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Extracorporeal shock wave treatment for defective nonunion of the radius: a rabbit model

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ABSTRACT

Purpose. To investigate the effect of extracorporeal shock wave treatment (ESWT) on bony union using volume analysis of the callus mass by computed tomography and histology.
Methods. Both radii of 13 rabbits were osteotomised and a defective bony nonunion created by placing a polyethylene pad between the osteotomy site for 40 days. Nonunion was confirmed by radiography using Lane-Sandhu criteria. ESWT (14 kW, 0.46 mJ/mm^2, 1000 shock waves) was applied to the right radius of the rabbits. The left radius served as a control. Five rabbits were killed 4 weeks after ESWT (group 1) and 8 after 6 weeks (group 2). Volume analysis of the callus mass was performed using computed tomography and the bone healing process was assessed by histology.
Results. In group 1, callus volume on the treated side was invariably greater than that on the control side; the difference being statistically significant (p=0.032). In group 2, the callus volume of the treated side was greater than that of the control side, except in rabbits 4 and 9. Only after excluding the findings from the latter did the difference attain statistical significance (p=0.020). Histology confirmed that the bone-healing process was faster in the treated side.
Conclusion. ESWT enhanced the bone-healing process by increasing both volume and speed of callus formation.

Key words: *bony callus; fracture healing; models, animal; pseudarthrosis*

INTRODUCTION

The management of long-bone defective nonunion is difficult and costly. Several defective nonunion models of various animals have been reported,[1–5] but what constitutes a standard model remains a matter of debate.

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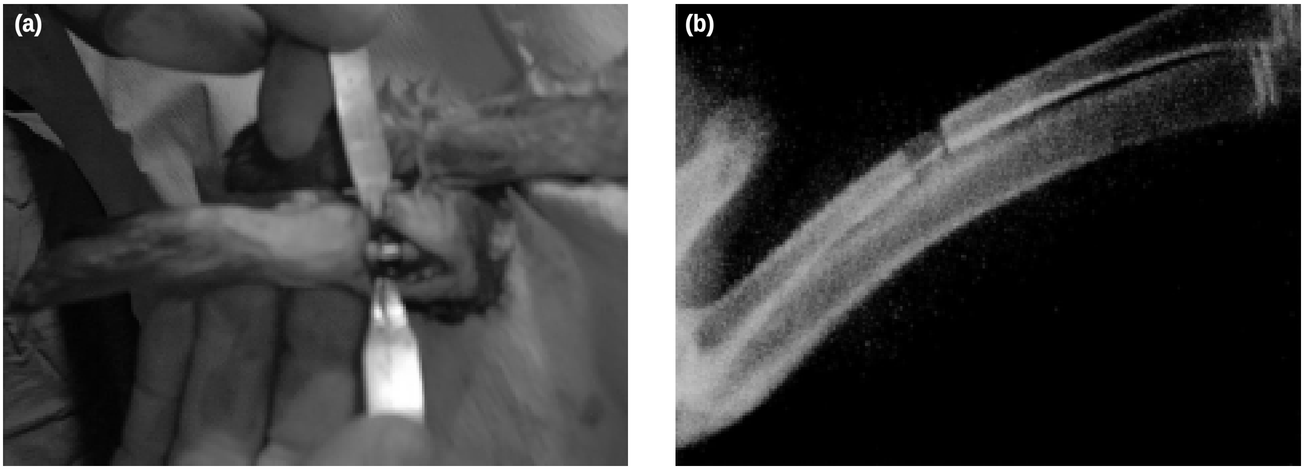

Figure 1 **(a)** Photograph and **(b)** radiograph showing osteotomy of the radius and insertion of polyethylene pads to create a bone defect.

Extracorporeal shock wave treatment (ESWT) has been used in the treatment of pseudarthrosis since 1988, with a success rate ranging from 60 to 85%.[6–8] More satisfactory results have been obtained in hypertrophic nonunion than atrophic nonunion.

Schaden et al.[9] reported a 76% success rate with ESWT in 115 patients with nonunion caused by fractures that were treated nonoperatively. They suggested that ESWT was a better method for treating nonunion with a defect smaller than 5 mm.

We aimed to create a superior, standard experimental model of defective nonunion in the radius of a rabbit, with a view to performing further studies to measure callus volume using computed tomography (CT) and assess the bone-healing process by histology.

Figure 2 A radiograph taken 40 days after osteotomy with polyethylene pads removed showing a defective nonunion.

MATERIALS AND METHODS

In May 1999, 15 New Zealand rabbits (age range 6–8 months and weighing 2850 to 3350 g) were chosen for this experiment.

Intramuscular injections of 3 mg/kg of xlazine hydrocloride (Rompun; Bayer, Monheim, Germany) and 10 mg/kg of ketamine hydrochloride (Ketalar; Pfizer, New York [NY], USA) were administered for general anaesthesia. Both forelimbs were shaved and washed with 10% polyvinylpyrolidon (Poviod; Saba, Redwood Shores [CA], USA). A 3-cm dorsal incision was made on each forelimb and the radius exposed. An osteotomy site of 6 mm was made on both radii together with the periosteum layers using a high cycle burr (3600 rpm). Polyethylene pads (Romsons, No. 8, nontoxic, pyrogen-free gastric tube) were placed into the bone defect to create an experimental nonunion (Fig. 1).

