EXPERIMENTAL STUDY

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Adjunctive hyperbaric oxygen therapy in the treatment of atrophic tibial nonunion with Ilizarov external fixator: a radiographic and scintigraphic study in rabbits*

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Objective: The aim of this experimental study was to determine the effects of adjunctive hyperbaric oxygen therapy (HBO) on atrophic tibial nonunion treatment using Ilizarov external fixator.

Methods: Twenty New Zealand white rabbits were randomly divided into two equal groups. A circular external fixator was applied to the right tibia of all the rabbits. A 5-mm bone block was resected and a tibial pseudarthrosis was obtained after a 6-month waiting period. The experimental group rabbits (n=10) underwent daily 2.5 ATA HBO therapy for 2 hours for 20 days and the control group rabbits (n=10) did not receive any corresponding treatment. Osteoblastic activity was evaluated with bone scintigraphy on days 30 and 90. Fracture healing was evaluated by plain radiographs on days 30 and 90.

Results: On Day 30, radiological scores were statistically similar in both groups (p=0.167). However, on Day 90, the experimental group displayed significantly higher radiological scores (p<0.001). Osteoblastic activity was also higher in the experimental group on both scintigraphic assessments (p=0.005 and p=0.001).

Conclusion: The results of this study suggest that HBO can be used as a supplementary therapy in the management of atrophic tibial nonunion.

Key words: Fracture; hyperbaric oxygen; Ilizarov method; nonunion.

Despite recent developments in fracture stabilization and better understanding of the biologic requirements of fracture healing, nonunion continues to pose one of the most challenging problems in orthopedic surgery. It is estimated that approximately 5 to 10% of fractures result in delayed union or nonunion.[1] The basic principles of atrophic nonunion treatment involve resection of nonviable bone, autogenous grafting, and skeletal stabilization. However, extensive surgical approaches further endanger an already impaired blood flow at

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the nonunion site.[2] The biomechanical properties inherent to circular external fixation and the techniques of compression-distraction and internal bone transport are important innovations that will help surgeons meet the challenges of this difficult problem.[3,4]

On the other hand, prolonged time spent on external fixation is a disadvantage that can cause complications, such as pin tract infection, loosening, muscle weakness and contractures.[5] Prolonged framing time may also decrease patient compliance, causing psychological and behavioral problems.[6] Therefore, various supplementary procedures have been used to promote fracture healing in the management of nonunions, such as electric stimulation, extracorporeal shock wave therapy, bone marrow injection, autologous platelet gel supplementation, low-intensity pulsed ultrasound, and bone growth factors.[7-12]

Hyperbaric oxygen (HBO) therapy is the intermittent inhalation of 100% oxygen at pressures greater than 1 atmosphere absolute (ATA). Adjunctive HBO therapy has been used as a treatment alternative for various orthopedic problems, such as chronic osteomyelitis, nonunion of fractures, acute traumatic ischemia of the extremities and compromised grafts for several decades. HBO therapy in fracture management aims to increase the oxygen supply to the fracture site and thereby improve fracture healing.[13]

The purpose of this experimental study was to determine the effects of adjunctive hyperbaric oxygenation on atrophic tibial nonunion treated with circular external fixation.

Materials and methods

Twenty adult New Zealand white rabbits (mean weight: 1800 g., range: 1500 to 2000 g.) were used in this study. The animals were housed in individual standard cages in a temperature controlled room (20 to 22 ºC) and fed a standard laboratory diet and water during a 12-hour day/night cycle. Before initiation of the study, approval from the Local Ethics Committee was obtained. The study was carried out in the "Center for Experimental Animals" in the same institution. The rabbits were randomized into experimental and control groups, each consisting of 10 animals.

The rabbits were anesthetized with 0.2 mg/kg xylazine (Rompun®; Bayer, Leverkusen, Germany) and 20 mg/kg ketamine hydrochloride (Ketalar®; Eczacıbaşı, İstanbul, Turkey). An infection prophylaxis of 20mg/kg/day cefazolin sodium (Cefozin®; Bilim, İstanbul, Turkey) was administered preoperatively and two days postoperatively. When the appropriate depth of anesthesia was achieved, a pre-constructed, four-ring circular external fixator was applied to the right tibia of all rabbits. 5/8 rings were used at the distal and proximal metaphyseal regions of the tibia and two complete rings were used at the proximal and distal ends of the defective area. Each ring was connected with three rods. Two Kirchner wires (1 mm in diameter) crossing each other at an angle of 45º to 60º were applied at every level. After the application of the circular external fixator, the skin subdermis and periosteum were exposed with an anteromedial longitudinal incision and a 5-mm bone block was resected with the surrounding periosteum from the middle-third of the tibia using a Gigli saw. The periosteum covering the remaining fragment ends were also stripped and resected. Subsequently the skin was closed (Fig. 1). After a six-month waiting period, radiographs were evaluated using the Lane and Sandhu five-point grading scale (Table 1).[14] No evidence of bone formation (Grade 0) was set as radiographic confirmation of atrophic pseudarthrosis.

