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The Effects of Electromagnetic Field on Distraction Osteogenesis

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The effects of electromagnetic field on distraction osteogenesis and new bony tissue were investigated.

Twenty-five New Zealand rabbits were divided into an experimental (12 rabbits) and a control (13 rabbits) group. An osteotomy was performed on the right tibia in the diaphyseal region in both groups. The experimental group was exposed to a magnetic field of 50 Hz 1.0 mT for 3 hours a day for 13 weeks. The control group was kept in a similar environment but with no electromagnetic field. The distraction was continued until an increase of 10 mm was achieved. At weeks 4, 8, and 13, radiography, scintigraphy, and a biopsy were performed in both groups, and the results were statistically analyzed.

The X-ray results were similar in both groups at all times. On the other hand while the scintigraphic and histopathological results were similar at weeks 4 and 13 in both groups, the osteoblastic activity was significantly greater in the experimental group at week 8 ($p<0.01$).

In conclusion an electromagnetic field increases the osteoblastic activity and osteogenesis, but has little effect during the remodeling phase.

Key Words: Distraction osteogenesis, magnetic field

INTRODUCTION

The callus distraction method has been successfully used for eliminating extremity length discrepancies and for treating large bone defects that develop as a result of etiological reasons such as infection, tumor, and trauma,[1-5] This treatment method has important advantages compared to conventional methods. The advantages of this technique are that it is biological, it has a lower complication rate than other methods it can be used to treat a defect of any length and diameter and it does not require long-term immobilization or thick autogenous bone grafts.[1,3] However, the periods of external fixation and bone maturation is long. This is main disadvantage of this treatment method.[6] The required period for bone recovery after a callus distraction is closely related to the extent of the defect or bone to be elongated. Therefore, the greater the bone defect or the length difference, the longer the period necessary for the external fixation and maturation.[6] Decreasing the treatment period by stimulating bone formation will significantly reduce the effect these disadvantages. Electromagnetic fields have been used successfully for treating several types of pseudoarthrosis and in a delayed union.

The aim of this study was to investigate the effect of electromagnetic fields on the newly formed callus tissue in rabbits in view of the concerned literature.

MATERIALS AND METHODS

This study was approved by the University of Dicle Ethical Committee for animal experimentation and conformed to the National Institutes of Health guidelines for the care and use of laboratory animals.

In this study, 5 to 7 months old New Zealand

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rabbits (1600 - 2400 gm) grown in The Dicle University Research Center of Health Sciences were used.

A total of 25 rabbits were divided into two groups. There were 12 and 13 rabbits in the experimental in the control group respectively. The rabbits in both groups were anesthetized with 10 mg/kg ketamine hydrochloride and Rompon 3 mg/kg administered intramuscularly. Prior to the surgery, 50 mg/kg/day of prophylactically 1st generation cephalosporin cephazoline sodium was administered intramuscularly. A total of four 1 mm Kischner wires, 2 in right tibia proximal and 2 in the tibia distal were placed in each rabbit. One 45 mm diameter circular ring (BTM) weighing 85 gram was held to the proximal and distal tibia by means of 3 rods. An osteotomy was performed on the tibia diaphysis of the subjects. First both cutenous and subcutenous tissues were passed through an incision. The periost was opened longitudinally and the osteotomy was then carried out after which the periost was then closed. Following a seven-day waiting period, the distraction was initiated in both groups at rate of 0.25 mm per 12 hour (0.5 mm/day). On the start date of the distraction, the 12 rabbits in the study group were placed in a magnetic field environment in 24 × 25 × 28 cm plexiglass boxes. The study group was exposed to a sinusoidal magnetic field of 50 Hz 1.0 mT for 13 weeks, 3 hours per day. The control group rabbits were also placed in a similar environment but without the magnetic field. The magnetic field was obtained by means of specially two-sidedly coiled two helmholtz spool. The field was measured at 1.5 tesla with a tesla-meter (Phywe) alternating field hall probe (230 V 10VA). X-rays were taken from all rabbits in both groups from the date when the distraction was started. The distraction proceeded until a total of 10 mm over a 20 day period was reached. After completing the elongation (4 th week) and on the 8 th and 13th week of the study radiographs, bone scintigraphy and a biopsy were taken from all rabbits in both groups (Fig. 1).