After 40 days, the polyethylene pads were removed under general anaesthesia. Plain radiographs were taken on both radii after a further 10 days (Fig. 2). Bone defects were evaluated according to the Lane-Sandhu radiographic criteria.[10]

ESWT was performed under general anaesthesia on the same day. An extracorporal shock wave lithotripsy device (PCK, Stonalith Smart Lithotripter)

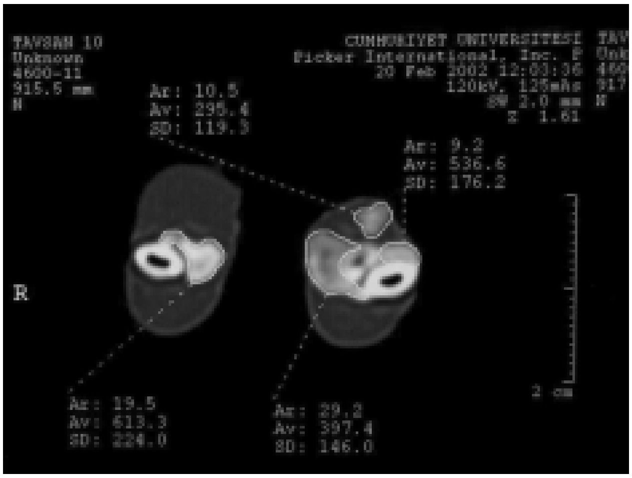

Figure 3 Analysis of the callus volume by free hand technique on computed tomographic scan.

was used and ESWT (14 kW, 0.46 mJ / mm^2, 1000 shock waves) was applied to the right radius by focusing the shock waves on the bone defect. The left side of each rabbit served as a control.

Five rabbits were randomly selected and killed 4 weeks after ESWT (group 1) and the remaining 8 rabbits were killed 2 weeks later (group 2). Callus formation was analysed using CT scan (Picker, PQS model) by taking 2-mm axial sections of the callus mass. The callus mass in every section with the border drawn using a 'free hand technique' was measured in cm^2 on the CT scan (Fig. 3). This value was multiplied by 0.2 cm (the distance between sections) to determine the section volume. Total callus volume was then calculated by combining the volume measurements of all callus areas.

Bone and callus tissue specimens from both the treated and control sides of groups 1 and 2 were examined under Jenamed II (Carl-Zeiss Jena, Germany) photomicroscopy, by taking 5 to 7 μm sections and staining them with hematoxylin and eosin.

Statistical analysis of the callus volume for the treated and control sides was performed using the one-tailed Wilcoxon signed rank test; a p value of <0.05 was considered significant.

The entire study was approved by the ethics committee of the Cumhuriyet University, Turkey.

RESULTS

Two rabbits were excluded from the study because of accidental ulnar fracture; 13 rabbits (26 radii) completed the study. Nonunion was confirmed by radiography 40 days following osteotomy and evaluated according to Lane-Sandhu criteria[10]: 24 defects were grade 0, one grade I, and one grade II.

In group 1, the callus volume of the treated side was always greater than that of the control side (p=0.032, Table 1). In group 2, the callus volume of the treated side was greater than that of the control side, except in rabbits 4 and 9. Only after excluding the findings from the latter did the difference attain statistical significance (p=0.020, Table 2).

Histological analysis of the control side of group 1 demonstrated that the bone comprised outer and inner layers bound to each other with trabeculae that were rather thin and surrounded by a single layer of osteoblasts. There were matrix, osteocytes, and hypertrophic chondrocytes in the trabecular tissue of the mature bone. A distinctive basophilic callus around the defect zone was present. Intertrabecular spaces were filled with a vascular haemopoetic tissue and adipocytes. Trabeculae were thickened and

Table 1
Callus volume analysis of group 1

Rabbit No.	Callus volume (cm^3)	
	Treated side, n=5	Control side, n=5
10	0.5338	0.1444
12	1.4704	1.0360
13	0.0400	0.0340
14	0.2078	0.1656
15	0.4678	0.1852
Mean (SD)*	**0.5439 (0.5548)**	**0.4083 (0.4084)**
Median	**0.4678**	**0.1656**

* p=0.032 (one-tailed Wilcoxon signed rank test)

Table 2
Callus volume analysis of group 2

Rabbit No.	Callus volume (cm^3)	
	Treated side, n=8	Control side, n=8
1	0.1276	0.0952
3	0.7764	0.0394
4	0.0370	0.0440
5	0.0460	0.0162
6	1.2420	0.1240
7	0.4598	0.2564
8	0.0554	0.0262
9	0.0026	0.0060
Mean (SD)*	**0.3433 (0.4530)**	**0.0759 (0.0833)**
Median	**0.0915**	**0.0417**

* p=0.020 (after excluding results from rabbits 4 and 9; one-tailed Wilcoxon signed rank test)