Two rabbits in the control group died during the first month of the waiting period. Nonunion was confirmed by radiography in the remaining 18 rabbits according to the Lane and Sandhu scale.

Acute compression was applied to each tibia, but full compression could not be achieved between the atrophic ends due to technical difficulty and the fibrotic and reactive bony tissues. Therefore, the frame number was reduced and the middle two frames were extracted to provide better compression. The experimental group received 2.5 ATA of hyperbaric oxygenation for 2 hours daily, beginning on the first day of compression and lasting for 20 days in a monoplace pressure cabinet with 210x70 cm dimensions (KRL, Turkey 2001). Oxygen was released into the pressure cabinet as free flow.

Radiographs taken on days 30 and 90 of the study were evaluated using the Lane and Sandhu scale by a single investigator, who was blinded to the assignment of study groups.

Before scintigraphic assessment, the rabbits were sedated with 10 mg/kg Ketalar®. In the scintigraphic study, 3±0.5mCi/0.5cc Technetium-99m methylene diphosphonate (Tc-99m MDP; Medi-Radiopharma, Budapest, Hungary) was injected into the ear vein of each rabbit. Three hours after the injection of the radiopharmaceutical, the rabbits were positioned laterally under the gamma camera (Millenium VG; General Electric, Milwaukee, WI, USA) which was equipped with a low-energy high-resolution collimator. Planar acquisition of 10 minutes was initiated using a 15%

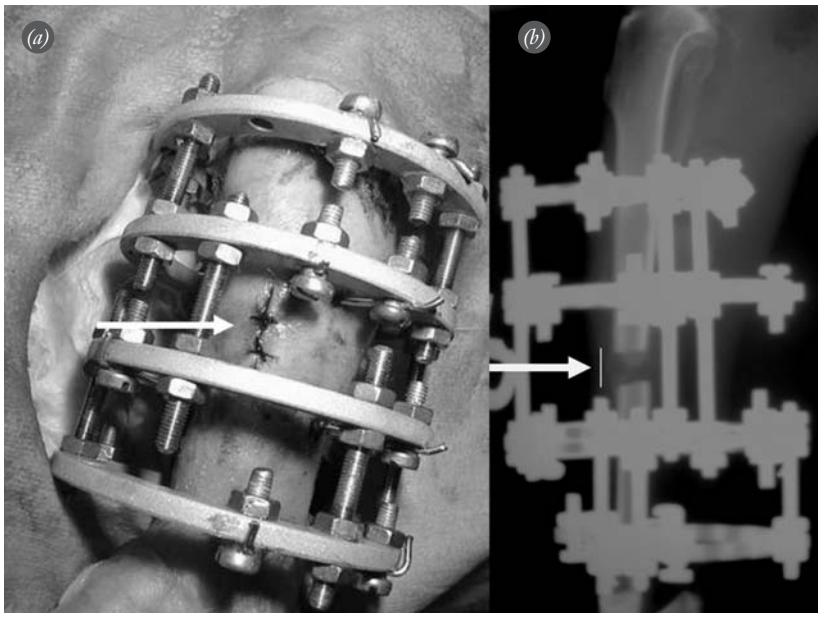

Fig. 1. **(a)** Photograph showing application of the circular external fixator and immediate postoperative sutures on the skin incision. **(b)** Radiological evaluation on the 3rd month demonstrating periosteal ossification on the defective area (white arrow).

window centered over the 140-keV photopeak. Rectangular regions of interest (ROIs) were drawn on both tibias (region of pseudarthrosis and contralateral healthy leg) at approximately similar localizations. Counts were derived from the both ROIs to calculate the osteoblastic activity ratio (count of the lesion / count of the contralateral side).

Table 1. Radiographic grading scale.