Radiology

Anterior-posterior graphics which were taken on the date when the distraction was started, after the distraction was completed (4th week), and on the 8th and 13th weeks were evaluated by a radiologist who was blinded to the study.

Scintigraphic method

Three-phase scintigraphy was performed 4, 8, and 13 weeks after completing the distraction. A Toshiba GCA 601E gamma camera equipped with a low-energy, general-purpose, parallel-hole collimator was used. The rabbits were sedated with 10 mg/kg intramuscular ketamine HCI and immobilized under the gamma camera in the supine position. Thirty four MBq/kg Tc-99m MDP was than injected in the ear vein. An angiogram (1s/

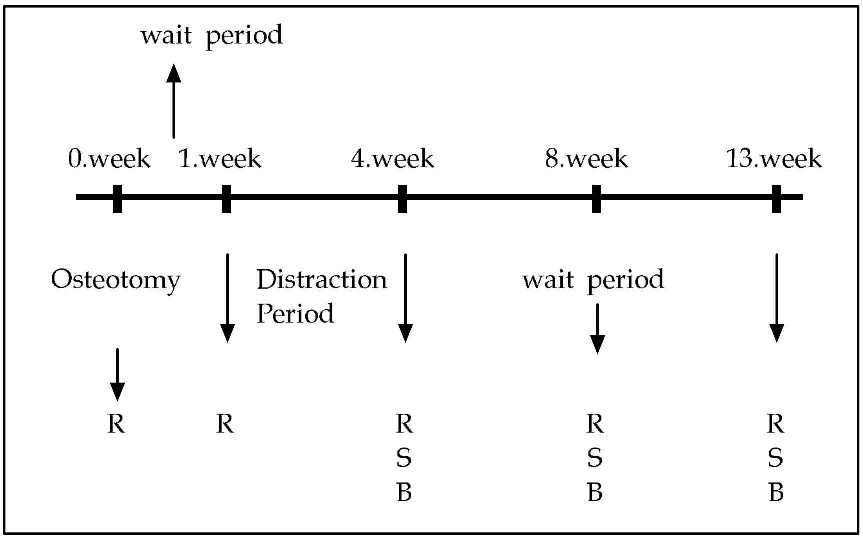

Fig. 1. Process diagram. (R, radiology; S, scintigraphy; B, biopsy)

frame, 64 × 64 matrix, 60 images), blood-pool (200000 counts/image), and 3 hours later, metabolic phase (200000 counts/image) images were taken in the anterior projection in order to include both lower extremities. All images were recorded on the gamma camera. Later, a rectangular region of interest (ROI) from the healthy leg was drawn on the blood-pool and metabolic phase images. For each image, the mean counts were calculated as the counts/pixel. The percentages obtained from both groups were calculated using the following equation: [(lesion count / contra lateral count) × 100]. Comparisons were made between the images from the 2 groups taken at the same time. In addition, the images taken at 4, 8, and 13 weeks from each group were compared.

Histopathological study

After the scintigrophic study the animals were anesthetized administered with 10 mg/kg ketamine HCL. A biopsy was then taken from the distraction zone. The specimens obtained after completing the distraction (4th week), and, 8th, and 13th weeks later were decalcified for a two day period in 1% HCI, fixed in 10% formaldehyde for 12 hours and then embedded in paraffin. Three micron thick, cross sections taken from the paraffin blocks were stained with hemotoxilene eosin and examined under an optical microscope (Olympus BH2). The cross-sections were evaluated by a pathologist blinded to their origin.

Statistical analysis

The Wilcoxon Signed Rank Test and Mann-Whitney U test coefficient test of nonparametric methods were used because the sample size was less than 30. The significance of the analysis is indicated by the p value in the relevant tables. Two-tailed hypotheses were used for all comparisons SPSS for windows 10.01 (Standard version, copyright SPSS inc. 1999) was used for statistical analysis.

RESULTS

One rabbit from the control group died after the surgery. No guinea pigs were included in this study. In the follow-up, a pin-tract infection developed in two rabbits in the study group, and 3 in control group. The infections were treated by administering 2 × 50 mg/kg/day cephasoline sodium. A fracture occurred where the K wire was placed in one rabbit of the study group following the distraction. This rabbit was consequently excluded from the study. The study was continued with 12 animals in the control group and 11 animals in the study group at the 8th and 13th weeks. A union was not observed in the 13th week in one rabbit from the control group. Sclerosis was found on the end of the fractures, and was therefore considered to be pseudoarthrosis. Complete union of the bones was observed in the 22 animals included into the study.