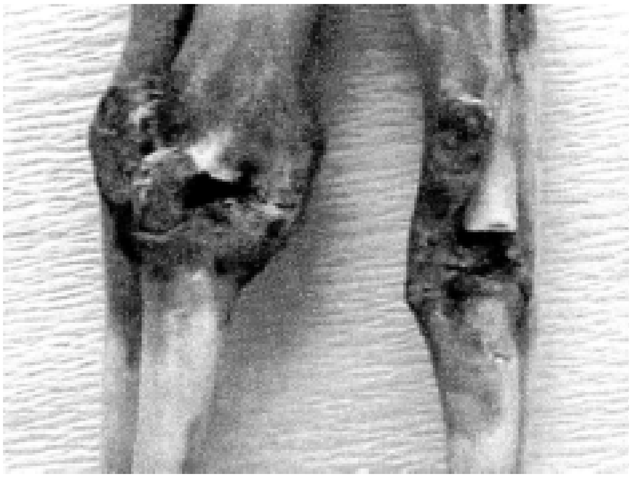

Figure 4 Macroscopic appearance of the control side (right) and treated side (left) in group 1. Note the increased callus volume on the left.

surrounded by osteoblasts. Cartilage islands and hypertrophic chondrocytes were also present in the trabeculae along with mature bone tissue. The callus tissue decreased and a small number of chondrocytes and rather small cartilaginous islands were present in the trabeculae. Bone remodelling increased and intertrabecular spaces decreased. Eosinophilic bone matrix and osteocytes were seen and the defect zone gained a mature bone appearance with its osteocyte. Histology revealed that healing of the defective bone on the treated side was more advanced in group 2 than in group 1. The faster bone healing was observed both macroscopically and microscopically: trabeculae were thicker and intertrabecullar spaces were narrower (Fig. 4). A small amount of callus tissue and cartilage islands with basophilic matrix in the fusion zone was present. The quality of the callus tissue was similar to that present in the normal bone-healing process.

DISCUSSION

Creating a defective nonunion of the long bone in an animal model is a challenge. This was achieved in all the rabbit radii using our method. Minimal new bone formation was seen in only 2 radii; neither bridging of callus tissue nor solid union was observed. Sclerosis, hypertrophy, atrophy, or cyst formation in the fractured ends were absent. The rabbit provides an experimental animal model that is readily available and cost-effective, and does not necessitate the use of external fixation devices such as casts or braces, because the load of the anterior part of the body can be borne by the ulna.[2,11] Many studies have shown that ESWT is highly effective in the treatment of pseudarthrosis and nonunion of the long bone.[6–9] In an experimental setting, quantitative analysis of fracture healing is essential to assess the efficacy of different treatments. Callus volume analysis is used, because it is superior for quantitative assessment of bone healing.[12,13] A simple method for analysis of callus volume was used; callus density measurement was not needed because callus density indicates the mechanical strength of callus tissue.[12,14] Volumetric analysis of callus tissue showed that the differences between the treated and control sides of groups 1 and 2 was significant (group 1, p=0.032; group 2, p=0.020). This is consistent with other studies.[2,15–17] Likewise, the bone-healing process was faster on the treated than control side.[2,15,18] Nonetheless, McCormack et al.[16] did not observe faster bone-healing process and remodelling phase, even though a greater callus volume was seen.

In group 1, histological evaluation of the treated side revealed thickened trabeculae surrounded by osteoblasts (more on the treated than untreated side). Whereas on the treated side of group 2, thickened trabeculae (more so than on the untreated side) were also present but less callue tissue was evident than in group 1. Wang et al.[17] demonstrated a great deal of cortical bone formation on the treated side, and a great deal of fibrous and fibrocartilaginous tissue formation on the control side 12 weeks after ESWT.

In the present study, callus volume on both the treated and control sides was much greater in group 1 than group 2. Moreover, 2 rabbits in the latter group had less callus on the treated than untreated side. These observations are consistent with the remodelling phase of the bone-healing process at the end of week 6, which was confirmed by histology. The presence of smaller amounts of callus tissue together with cartilage islands and basophilic matrix in the fusion zone in the specimens from group 2, indicated that healing was almost complete. Thus, the speed of bone healing appeared significantly enhanced by ESWT.

Although others have reported that satisfactory results were not obtained by ESWT in patients with nonunion or delayed unions greater than 5 mm,[9,19] our results showed that it had a favourable effect on defective nonunion of 6 mm. This may be due to the difference between nonunion in experimental studies and in human bodies. In the latter, fractures are caused by high-energy injuries that often damage soft tissue, bone, and local blood flow. Rompe et al.[8] suggested that ESWT was more effective in fractures caused by osteotomy than those caused by injury. Moreover, negative effects of ESWT such as reduced mechanical stability[20] and delayed fracture healing[21] in a sheep model, and delayed bone healing[22] and epiphyseal dysplasia[23] in a rat model, have also been reported. In the present experimental study, however, ESWT increased volume and speed of callus formation in the radius of rabbits with experimental bone defects.

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