Effect of hyperbaric oxygen therapy on tense repair of the peripheral nerves

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ABSTRACT

Background: After a peripheral nerve cut, tense repair of a nerve compromises circulation of the nerve at the injury site, making the site hypoxic. Hyperbaric oxygen might increase tissue oxygenation and therefore diminish the effects of injury. We investigated whether hyperbaric oxygen treatment affects peripheral nerve healing when repaired nerves are under tension.

Methods: Sixteen young female albino Wistar rats were used. Sciatic nerves of the animals were cut and a 3mm part of each nerve was excised. The animals were distributed into two groups: 1) The HBO2 group (n=8), which received surgical repair and HBO2 therapy; and 2) The Control group (n=8), which received only surgical repair. Walking track analysis was performed five times, on Days 12, 15, 18, 20 and 22 after surgery. The healing of sciatic nerves was evaluated by histopathological study and electrophysiological study. Pillai’s Trace test and Mann-Whitney U-test were used for statistical analysis.

Results: Walking track analysis: Sciatic function index (SFI) scores of HBO2 group were significantly higher than SFI scores of Control group (p:0.026). Electrophysiological study: A statistical difference was not found between groups. Histopathological study: Counts of HBO2 group axons were significantly greater than in the control group (p: 0.008).

Conclusions: In clinical practice, tension after nerve repair frequently occurs. However, neither grafting nor other current surgical methods are functionally perfect. Since primary end-to-end repair is known to be the best repair when possible, we think HBO2 allows for the use of primary repair even when nerve tension is foreseen.

INTRODUCTION

Peripheral nerve injuries accompany 5% of all traumas [1]. Following some injuries, nerve integrity is maintained, allowing nerves to heal spontaneously. However, if a nerve is transected, surgical intervention is required. There have been some advances in repair of peripheral nerves, but full functional recovery has not yet been achieved. Factors such as co-existing injuries, advanced age, delay of repair, proximity of lesion and tension on the repair site have negative effects on healing [2,3]. A tense nerve repair can be defined as:

1. the existence of a gap at the repair site; or
2. difficult coaptation maintenance even after two consecutive sutures are placed, with the extremity in the anatomical position and with the appropriate suture material.

In clinical practice, nerve gaps are fairly frequent complications, as the damaged ends of healthy nerve tissue must be resected before repair. Ischemia, edema and hypoxia resulting from nerve gaps are thought to be responsible for the poor healing of tense repairs [4].

In tense repair of peripheral nerves, the integral circulatory failure of the nerve due to epineural damage is worsened by the stretching of the surrounding capillaries that occurs as a result of coaptation. This stretching causes the capillaries to collapse, making the injury site hypoxic [1]. Following hypoxia, edema develops at the injury site. Therefore, it appears that hyperbaric oxy-
gen would be a reasonable adjunctive treatment after a primary repair under tension, since tissue oxygenation will be raised and the edema related to the injury will be diminished.

Hyperbaric oxygen (HBO₂) has been shown to promote healing in many types of injuries and repairs. HBO₂ has been used to help facilitate peripheral nerve repair, to effectively regenerate axons after transaction of a nerve, and to improve axon regeneration level after crush injuries [5,6]. In some of these studies, functional nerve recovery was tested and found to be faster with HBO₂ [7]. Likewise, HBO₂ was shown to improve nerve graft healing [8]. However, the effect of HBO₂ on the healing of peripheral nerves repaired under tension has not previously been studied.

This study investigated whether hyperbaric oxygen treatment has an effect on peripheral nerve healing under tension.

MATERIALS AND METHODS

Animals: The study was approved by Istanbul Faculty of Medicine’s local animal ethics committee. Sixteen young female albino Wistar rats, weighing 250-300 grams were used. These animals were distributed randomly into two groups: the HBO₂ group, which received only surgical repair; and the Control group, which received only surgical repair.

The animals were housed under temperature-controlled conditions (21±1°C), in separate cages with 12-hour daylight 12-hour night cycle and were fed ad libitum.