Radiology

After the osteotomy, the callus tissue was not observed in the radiography around the osteotomy of both groups.

After completing the elongation, callus tissue was found in proximal and distal segments in the graphics taken in the 4th and 8th weeks (Fig. 2).

Bony union was found in the callus tissues and

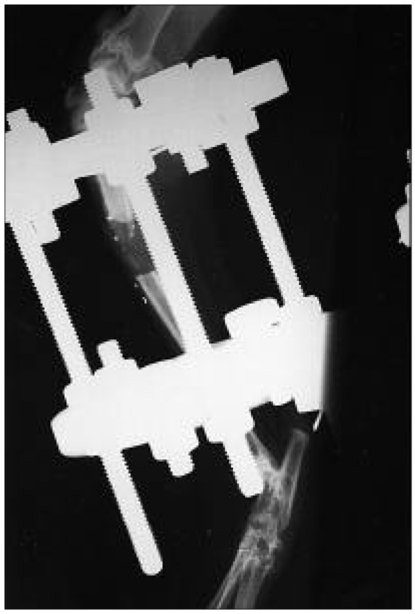

Fig. 2. Radiography image eighth weeks after the distraction in a rabbit group study.

remodeling was indicated in the graphics taken on the 13th week. However no difference between the groups was observed (Fig. 3).

Scintigraphy

The percentages from both group obtained at week 4 were similar ($p > 0.05$) (Table 1).

The Images obtained at week 8 showed that the counts from the blood-pool phase were similar, but there was a significant difference between groups in the counts from the metabolic phase ($p < 0.05$) (Table 1). In addition, a visual examination indicated that osteoblastic activity was more evident in the experimental group (Fig. 4 and 5).

At week 13, the counts from the blood-pool and metabolic phases in both groups were similar ($p > 0.05$) (Table 1).

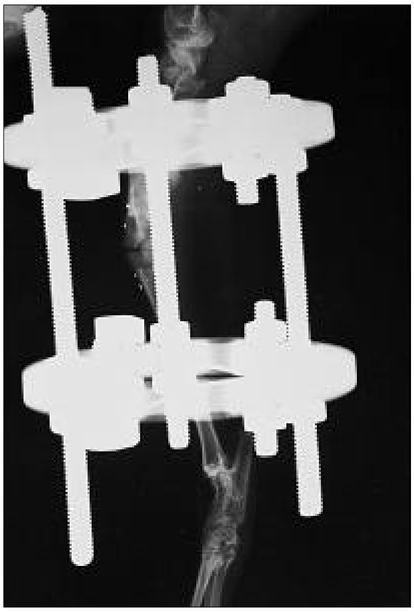

Fig. 3. Radiography image after 13 weeks after the distraction in a rabbit group study.

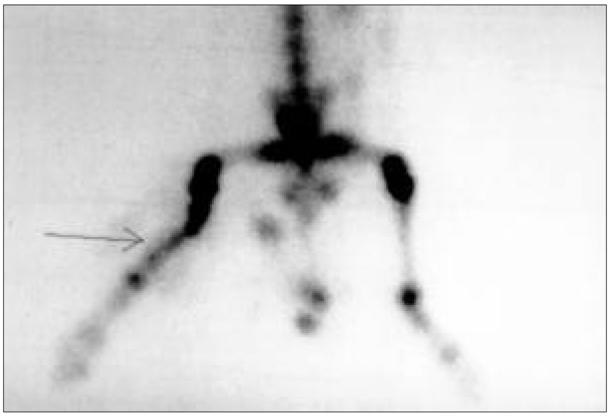

Fig. 4. Bone scintigraphy (blood pool phase) of a rabbit of the study group in week 8.

