

Comparison of the effects of hyperbaric oxygen and extracorporeal shock wave therapy on healing in osteotendinous junctional injuries: an experimental study of the rabbit model

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ABSTRACT

Objectives: To biomechanically and histopathologically compare the effects on healing in the osteotendinous region of the treatment methods of hyperbaric oxygen (HBO) and extracorporeal shock wave (ESW) on an injury created at the osteotendinous junction in an experimental rabbit model.

Methods: The study included 36 New Zealand rabbits. The patellar tendon tibial osteotendinous junction was cut and then repaired. Group 1 was evaluated as the control group, with no further intervention. In Group 2, a single session of ESW was applied at 14 hz/750 Impulse/10 mins to an area 1×1 cm² in the repaired area under fluoroscopy guidance. The animals in Group 3 were applied with 100% oxygen as HBO at 2.4 ATA/110 min for 7 days. At the end of 6 weeks, 6 animals from each group were applied with a tension test and 6 animals were processed for histopathological examination.

Results: In Group 1, the forces at the start of the separation of the osteotendinous junction, at a 2 mm separation and at full rupture were lower than those of Group 2 and Group 3 ($p = 0.001$ and $p = 0.001$, respectively). The highest separation force values were determined in Group 3 ($p = 0.001$). Histopathologically, a statistically significant difference was determined in respect of the fibrocartilage tissue formation, and fusion with new bone tissue between Group 1 and Group 2 and between Group 1 and Group 3 ($p < 0.05$). No statistically significant difference was determined between Group 2 and Group 3 ($p = 0.310$).

Conclusions: It was observed that the application of HBO and ESW improved biomechanical resistance and healing in the injuries in the osteotendinous junction by increasing the bone-tendon fusion in this area. The application of HBO and ESW increased biomechanical resistance compared to the control group. In the histopathological evaluation, the ESW group was better than the control group and the HBO group was evaluated as the best.

Keywords: Tendon, hyperbaric, oxygen, shock wave, healing

Some studies have reported that the results are not pleasing after surgical interventions of the osteotendinous junction injuries. Healing of the osteotendinous junction is depend on both the bone and the surrounding soft tissues [1]. Osteotendinous junction injuries are require an aggressive multidisciplinary

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physical therapy and rehabilitation program because of the complex anatomic structure of this region. Failure rates have been reported at 20%-25% after rotator cuff repair in the shoulder, and at 10%-22% after reconstruction with tendon autograft in anterior cruciate ligament ruptures [2, 3]. For effective and successful treatment, in addition to early surgical intervention; biological or supplementary treatments are needed to start with an early rehabilitation program that cannot be achieved in routine clinical practice generally.

Biological treatments such as Transforming Growth Factor-beta (TGF- β), Epithelial Growth Factor combined with TGF, Recombinant Human Bone Morphogenic Protein (rhBMP-2), Bone Growth Factor, Granulocyte Colony Stimulating Factor, Periosteal Cells and Autologous Mesenchymal Stem Cells have not routinely applied because of the high costs and difficulties of their acquisition [4, 5-8]. However, supplementary treatments such as Hyperbaric Oxygen Treatment (HBO), Extracorporeal Shock Wave (ESW), Electric Stimulation and Low-intensity Pulsed Ultrasound Stimulation (LIPUS) are currently used in chronic musculoskeletal system diseases [9-13].

Hyperbaric oxygen (HBO) therapy is the inhalation of 100% oxygen at a pressure of at least 1.4 atmospheres absolute (ATA), 140 kPa, in the hyperbaric chamber [1-8]. HBO was first used in decompression sickness [14]. Subsequently, with understanding of the physiological and biomechanical effects, HBO has been widely used in the supplementary treatment for gas embolism, carbon monoxide poisoning, diabetic foot ulcers, peripheral vascular diseases, thermal burns, skin grafts and flaps, purpura fulminans, osteomyelitis, osteoradionecrosis, cerebral and retinal oedema [15-18].

In the literature, efficacy of ESW has been investigated by both experimental and clinical studies, as a result started to use in chronic musculoskeletal system disorders such as tennis elbow, adhesive capsulitis, heel spur, patellar tendinopathy, Achilles tendinitis, delayed bone union, osteochondritis dissecans and avascular necrosis of the femoral head [19-28].

The aim of this study was examined and compared the effects of HBO and ESW biomechanically and histopathologically as a supplementary treatment methods that help to healing of osteotendinous injury on an experimental rabbit model after surgical intervention.