Surgical procedure: The animals were anesthetized with intraperitoneal xylazine (5 mg/kg) and ketamine HCL (50 mg/kg). The animals were placed in the prone position, and the right sciatic nerve was exposed from the sciatic notch to the point of trifurcation via posterolateral approach. Once the sciatic nerve was reached, it was marked with a 10/0 epineural microsuture 10mm distal to the sciatic notch. A second marking suture was placed on the epineurium precisely 3mm distal to the first one and a “clean-cut” transection was performed. Thus, a 3mm piece of nerve was excised in order to create a tensile repair model for our study. We excised a 3mm piece of nerve which causes 3.3±/1.09 g of tension and corresponds to mild tension in the rat sciatic nerve [4,9]. Afterwards these marking sutures were aligned and used as a guide for coaptation during the repair. An end-to-end repair was performed with 10/0 nylon (10/0 Daylon, Dogsan©) suture material, placing six epineural sutures under microscope magnification. The repair was checked by stretching the leg, and the wound was closed.

Hyperbaric oxygen therapy (HBO₂): All treatments were carried out in Istanbul Faculty of Medicine, Department of Underwater and Hyperbaric Medicine animal hyperbaric chamber. When the animals were placed in the chamber, before starting treatments, the chamber was ventilated with 100% oxygen for 10 minutes, and then compressed to 2.5 ATA in six minutes. After a 60-minute HBO₂ treatment, the chamber was decompressed, again taking six minutes. HBO₂ was administered three times a day for the first 72 hours, two times a day for the next 72 hours and then daily for a total treatment session period of three weeks. The first treatment was given at two hours post-op to all HBO₂ group animals.

Evaluation: Walking track analysis was performed five times, on Days 12, 15, 18, 20 and 22 after surgery. After the hind feet were dipped into play paint, the animals were placed on a walkway that was 20 cm wide and 100 cm long, with a dark cabin at the end. Graphic paper was placed on the track to obtain the footprints. From each paper, distance from tip of the third toe to heel (print length), distance between the first and fifth toes (toe spreading) and second and fourth toes (intermediary toe spreading) of both the operated and non-operated foot were measured by an assessor blinded to the groups. With these values, sciatic function index (SFI) scores were calculated according to Bain’s formula [10]. On the SFI scale, 0 indicates normal function and -100 or less shows disability.

Electrophysiological study: All measurements were done in Istanbul University, Istanbul Faculty of Medicine Physiology Laboratory. On post-op Day 22, all animals were reanesthetized in the same manner as before, and the sciatic nerves that had surgery were exposed. The prepared electrodes were placed so that one group electrode was on the proximal and one group electrode was on the distal end of the repair line. The proximal electrode was then connected to a stimulator (Bio Science 10550 Kymograph + Stimulator); the distal electrode was connected to the recording system. The stimulating current was chosen to be submaximal; duration was 0.14 milliseconds. The compound field potentials were screened (National Instruments ETH-255 Bridge/Ampifier) and sent with a speed of 5000 specimen/second to the recording program (Lab Scribe). From these recordings, first and second wave latencies were determined.
Histopathological study: After the electrophysiological evaluations had been completed, the animals were sacrificed with high dose intracardiac xylazine and ketamine HCL. Repaired sciatic nerves were dissected out and placed in 10% formaldehyde. A 0.5-cm length of each nerve that included the repair site was sectioned. These specimens underwent the routine histology procedure and were stained with hematoxylin-eosin stain. The axons were counted from five zones distal to the repair site and the averages were recorded using a light microscope under 400X magnification.

Statistics: All data were collected as Microsoft Excel© folders and were analyzed with the SPSS 5.0 program©. SFI scores were compared with Pillai’s Trace test. In addition, mean values for each animal were compared with Mann-Whitney U-test. Latency values and the axon counts were also compared with Mann-Whitney U-test. A level of $p<0.05$ was regarded as significant.