Grade 0	No evidence of bone formation
Grade 1	Bone formation occupying 25% of the defect
Grade 2	Bone formation occupying 50% of the defect
Grade 3	Bone formation occupying 75% of the defect
Grade 4	Full gap bone formation

Schematic representation of the experimental plan is shown in Fig. 2.

The Wilcoxon signed-rank test was used for analysis of repeated measures of the same group, and the Mann-Whitney U test for comparison of different groups. Statistical significance was set at $p<0.05$ in consideration of 95% confidence interval.

Results

One of the rabbits in the experimental group died on the 5th day of HBO therapy. Data from the remaining 17 rabbits (9 in the experimental group, 8 in the control group), were collected without any complications. Radiological scores on Day 30 were statistically similar in both groups ($p=0.167$). However, on Day 90, when compared with the control group, the experimental group displayed significantly higher radiological scores ($p<0.001$) (Fig. 3). Full bone union was seen in 6 of 9 rabbit tibia (full gap bone formation) in the experimental group, whereas there was no full union in any tibia in the control group at the final radiographic assessment (Day 90). Osteoblastic activity was significantly

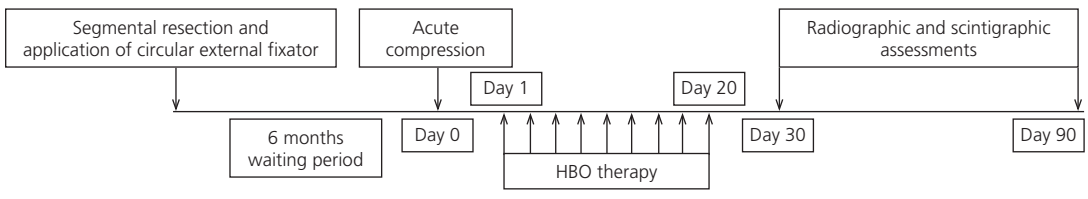

Fig. 2. The schematic representation of the experimental plan.

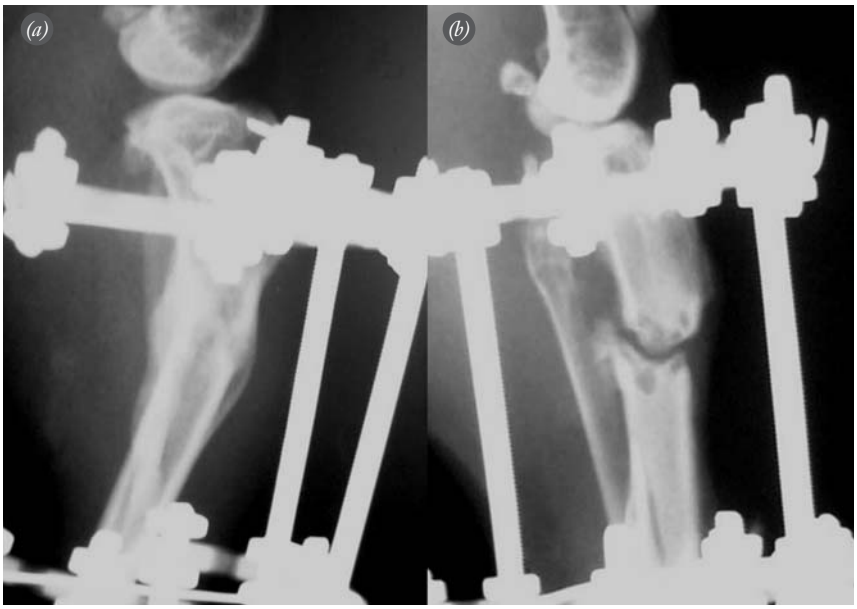

Fig. 3. Radiographs on the 3rd month of compression (final radiographic evaluation). **(a)** Complete union at the pseudarthrosis site was achieved in experimental group, **(b)** atrophic pseudarthrosis was still present in control group.

higher in the experimental group on both (days 30 and 90) scintigraphic assessments (p=0.005 and p=0.001). The results are summarized in Tables 2 and 3.

Discussion

Cellular evidence of the potential applications of HBO therapy in fracture healing has previously been investigated. Wu et al. suggested that HBO enhances the differentiation of osteoblasts towards the osteogenic phenotype in vitro.[15] Milovanova et al. showed that HBO stimulated vasculogenic stem cell growth and differentiation in vivo.[16] Recent research has mostly focused on the use of adjunctive HBO therapy in fresh fracture healing. However, there is little published data regarding the use of HBO in the treatment of nonunions, which is much more challenging than fresh fracture

Table 2. Bone scintigraphy results of the groups.