METHODS

Approval for this experimental study was granted by the Animal Experiments Local Ethics Committee. Number of animals was decided with power analysis and a total of 36 adult, female, New Zealand White (*Oryctolagus cuniculus* L) rabbits weighting mean 3.10 kg (range, 2.85-3.36) and with a mean age of 6 months with simple randomisation separated into 3 groups as 12 as control group (Group 1), ESW group (Group 2) and HBO group (Group 3) and included in the study.

Surgical Procedure

Under general anaesthesia, the rabbits in supine position were performed to 5 centimeter (cm) longitudinal incision on the patellar tendon. After exposure of the patellar tendon and the proximal tibia, with 45 degree angle to tibia, a full layer cut was made with a lancet at the bone-tendon junction. Then, immediately below the cut, a tunnel was opened vertical to the tibia long axis, using a 2 mm drill. The bone junction tendon was anatomically repaired by passing 3.0 prolene sutures through the created tunnel with the Krakow method. To prevent breakage, a quadriceps tenotomy was made to eliminate the extensor power of the quadriceps tendon over the repaired osteotendinous junction. After ensuring the stability of the repaired osteotendinous junction, the layers were anatomically closed [6].

A polyethylene long-leg brace was applied with the hip joint in 30° flexion, the knee joint in full extension and the ankle free, and thus immobilisation was provided for 6 weeks. In Group 1, as the control group, the repair was made and no additional treatment method was applied. In Group 2, sedation was applied and with an EMD/E 1000 ESW scopy device, a single session of ESW was applied at 14 Hertz (Hz)/750 Impulse/1 centimeter.square (cm²)/10 minutes (min) to the osteotendinous junction.

The animals in Group 3 were placed in a pressure chamber (Hiperteks Neoks Arbe Chamber) and were applied with 100% oxygen at 2.4 ATA/110 mins for 1 session each day throughout 7 days.

Biomechanical and Histopathological Evaluation

At the end of 6 weeks, all animals were sacrificed by same surgical team and samples were separated

randomly into 2 equal subgroups for biomechanical and histopathological evaluations. Entering over the old incision, the cut was extended proximally and distally and an osteotomy was applied with an osteotome to the centre of the femur and tibia diaphysis. Soft tissues were removed from the patella-patellar tendon-tibia proximal third and the samples were placed in saline and kept at 4°C until the biomechanical tests were applied on the same day.

The materials taken for histopathological examination were placed in a sterile dishes in 10% formaldehyde solution and stored until tested. The whole material was evaluated macroscopically in respect of rupture and separation and the results were recorded. For the biomechanical evaluations, tension tests were applied using an Instron 4301 test device in the Physics Laboratory of the Textile Engineering Department of Uludag University (Fig.1).

The tension test procedure was started at a fixed rate of 25mm/min and was continued. The tension forces (Newton units/kN) were determined at the moment when separation started, when there was a 2 mm separation, and at the moment of complete rupture, and these values were recorded. Histopathological examinations were conducted in the Pathology Laboratory of Uludag University Hospital. After removal of the sutures in the osteotendinous junction area, a longitudinal cut was made from 2 different points and routine follow-up procedures were applied to the two corresponding pieces separately. After the routine procedures, the pieces were embedded in paraffin blocks and sections were cut 4-

5 microns in thickness. These sections were stained with haematoxyline and eosin and evaluated by a pathology specialist blinded to the study. In the histopathological examination, the fibrocartilage tissue, new bone formation and tendon-bone tissue fusion in the healed area of the osteotendinous junction were evaluated using the histological scoring system described by Weng *et al.* [29]. Each parameter was scored with points.

Statistical Analysis

Statistical analyzes were carried out with SPSS v.16.0 software (SPSS Inc., IBM Corporation, Armonk, New York, USA) and power analysis was carried out with G-Power v.3.1.9.1 software [Franz Faul, Universitat Kiel, Germany]. For this study, alfa error was accepted as %5 and $p < 0.05$ values were accepted as statistically significant. As there were a total of 3 paired comparisons, Bonferroni correction was applied because of the group analysis, the p value was calculated as $0.05/3 = 0.017$. Before the study, power analysis was performed. Due to similar studies at the literature statistical power ($1-\beta$) was accepted %80 and total sample size was determined as 36 animals. Demographic datas of rabbits as age and weight were evaluated with the Chi-square test. Because of small sample groups, results were accepted to non-normally distribute so median, minimum and maximum values are determined and non-parametric datas were compared with Kruskal-Wallis and Mann Whitney U-tests.

