ORIGINAL RESEARCH

Distraction osteogenesis is inhibited by cancer radiotherapy with Co 60

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Abstract

Aim: To investigate the effects of radiotherapy on distraction osteogenesis performed on the same bone in an area that has not received radiation. Radiotherapy (Co60) was carried out in a region where tumors may develop, and then, on the assumption that the tumoral region had been removed, distraction osteogenesis was carried out, and the effects were investigated.

Method: Thirty New Zealand rabbits were randomized into two groups, a study group (15 rabbits) and a control group (15 rabbits). In the study group, Co60 was administered by teletherapy to the distal half of the left tibia. Rabbits in the control group were kept in the same environment for the same period, but were not subjected to radiotherapy. Four weeks after radiotherapy, osteotomy was performed on the proximal part of the left tibia of all subjects, and distraction was carried out until 10mm. After distraction was completed, the outcomes were evaluated radiologically, scintigraphically, and histopathologically, and the results were compared.

Results: New bone formation achieved through distraction osteogenesis in the study group animals was inadequate, while new bone tissue achieved in the control group was superior ($P < 0.001$).

Conclusion: Radiotherapy has a negative effect on distraction osteogenesis, even if performed on a different part of the bone.

Bone defects may occur for a variety of reasons, including tumor resection, trauma, and bone infection. Bone defects may be treated by various methods, such as autografts, allografts, and distraction osteogenesis. Each has advantages and disadvantages¹⁻⁵. Osteogenesis, developed by Ilizarov, is more advantageous than the other methods, and hence is frequently used. Distraction osteogenesis is a biological process, and its complication rate is low compared with other methods. Furthermore, it can be used in the treatment of a broad array of defects. Resection surgery and chemotherapy, radiotherapy surgical treatment alone, and the combination of radiotherapy chemotherapy and surgical therapy are also in use.^{6,7} Radiotherapy decreases osteogenic capacity.^{8,9} In experimental and clinical trials, distraction osteogenesis performed in

the same region of the mandible treated by radiotherapy is adversely affected.^{8,10,11} Studies have been carried out to determine factors affecting distraction osteogenesis^{2,12,13} but we have found no experimental or clinical studies investigating whether distraction osteogenesis performed in an extremity that has received radiotherapy is affected.

In the present study, we performed radiotherapy (Co60) in a region of a bone where tumors may develop and, assuming that the tumorous region had been removed, then performed distraction osteogenesis in the same bone on a region that did not receive radiation, in order to determine whether osteogenesis was affected.

Materials and Methods

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

Thirty male New Zealand rabbits were used in this study. The average age of the rabbits was 5-7 months. Their weight ranged from 1600 to 2400 g. Animals were randomized into a control group (15 rabbits) and a study group (15 rabbits).

Radiotherapy

The irradiation field was simulated on each of the 15 experimental rabbits to fix its precise location, and to mark the exposed skin for placement. Each of the animals was fixed in a prone position on a Perspex jig, with its thighs extended laterally at a right angle to the body. In order to deliver the most effective amount of radiation to the bone cells, the distal half of the left tibia with the adjacent fibula of each animal was irradiated with a Co60 teletherapy machine. The distance between the source of radiation and the skin was adjusted to 80cm, and 1cm of tissue-equivalent bolus was applied to the treatment field. The total irradiation dose was 30 Gy, administered in 10 doses over a pe-

riod of two weeks. Each animal was with 0.10 mg/kg ketamine i.m for the simulation and the radiation therapy. All the animals were kept in the same environment during the experiment (Figure 1).