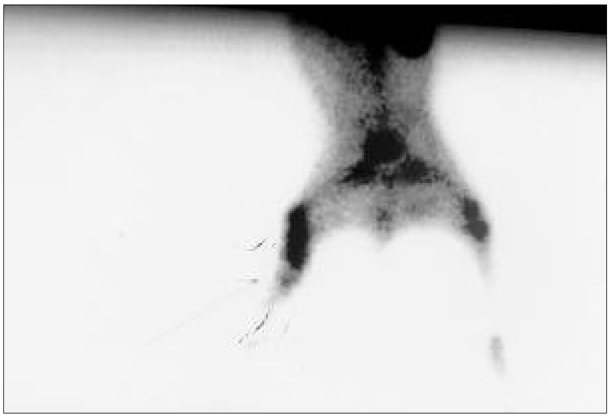

Fig. 5. Bone scintigraphy (metabolic phase) on week 8 in a rabbit group study.

Table 1. Comparison between the Experimental and Control Group by the Mann-Whitney U Test

Scintigraphy Phase	Weeks	Median of the percentages (experimental group-control group)	p
Bood Pool	4 week	2.615 - 2.456	0.270 > 0.05
	8 week	2.805 - 2.930	0.916 > 0.05
	13 week	1.745 - 1.540	0.793 > 0.05
Metabolic phase	4 week	3.605 - 3.420	0.066 > 0.05
	8 week	5.475 - 2.470	0.001 < 0.05
	13 week	2.545 - 2.505	0.834 > 0.05

Comparisons were made between the results from the images taken at weeks 4, 8, and 13, within the experimental group, and differences in the blood pool and metabolic phases were found. The increase was greatest in the images taken at week 8, and the activity at week 4 was greater than at week 13 ($p < 0.05$).

Comparisons were made between the results obtained at weeks 4, 8, and 13 within the control group, and a difference in the blood pool phase between weeks 4 and 8 was found but this was not statistically significant ($p > 0.05$). Statistically significant differences were observed between the results obtained at weeks 4 and 13 and weeks 8 and 13 ($p < 0.05$). In the metabolic phase, there was no statistically significant difference between the results obtained at weeks 4 and 8 or weeks 8 and 13 ($p > 0.05$), and a significant difference between the percentages in images taken at weeks 4 and 13 ($p < 0.05$).

Histopathology

In the first biopsies taken in the 4th week, the osteotomy field of both groups was surrounded by cartilage callus and fibrous tissue. The field was full of immature bone trabeculae and callus.

In the second biopsies taken in the 8th week, the callus reached the proximal and distal tips. The fibrous tissue that enclosed the new blood veins placed longitudinally on the elongation space was filled with tissue. The tissue encompassed the chondroid fields and the bone fields on the surface. The osteoblasts were more intensive in the first group (study group) than in the second (Fig. 6a and b).

In the final biopsies (13th week), the cartilage callus tissue was being resorbed, and took the place of the bone tissue. These new bones lay longitudinally between the proximal and distal tips. Cells such as osteoblasts, preosteoblasts and fibroblasts surrounded the bone trabeculae. There was no histological difference between the groups.

DISCUSSION

The elimination of length discrepancies and the treatment of large segmental defects associated with an infection, trauma, and tumor surgery are major problems faced by orthopedic surgeons.[7] Currently, a callus distraction is practiced to both eliminate extremity length discrepancies and treat segmental bone defects.[2-4,6,8] The advantages of this technique are that it is biological, it has a lower complication rate than other methods it can be used to treat a defect of any length and diameter and it does not require long-term immobilization or thick autogenous bone grafts.[1,3] However, there are disadvantages, such as a long external fixation period. Research on techniques to shorten the treatment period and the external fixation period, including a callus distraction with a combination of an intramedullary nail and an external fixation are underway.[9] However, this technique has disadvantages, such as its high cost, the need

