection of methylprednisolone (20 mg/kg body weight) intramuscularly into the gluteus medius muscle after 1 week; (3) an untreated group ($n = 5$); and (4) an electromagnetism-only group ($n = 5$), which received 5 weeks of electromagnetic exposure (10 hours/day). Steroid-induced ON was produced by an intramuscular injection of methylprednisolone (20 mg/kg body weight). This experimental model induces ON in the humeral and femoral bones of approximately 70% of treated rabbits [13–16]. The animals were housed at the Animal Center of Kyoto Prefectural University of Medicine, fed nutritionally adequate food daily, and had free access to clean drinking water. This study followed the guidelines of the Kyoto Prefectural University of Medicine Animal Care and Use Committee fully.

A study using the same rabbit model reported a reduction in the frequency of ON to 30% to 40% with an anticoagulant drug (warfarin) or a lipid-lowering agent (probucol) [16]. The sample size calculation was based on an estimated effect size of 42% for electromagnetism with steroid administration and 70% for steroid administration alone. With an alpha level of 0.05 and a test power of 0.8, the calculated sample size (ie, the minimum number of rabbits required) was 39 for each group.

The protocol of pulsed electromagnetic field stimulation in this study was chosen according to that reported effective for bone fracture healing [2]. One study reported acceleration of angiogenesis within 1 week of commencing electromagnetic therapy [27]. Another study reported ischemia in the bone occurs within 5 to 6 days after corticosteroid administration [10]. Therefore, we began pulsed electromagnetic field stimulation 1 week before the administration of corticosteroid. The pulsed electromagnetic fields were generated by an EBI Bone Healing

Fig. 1 The pulsed electromagnetic field was generated using the EBI Bone Healing System[®]. It consists of a portable pulse-shaping circuit (right) and a coil that is affixed to the cast surface (left).

System[®] (Biomet Osteobiologics, Parsippany, NJ), which delivered uniform time-varying fields consisting of asymmetric 4.5-ms pulses repeated at 15 Hz (Fig. 1). A coil was installed in each cage to generate an electromagnetic field on the gluteofemoral area of the rabbit. During the electromagnetic treatment, the rabbits had free access to water and food.

All rabbits were euthanized after 5 weeks via intravenous injection of a large dose of pentobarbital sodium. We excised bilateral femurs of all rabbits and obtained a tissue section at the proximal third and distal third of the femur in the coronal plane (total four sections). The specimens were stained with hematoxylin and eosin.

Osteonecrosis and all other histologic measures were assessed blindly by two independent authors (MI, MF). A positive diagnosis was based on the presence of empty lacunae or pyknotic nuclei of osteocytes in the bone trabeculae, accompanied by surrounding bone marrow cell necrosis. Only bone marrow cell necrosis showing tissue debris consisting of both hematopoietic cell necrosis and fat cell necrosis and included no bone trabeculae qualified as ON. Lesions consisting of only empty lacunae in normal bone trabeculae and/or fat cell necrosis without bone marrow cell necrosis were excluded from the assessment of ON in this study. If the diagnoses of the two examiners differed, consensus was reached after discussing the histologic findings without unblinding of the group. Rabbits with at least one osteonecrotic lesion in the four areas examined were considered to have ON [29]. The effect of electromagnetic treatment on the prevention of steroid-induced ON was evaluated as the difference between the proportions of rabbits that acquired ON in the steroid-only group and the steroid-electromagnetism group.

In this rabbit model, ON does not occur later than 4 weeks after the methylprednisolone injection [9], and the repair process of necrosis is incomplete at 4 weeks [29]. Therefore, histopathologic evidence of ON is obtainable 4 weeks after the methylprednisolone injection. We histologically evaluated the effects of methylprednisolone administration and pulsed electromagnetic field stimulation on steroid-induced ON 4 weeks after the injection. To determine whether electromagnetism could suppress multiple occurrences of ON, the necrotic lesions in the proximal third and distal third of the femurs of affected rabbits were counted in each group, and the average number was compared among the groups. The effects of electromagnetism on the location of ON were assessed by counting and comparing the number of lesions in the epiphyseal area, proximal third of the femur, and distal third of the femur. The influence of electromagnetic field stimulation on the size of osteonecrotic lesions was examined by measuring the affected area. The ON-affected area was expressed as the ratio of the affected area to the total area in the proximal third of the femur. The area was calculated from computerized microscope images using NIH image software (US National Institutes of Health, Bethesda, MD). The osteonecrotic area was defined as the area containing necrosis of osteocytes or bone marrow cells. Areas undergoing any repair process were not regarded as necrotic [29]. To determine the influence of pulsed electromagnetic field stimulation on the repair process, we examined the bone samples for the presence of histologic features of repair, including granulation tissue, fibrosis, and/or appositional bone formation against necrotic tissue, and findings in the steroid-only and steroid-electromagnetism groups were compared.