Surgical Methods

The animals underwent surgery 4 weeks after the completion of radiotherapy. Rabbits in both groups were anaesthetized with 10mg/kg ketamine hydrochloride and 3mg/kg xylazine hydrochloride (Rompun®, Bayer, Istanbul, Turkey) im. Prior to surgery, 10mg/kg/day of cefazolin sodium im, was administered. Four 1mm-thick Kirshner wires were inserted in the left tibia of each of the rabbits, two in the proximal and two in the distal, two rings (BTM) by three rods with an average weight of 85 gm and a diameter of 45 mm joined together were placed and fixation was achieved. Then, six layers of soft tissue were penetrated by incision close to the 1/3 proximal of the tibia and the periosteum was reached, in order to perform osteotomy in such a way that the tuberosity of the tibia would remain beneath the tibia. The periosteum was opened longitudinally, and osteotomy was performed with a jigsaw. Afterwards, the periosteum, soft tissues, and skin were closed with a suture. After a seven-day waiting period following osteotomy, 0.25mm distraction was performed every 12 hr. Post-osteotomy radiographs were taken of all rabbits in both groups under anesthesia. Distraction was continued for 20 days, for a total of 10mm. Imaging by radiography and bone scintigraphy was carried out at weeks 4, 8 and 12 after completion of distraction. At the end of week 12, rabbits in both groups were sacrificed, the tibias were removed, and histopathological analysis of the distraction region was performed.

Radiology

Radiographs were taken after osteotomy and at weeks 4, 8 and 12 following the completion of distraction (Figure 1). The radiographs were evaluated by a radiologist unfamiliar with the study. Results were evalu-

FIGURE 1. Process diagram. (R: radiology, S: scintigraphy, B: biopsy)

ated radiologically by the scoring system of Huddleston et al.¹⁴.

Scintigraphic Method

Three-phase scintigraphy was performed 4, 8 and 12 wk after completing the distraction (Figure 1). A Toshiba GCA 601E gamma camera equipped with a low-energy, general-purpose, parallel-hole collimator was used. The rabbits were sedated with 10mg/kg ketamine HCl im and immobilized under the gamma camera in the supine position. Thirty-four MBq/kg Tc-99m MDP was then injected in the ear vein. An angiogram (1s/frame, 64x64 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: $[(\text{lesion count} / \text{contralateral count}) \times 100]$. Comparisons were made between the images from the 2 groups taken at the same time. In addition, the images taken at weeks 4, 8 and 12 from each group were compared.

Histopathology

After the scintigraphic study (12 wk after distraction), the animals were anesthetized with 10mg/kg ketamine-HCl, and a biopsy was taken from the distraction zone. The specimens were decalcified for two days in 1% HCl, fixed in 10% formaldehyde for 12 hours, and embedded in paraffin. Three micron-thick cross sections taken from the paraffin blocks were stained with Hematoxylin-Eosin (H&E), and examined under an optical microscope (Olympus BH2). The cross-sections were evaluated by a pathologist blinded to their origin (according to the modified grading system of Lane and Sandhu).¹⁵

Statistical Analysis:

The non-parametric Friedman test and Mann-Whitney U tests were used because the sample size was < 30 . The significance of the analysis is indicated by the P value in the relevant tables. Two-tailed hypotheses were taken into account for all comparison. SPSS for Windows XP 12.00 (base version, copyright SPSS inc. 2003) was used for statistical analysis.

Results

Following surgery, one rabbit in the control group died and was not replaced in the study. On follow-up, 3 rabbits in the study group and 4 in the control group developed pin-tract infection. Infections were treated with cefazolin sodium in doses of $2 \times 50 \text{ mg/kg/day}$, and with daily changing of dressing. During the distraction process, a fracture developed in one rabbit at the Kirschner-wire pin site and displacement occurred. The animal was excluded from the study, and a replacement rabbit was not admitted. The study continued with 14 rabbits in the control group and 13 in the study group.

Radiology

There was no callus formation in either group in the osteotomy zone on images taken after osteotomy, and the osteotomy lines were observed to have been completed. There was no difference in scoring between groups.

TABLE 1. Histological and radiological results

	Group 1(n=13)	Group 2(n=14)	
	Mean Scores	Mean Scores	P
Histological scores	6 ± 3.18	13.86 ± 2.656	0.001
Radiological scores at 4th Week	2 ± 1.414	4.43 ± 1.158	0.001
Radiological scores at 8th Week	2.54 ± 1.761	6 ± 0.961	0.001
Radiological scores at 12th Week	3.08 ± 1.891	8.21 ± 0.699	0.001

Images taken 4 weeks after completion of extension, callus tissue formation was observed in the distraction gap in the control group, but no callus formation was observed in the distraction region in the study group. On Huddleston's scoring, there was a statistically significant difference between groups ($P < 0.001$) (Table 1).