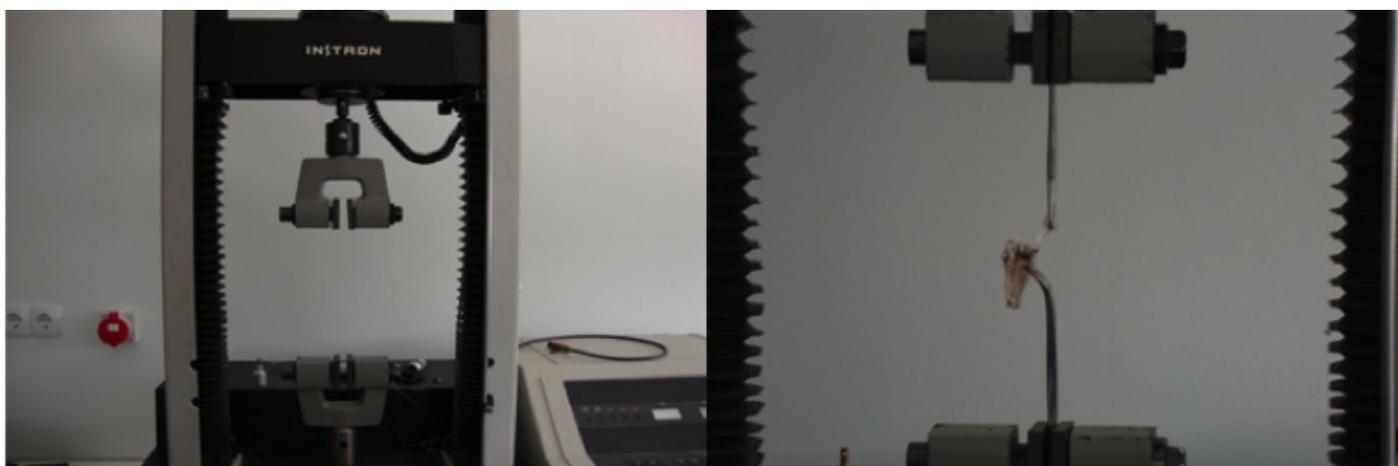


Fig. 1. Application of the tension test with the Universal Instron 4301 test device.

RESULTS

A significant difference was observed with comparisons of the force at the start of the separation of the healing area of the osteotendinous junction, the force creating the 2 mm separation and the force providing full rupture ($p = 0.001$) (Table 1).

In Group 1; the separation at the healing area of osteotendinous junction was started first, biomechanical resistance to 2 mm separation and full rupture were significantly lower than Group 2 and Group 3 ($p = 0.001$ and $p = 0.001$, respectively). The highest biomechanical resistance to separation were determined in Group 3 ($p = 0.001$) (Table 1).

The fibrovascular tissue, collagen bundles, fibrocartilage tissue and new bone formation were examined in the materials taken from the osteotendinous junction of the other half of the subjects. Fibrovascular tissue and new bone formation were more evident in the treatment groups compared to the control group (Fig. 2a, 2b and 2c). Collagen alignment was more regular and mature in the treatment groups than in the control group. Osteotendinous fusion, fibrocartilage tissue formation, new bone evaluation formation and total histological score were evaluated (Fig. 3a, 3b and 3c). Except fibrocartilage tissue formation ($p = 0.049$); in histological evaluations, there was a statistically significant difference between control group and treatment groups but no difference between treatment groups. (Table 1).

DISCUSSION

Although osteotendinous junction injuries are often encountered, because of the complex anatomic

structure of the region, the expected successful results cannot always be obtained even if early and optimum surgical treatment has been applied [2, 7]. As postoperative immobilisation of the extremity is required and no consensus has yet been reached in literature on the treatment method, there is still not a full understanding of the biomechanical function and histological properties of the anatomic structure of the osteotendinous region and this has rendered treatment of injuries in this region complicated [1, 2].

Similarly, in a rabbit model of delayed osteotendinous junction injuries, Qin *et al.* [12] reported that in the group applied with ESW, there was more bone formation in the healing area of the patella-tendon junction at 8 weeks (293.4%) and 12 weeks (185.8%) compared with control group and histologically collagen bundles were more mature and fibrocartilage tissue was thicker and more regular. Wang *et al.* [13] reported that ESW accelerated neovascularisation in the Achilles tendon-bone junction in rabbits and this effect lasted for up to 12 weeks. In those studies, an increase was determined in angiogenic markers in the tissue such as VEGF and endothelial nitric oxide (eNOS).