To evaluate bone marrow fat cell enlargement caused by corticosteroid administration, the size of the fat cells was calculated and compared between the steroid-only group and the untreated group. The effect of pulsed electromagnetic field stimulation on corticosteroid-induced bone marrow fat cell enlargement was assessed by comparing fat cell size in the steroid-electromagnetism group and the steroid-only group. Bone marrow fat cell size was calculated as the average of the largest diameters of 25 fat cells in each of four randomly selected fields (one field = $25 \times 10^{-8} \text{ m}^2$) in nonnecrotic regions in the proximal third of the right femur using NIH imaging software [22, 28].

All values were expressed as the mean $\pm$ standard deviation. Each variable was compared using parametric or nonparametric methods with or without the normality of distribution of data. The proportions of rabbits in the steroid-only and steroid-electromagnetism groups that acquired ON were compared nonparametrically using the chi-square test. The number of necrotic lesions per rabbit that acquired lesions was compared between the

steroid-only and steroid-electromagnetism groups. The difference between groups was evaluated with the Mann-Whitney U test. The unpaired t test was used to test for a difference between the mean area of lesions in the proximal femur in the steroid-only group and the steroid-electromagnetism group. The mean diameter of 100 fat cells from each of five rabbits from each group was compared among the four groups. One-way analysis of variance was used to determine the overall difference among the groups. If the difference was significant at $p < 0.05$, Scheffe's post hoc tests were performed to test the significance of individual comparisons. Values with $p < 0.05$ were considered significant.

Results

Electromagnetism reduced ($p = 0.01$) the frequency of ON in the steroid-electromagnetism rabbits compared to the steroid-only rabbits (15 of 40, or 37.5% versus 26 of 40, or 65%, respectively) (Figs. 2A–C, 3A–B). None of the rabbits in the untreated group and the electromagnetism-only group had features of ON.

Rabbits treated without and with electromagnetism had similar severities of steroid-induced ON (Table 1). The number of osteonecrotic lesions per affected rabbit was 1.1 ± 0.3 and 1.2 ± 0.4 , respectively. Lesions in the proximal third of the femur occurred in 15 of 26 and 10 of 15 affected rabbits in the steroid-only and steroid-electromagnetism groups, respectively; lesions occurred in the distal third of the femur in 14 of 26 and eight of 15 affected rabbits in each group, respectively. The epiphysis did not display bone necrosis. The extent of ON was similar in the two groups ($2.8 \pm 1.9\%$ in the treated and $2.7 \pm 1.8\%$ in the untreated). In the steroid-only group and the steroid-electromagnetism group, we observed similar amounts of fibrous and cellular-rich granulation tissue (reparative tissues) and they were found on the margins of osteonecrotic lesions. There was no appositional bone formation.

Mean bone marrow fat cell size in the steroid-only group was larger ($p < 0.01$) than that in the untreated group. We observed no difference in fat cell size after corticosteroid administration with or without electromagnetism ($p = 0.43$), ie, $61 \pm 4.5 \mu\text{m}$ in the steroid-only group, $56.9 \pm 5.4 \mu\text{m}$ in the steroid-electromagnetism group, $47.6 \pm 3 \mu\text{m}$ in the untreated group, and $46.1 \pm 3.4 \mu\text{m}$ in the electromagnetism-only group.

Discussion

Pulsed electromagnetic field stimulation induces angiogenesis [11, 19, 27] and vasodilatation [23], but its clinical

Fig. 2A–C (A) The osteonecrotic lesions (arrows) in this rabbit from the steroid-only group showed pyknosis (stain, hematoxylin and eosin; original magnification, $\times 100$). (B) Bone marrow cells had necrosis and stained acidophilic (arrowheads). The nuclei of bone marrow cells display pyknosis and karyorrhexis. Some cells went through karyolysis and uniformly stained acidophilic (arrows). The cellular structure of fat cells also collapsed (stain, hematoxylin and eosin; original magnification, $\times 200$). (C) Bone cells in the bone trabeculae showed pyknosis and empty lacunae (arrows) that were associated with necrotic changes of the surrounding bone marrow cells (stain, hematoxylin and eosin; original magnification, $\times 200$).