In images taken 8 wk after distraction, no callus formation was observed in the distraction region in the study group. In the control group, on the other hand, callus tissue formation was salient ($P < 0.001$).

On images taken at week 12 following completion of distraction, no union was observed in the distraction gap in the study group (Figure 2). In the control group, on the other hand, there was callus formation in the distraction gap, and union was observed to be completed (Figure 3). Scores showed a difference between groups ($P < 0.001$).

Scintigraphy

On images taken 4 wk after completion of distraction, there was no difference between groups in terms of blood-pool phase but there was a difference in values obtained during the metabolic phase ($P < 0.05$). Osteoblastic activity was observed to be higher in the control group.

On images taken 8 wk after completion of distraction, there were differences between groups in both blood pool and metabolic phases ($P < 0.001$). Osteoblastic activity was higher in the control group.

There was a difference between groups in values obtained 12 wk after completion of There was also a difference between groups in values obtained from images taken during the metabolic phase ($P < 0.001$).

Histopathology

Samples taken after the animals were sacrificed at the 12th week were evaluated histopathologically. In the study group, new longitudinally extending veins had formed in the distraction zone, and that the surrounding area was filled with fibrous tissue with very few

FIGURE 2. The radiography image at 12th week after the distraction in a rabbit group control. The union was complete in 12 week.

FIGURE 3. The radiography image at 12th week after the distraction in a rabbit group study. There was not union.

osteoblasts (Figure 4). In the control group, cartilage tissue had begun reabsorption in the distraction zone and bone tissue had begun to replace cartilage. New bone tissue extended longitudinally between the proximal and distal ends in the distraction zone. Osteoblasts, preosteoblasts, and fibroblasts were seen around the trabecular bone (Figure 5). On Lane-Sandhu (modified Heiple) scoring, there was a statistically significant difference between the two groups ($P < 0.001$) (Table 1).

Discussion

In this study we used a scoring system developed by Huddleston^{14,16} for radiological evaluation. There

were differences between the two groups indicating that new bone formation (callus tissue) achieved by distraction osteogenesis is slowed down or inhibited by radiotherapy.

Scintigraphic techniques^{2,3,16,17} were used in the evaluation of distraction osteogenesis. The greater osteoblastic activity in the control group than in the study groups at wk 4 and 8 after distraction shows that osteoblastic activity is adversely affected by radiotherapy during this period. However, at week 12 the difference between groups was not significant statistically, and the decrease in the study group since wk 4 and 8, shows that the study group was in the remodeling phase. Scintigraphic results also indicate that dis-

FIGURE 4. There are fewer osteoblasts in the study group.

FIGURE 5. There are many osteoblasts surrounding the bone trabecule in the control group.

traction osteogenesis was adversely affected by radiotherapy in the study group.

Histopathology was used in the evaluation of distraction osteogenesis.¹⁸ We used a modified Lane-Sandhu scoring system¹⁵, and the results indicated that radiotherapy slowed or inhibited new bone formation in the study group.

We found no previous experimental or clinical studies on extremities that investigated the effects of radiation on distraction osteogenesis.^{10,11} There are a few experimental and clinical studies on the mandible in maxillofacial surgery that showed that distraction osteogenesis was adversely affected by radiotherapy. Our study differs from these in that it is a study on an extremity, and that the radiotherapy was carried out not on the distraction area but on an area in the same bone distant from the distraction area. Thus, this study was prepared with a bifocal osteogenesis in mind to be carried out after the removal of the tumorous region.

In conclusion, when radiotherapy is performed on bone undergoing osteogenesis, even if carried out at a different site, it decreases or inhibits the formation of new callus tissue. There is a need for both clinical and

experimental studies to determine whether osteogenesis should be performed after radiotherapy.

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