In an Achilles tendon partial rupture model in rats, Orhan *et al.* [30] compared subjects applied with ESW (500 shock/15 kV) with a control group and reported that in the ESW group, neovascularisation was increased, there were fewer adhesions and a mechanically stronger tissue was obtained.

In a patellar tendinitis model created in rabbits by Hsu *et al.* [31], the application of ESW at 0.29Mj/mm² energy was seen to increase collagen synthesis in the healing tissue, accelerated neovascularisation and increased tensile strength of the tissue. Similar to these findings in literature, accelerated collagen synthesis

Table 1. Biomechanical and histological evaluations

	Initial Sep. ³	2mm Sep. ⁴	Full Sep. ⁵	OF ⁶	FTF ⁷	NBF ⁸	TS ⁹
K-W ¹	0.001	0.001	0.001	0.016	0.049	0.016	0.014
MW-U ²							
Group 1-2	0.002	0.002	0.002	0.015	0.093	0.014	0.093
Group 1-3	0.002	0.002	0.002	0.015	0.026	0.009	0.002
Group 2-3	0.002	0.002	0.002	0.310	0.818	0.240	0.310

¹Kruskal-Wallis Test, ²Mann Whitney-U Test, ³Initial Separation (k/N), ⁴2mm Separation (k/N), ⁵Full Separation (k/N), ⁶Osteotendinous Fusion, ⁷Fibrocartilage Tissue Formation, ⁸New Bone Formation, ⁹Total Score

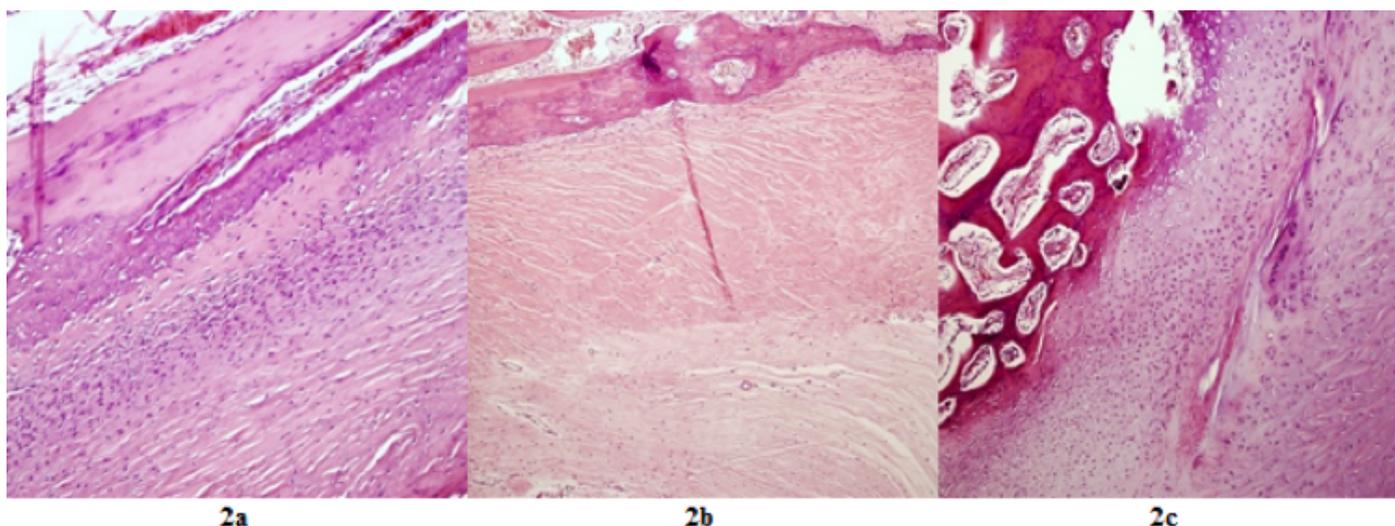


Fig. 2 (a, b and c). Histopathological examination images of the fibrovascular tissue, collagen bundles, fibrocartilage tissue and new bone formation of the groups.

and maturation; osteotendinous fusion and new bone formation were observed significantly higher in the group 2 (Table 1 and 2) ($p = 0.015$ and $p = 0.014$). As a result of these histological changes, the osteotendinous fusion was increased and biomechanical strength of the tendon was increased.

In the group 3 as the other supplementary treatment method, the biomechanical resistance was determined to be statistically significantly greater than other groups ($p = 0.002$). The results of the histological evaluation showed a similar statistically significant higher osteotendinous fusion and new bone formation but no significant difference found at

fibrocartilage tissue formation ($p = 0.049$).