Fig. 3A–B (A) The osteonecrotic lesions (arrows) in this rabbit from the steroid-electromagnetism group stained less intensively (stain, hematoxylin and eosin; original magnification, $\times 100$). (B) Bone

marrow cells showed cytolysis and pyknosis, and there was bone marrow cell necrosis (stain, hematoxylin and eosin; original magnification, $\times 200$). Nec = necrotic zone; Liv = living bone marrow tissue.

Table 1. Number, location, and extent of osteonecrosis in affected rabbits of the steroid only and steroid-electromagnetism groups

Group	Number with osteonecrosis*	Proximal third of femur	Distal third of femur	Ratio of osteonecrosis area (%)*
Steroid only (n = 26)	1.1 $\pm$ 0.3	15 (58%)	14 (54%)	2.8 $\pm$ 1.9
Steroid-electromagnetism (n = 15)	1.2 $\pm$ 0.4	10 (67%)	8 (53%)	2.7 $\pm$ 1.8

* Values are expressed as mean $\pm$ standard deviation.

effects on ON have not been clarified [3, 12, 24–26]. We therefore asked whether electromagnetism would influence the risk and severity of ON and if so whether it would influence the size of fat cells since enlargement has been proposed as a mechanism to induce ischemia.

The major limitation of the current study is that osteonecrosis in the rabbit model is different from steroid-induced bone necrosis in humans [29]. The rabbit model is different from human ON in that (1) bone necrosis in rabbits frequently occurs in the metaphysis, not in the epiphysis, and (2) the model does not lead to collapse and the lesions resolve spontaneously. However, the pathophysiology of ON in the rabbit model is characterized by empty lacunae accompanied by surrounding bone marrow cell necrosis, and that of reparative changes as granulation tissue and appositional bone formation are thought to be closer to steroid-induced ON in humans [29]. Therefore, the rabbit model has been widely used in various studies [9, 10, 13–16].

Histopathologic evidence of ON is obtainable 4 weeks after the steroid injection as described in Materials and Methods. We examined bone necrosis at one time point, and did not evaluate chronological effects of electromagnetism on steroid-induced ON, therefore bone necrosis should be examined in earlier and later stages of the experiment in another study. We used a protocol (dosage and frequency) for electromagnetic field stimulation used to treat adult bone nonunion (ie, exposure to 15-Hz electromagnetic stimuli for 10 hours/day) [2], but the optimal electrical stimulation for the augmentation of growth factors varies according to cell type [11]. Thus, the optimal protocol of pulsed electromagnetic stimulation for the prevention of ON should be investigated in another study.

Our data suggest the number of rabbits with ON 4 weeks after steroid administration was lower in the group that received pulsed electromagnetic field stimulation than the group that did not. We did not determine the mechanism of the pulsed electromagnetic field benefit, but we speculate acceleration of angiogenesis and vasodilatation caused by the electromagnetic waves could suppress ischemia in the bone after corticosteroid administration. Fat cells were not affected by electromagnetism, so we presume that is not the mechanism.

We found no influence of electromagnetism on histologic factors relating to the severity of necrosis (ie, number of osteonecrotic lesions per rabbit), size, location, and repair level of bone necrosis. This suggests this treatment can help prevent ON, but if it occurs, the treatment does not reduce its severity. This same conclusion has been reached for the prevention of steroid-induced ON with chemicals [16].

Several researchers have shown steroid-induced ON can be prevented by medications [6, 17, 18], and a study that

used the same rabbit model as ours reported a reduction in ON frequency to 30% to 40% [16]. Our study showed similar suppressive effects, and no rabbits developed tissue damage due to electromagnetism. This point also indicates electromagnetism could be a useful prophylactic therapy for steroid-induced ON.

Miyanishi et al. [15] reported a larger bone marrow fat cell size in rabbits that developed ON than in those without ON after corticosteroid administration. They hypothesized corticosteroids become a cause of ON through enlargement of bone marrow fat cells and an increase in intraosseous pressure, resulting in a disturbance of intraosseous circulation. Another study proposed prevention of fat cell enlargement could also prevent steroid-induced ON [16]. We found bone marrow fat cell size was larger in rabbits that received corticosteroids than in those that did not, but electromagnetism did not affect bone marrow fat cell size. Therefore, we assumed its preventative effect on steroid-induced ON occurs via a mechanism independent of lipid metabolism.

Our preliminary data suggest electromagnetic fields could be a preventative method, particularly since it is noninvasive [1, 2]. Further studies are necessary to clarify the optimal protocol of pulsed electromagnetic fields for the prevention of ON and to apply this treatment in clinical trials.

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