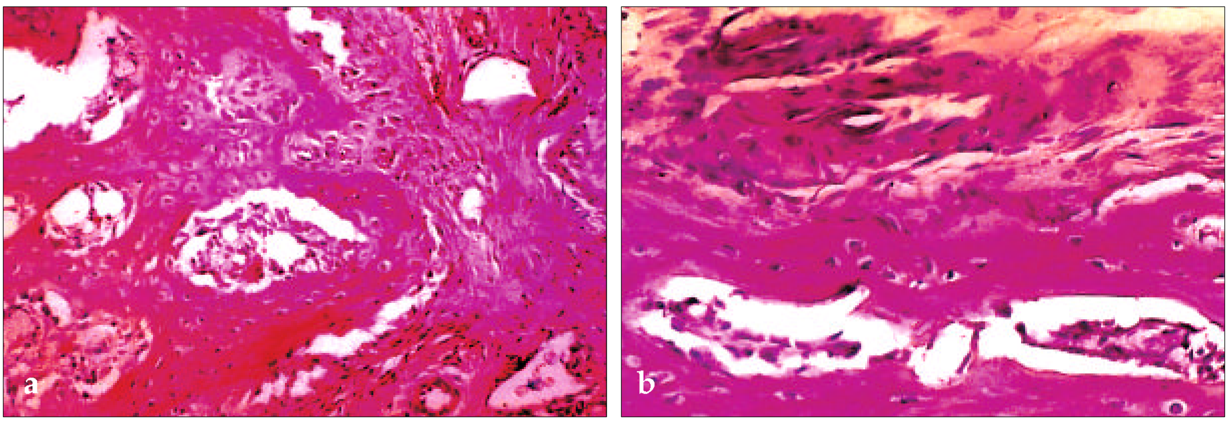

Fig. 6. a-There are many osteoblasts surrounding the bone trabecule in the study group. b-There are fewer osteoblasts in the control group than in the study group.

for a second operation, and the risk of infection. Furthermore, while it shortens the external fixation period, it does not promote bone maturation.

In order to reduce the treatment period, the bone maturation period must be reduced. However at present there is no such technique for achieving this. Previous studies have shown that electromagnetic fields induce cartilage differentiation[10] and increase both DNA synthesis and transcription.[11] Further research also claimed that the osteoblastic activity increased[12] and alcalic phosphatase enzyme (ALP) level rose by 100% as a result of a magnetic field.[13] An increase in factors such as bone maturation, bone volume and bone formation were also reported to be due to an electromagnetic field.[14] A number of studies have reported the successful treatment of acquired or congenital pseudarthrosis and avascular necrosis of the femoral head using an electromagnetic field.[15] Mammi et al. used an electromagnetic field to promote spinal fusion, and found that it occurred sooner in the experimental group.[16] Borsalino et al. used electromagnetic field therapy after an intertrochanteric osteotomy, and reported union in an earlier phase in the experimental group than in the control group.[17] Giannini reported that an electromagnetic field may be effective in the early stage of bone mending and during the cartilage-to-bone stage, but that it was not effective in the later stages.[18] Roermund studied the effects of electromagnetic fields on distraction epiphysiolysis bone formation and remodeling, and found no difference between the control and experimental groups.[7] The effects of electromagnetic fields on distraction osteogenesis in a rabbit model were investigated radiologically, scintigraphically, and histopathologically.

Although It is possible to collect data about the callus formation by direct radiography, but it is not adequate for a reliable estimation of the recovery stage.[2] In this study, there was no clear visible difference on radiological images taken from the study and control groups at different times.

Blood flow and osteoblastic activity in the callus tissue can be measured and evaluated *in vivo* by 3-phase Tc99-MDP scintigraphy.[19-22] The major indicators of the location of Tc99-MDP in bone are the blood flow, osteoblastic activity, and extraction activity. The bone uptake of a radiopharmaceutical depends on the region's blood flow and the extraction of the radiopharmaceutical.[20-22] These events are directly related to the metabolic activity, capillary permeability, and extracellular liquid volume.[19] In this study the scintigraphy performed at week 8 after completing the distraction, showed significant differences between the experimental and control group. In the experimental group there were significant differences in both the blood pool phase and the metabolic phase at week 8 compared to weeks 4 and 13, showing that the electromagnetic field increased the osteoblastic activity and the rate of bone formation. However, the scintigraphy performed on week 13 showed no marked difference between groups in either the blood pool or the metabolic phase, suggesting that the remodeling phase was unaffected by the electromagnetic field. The results of this study were consistent with those reported by Gianni.[18]

The histopathological findings were consistent with the scintigraphic findings. The biopsies taken 8 weeks after completing the distraction, showed the osteoblastic activity to be better and more advanced bone formation in the experimental group, indicating that the electromagnetic field increased the osteoblastic activity during this period. In contrast the biopsies taken at week 13 indicated the presence of mature bony tissue in both groups and the absence of a marked difference between groups showed that the electromagnetic field had little effect during this period.

These results suggest that post-callus-distraction electromagnetic flow increases callus formation but does not affect the remodeling phase. Therefore there is a need for further experimental and clinical studies In order to increase the rate of bone formation and shorten the treatment period.

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