In the literature, several studies were researched to effects of HBO with biological and biomechanical datas and concluded that, HBO treatment was accelerated the fibroblast proliferation, increased the structural protein synthesis, inhibit to TGF- β 1 synthesis, inhibited the metalloproteinase effects as a result inhibited the degradation also tendon-bone healing and biomechanical strength of tendon was significantly increased [29, 32, 33].

In the current study, the histopathological and biomechanical findings of the animals applied with supplementary treatments were found statistically

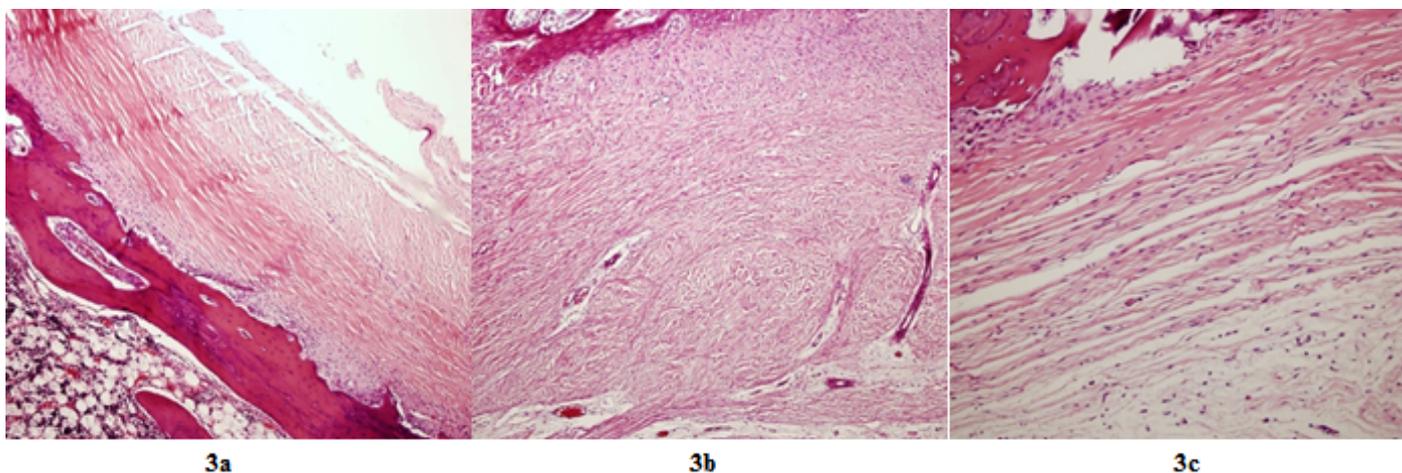


Fig. 3 (a, b and c). Histopathological evaluation images of the collagen alignment and maturation and the formation of fibrocartilage tissue of the groups

Table 2. Biomechanical results

		Initial Separation (k/N)	2 mm Separation (k/N)	Full Separation (k/N)
Group 1	n	6	6	6
	Median	173.50	193.00	205.50
	Minimum	168	188	200
	Maximum	179	199	209
Group 2	n	6	6	6
	Median	244.50	261.50	296
	Minimum	236	252	288
	Maximum	252	266	300
Group 3	n	6	6	6
	Media	291	326	345
	Minimum	280	310	330
	Maximum	296	338	352
Kruskal-Wallis	p value	0.001	0.001	0.001

significant to compared with group1 except fibrocartilage tissue formation (Table 1 and 2). Osteotendinous fusion, new bone formation and biomechanical strenght were observed significantly increased to compared with group 1 ($p < 0.05$). Comparison between group 2 and 3; the biomechanical strength was significantly increased in group 3 ($p = 0.002$)but there were no significant difference in histopathological findings ($p > 0.05$).

CONCLUSION

In conclusion, the results of this study concluded that the supplementary treatments were accelerated to healing of osteotendinous junction injuries with increased new boneformation, osteotendinous fusion and biomechanical strength. Comparison between ESW and HBO, except the biomechanical strenght was significantly increased in HBO; there were significant difference. In the light of these results,

ESW and HBO treatments can be considered beneficial in the early postoperative healing of osteotendinous junction injuries and as increased strenght of the bone-junction tendon, could make a positive contribution to the rehabilitation process and have a positive effect on outcomes. Also further well-designed randomise clinical trials are required.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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