	Experimental group (n=9) Count of the lesion / Count of the contralateral side (±SD)	**Control group (n=8)** Count of the lesion / Count of the contralateral side (±SD)	**P value**
30th day	1.62±0.21	2.70±0.89	0.005
90th day	1.00±0.08	4.22±0.89	0.001
P value	0.012	0.011	

P values at the last column demonstrate the statistical difference between groups calculated with Mann-Whitney U test. P values at the last row demonstrate the statistical difference of repeated measurements of the same group calculated with Wilcoxon signed-rank test.

Table 3. Radiographic scoring of the groups.

	Experimental group (n=9) (±SD)	**Control group (n=8) (±SD)**	**P value**
30th day	0.77±0.44	0.37±0.51	0.167
90th day	3.66±0.50	1.62±0.74	0.000
P value	0.004	0.008	

P values at the last column demonstrate the statistical difference between groups calculated with Mann-Whitney U test. P values at the last row demonstrate the statistical difference of repeated measurements of the same group calculated with Wilcoxon signed-rank test.

treatment. There have been case reports of clinical improvement following the application of HBO therapy in individuals with established nonunions treated with aggressive debridement and internal bone transport through distraction osteogenesis.[17] A recent Cochrane review assessed the evidence concerning the beneficial effects of HBO therapy in treating delayed union and established nonunion of fractures. The authors concluded that there was insufficient evidence to support or refute hyperbaric oxygenation for the treatment of established nonunion as they failed to locate any randomized clinical trial.[18] To the best of our knowledge, there is no experimental study that has investigated the bone healing process in established tibial nonunions treated with circular external fixation and HBO therapy.

Traditionally, tibial nonunions are classified as hypertrophic or atrophic. Atrophic nonunion reflects inadequate or poorly vascularized nonunion with a very low potential of bone formation with a gap usually found between the bony fragments.[2] Therefore, treatment of atrophic tibial nonunion must be addressed both biologically and mechanically, i.e. with provision of stability and osteogenic environment. In this regard, the Ilizarov method offers solutions for problems in atrophic tibial nonunion. Fracture union is promoted by the removal of non-viable bone through subperiosteal corticotomy and intermittent compression-distraction in most cases.[3] However, this technique remains controversial due to the disadvantages of prolonged framing time, the need for good patient tolerance, the fact that frame application is a technically demanding procedure, and the need for close follow-up and frequent frame adjustment.[19] Therefore, in these cases, extra interventions to promote healing are beneficial. Accordingly, in this study, the effects of additional HBO therapy on the outcome of this surgical method were investigated.

In our study, contrary to what has been described, the atrophic bone ends were not opened or cleaned, and only acute compression was applied. This method is an easier and more physiological surgical protocol than resection of the non-viable bone followed by the intermittent compression-distraction method. Opening the area of nonunion is more detrimental to any weakened vascularity. HBO therapy achieved union by increasing osteoblastic activity and vascularization in the non-living bone tissue at the fracture ends. Although no radiological difference was seen between the two groups on Day 30, scintigraphy showed a significant increase in osteoblastic activity in the experimental group. The reason for this is thought to be that newly-formed callus tissue cannot be clearly seen on radiographs. By Day 60, the difference of mature bone replacing callus tissue was visible radiologically.

Our results showed that HBO enhanced new bone formation, provided faster consolidation and remodeling compared to controls. Six rabbit tibia (66.7%) in the experimental group showed complete union compared to none (0%) in the control group. Furthermore, adjunctive HBO therapy eliminated the above mentioned disadvantages of the Ilizarov method. Acute compression was performed without freshening the fracture ends. In this manner, the need for additional surgical intervention or frame adjustment was avoided.

However, there were some limitations to this study. To create an atrophic nonunion of the long bone in an animal model is quite a challenge.[20] For this study, it was achieved with segmental bone block resection; and the atrophic tibial nonunion model was determined by radiological criteria alone. However, it is still debatable whether this model reflects real physiological conditions in atrophic nonunion. Moreover, no histological or biomechanical evaluations were made.

In conclusion, adjunctive HBO therapy can enhance fracture healing in an atrophic tibial nonunion model and can therefore be used as supplementary therapy. Further clinical studies with different HBO protocols are necessary to confirm the effects on human subjects.

Conflicts of Interest: No conflicts declared.

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