RESULTS

Generally, the animals treated with HBO₂ were more active and appeared more comfortable during walking analysis. Until the fifteenth day of the project, self-mutilation was not observed, but that day a wound was seen on the operated foot of a control group animal. During electrophysiological evaluation we noted that this animal’s repair had failed and the nerve ends were separate. Therefore all the overall statistical analysis was done for only seven of the animals in the control group.

Walking track analysis

Both HBO₂ and Control groups went through walking track analysis on Days 12, 15, 18, 20 and 22, and SFI scores were calculated. All the values recorded were compared with Pillai’s Trace test; SFI scores of the HBO₂ group were found to be significantly higher than SFI scores of the Control group ($p<0.026$). Mean SFI scores for each evaluation day (Table 1, above right) were also compared with Mann-Whitney U test. The results on post-op Day 12 showed no statistically significant difference ($p=0.294$). On post-op Days 15, 18, 20 and 22 the HBO₂ group SFI values were significantly higher than the control group (Table 2, right).

Electrophysiological study

The mean latency values gathered from compound field potential curve are shown in Table 3 (right). The values were compared with Mann-Whitney U-test; a statistical difference was not found for either Latency 1 or 2 ($p_{1}: 0.219$ and $p_{2}: 0.562$).

**TABLE 1: Sciatic Function Index scores for HBO₂ and Control groups**

<table>
<thead>
<tr>
<th>Day</th>
<th>HBO₂-1</th>
<th>HBO₂-2</th>
<th>HBO₂-3</th>
<th>HBO₂-4</th>
<th>HBO₂-5</th>
<th>HBO₂-6</th>
<th>HBO₂-7</th>
<th>HBO₂-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 20</td>
<td>-87.052</td>
<td>-85.228</td>
<td>-95.898</td>
<td>-81.339</td>
<td>-98.056</td>
<td>-104.547</td>
<td>-87.85</td>
<td>-91.164</td>
</tr>
</tbody>
</table>

**TABLE 2: Mean Sciatic Function Index scores, Standard Deviation values and p values for both groups**

<table>
<thead>
<tr>
<th>Day</th>
<th>HBO₂ Mean</th>
<th>Group SD</th>
<th>Control Mean</th>
<th>Group SD</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 12</td>
<td>-118.58</td>
<td>10.58</td>
<td>-114.89</td>
<td>6.67</td>
<td>0.294</td>
</tr>
<tr>
<td>Day 15</td>
<td>-98.78</td>
<td>7.99</td>
<td>-108.79</td>
<td>5.54</td>
<td>0.012</td>
</tr>
<tr>
<td>Day 18</td>
<td>-98.30</td>
<td>4.89</td>
<td>-122.78</td>
<td>6.38</td>
<td>0.001</td>
</tr>
<tr>
<td>Day 20</td>
<td>-91.39</td>
<td>7.63</td>
<td>-112.78</td>
<td>5.41</td>
<td>0.004</td>
</tr>
<tr>
<td>Day 22</td>
<td>-91.61</td>
<td>3.04</td>
<td>-106.94</td>
<td>5.95</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>HBO₂ Mean</th>
<th>Group SD</th>
<th>Control Mean</th>
<th>Group SD</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 12</td>
<td>-118.58</td>
<td>10.58</td>
<td>-114.89</td>
<td>6.67</td>
<td>0.294</td>
</tr>
<tr>
<td>Day 15</td>
<td>-98.78</td>
<td>7.99</td>
<td>-108.79</td>
<td>5.54</td>
<td>0.012</td>
</tr>
<tr>
<td>Day 18</td>
<td>-98.30</td>
<td>4.89</td>
<td>-122.78</td>
<td>6.38</td>
<td>0.001</td>
</tr>
<tr>
<td>Day 20</td>
<td>-91.39</td>
<td>7.63</td>
<td>-112.78</td>
<td>5.41</td>
<td>0.004</td>
</tr>
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<td>-91.61</td>
<td>3.04</td>
<td>-106.94</td>
<td>5.95</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**TABLE 3: Mean latency and p values for HBO₂ and Control groups**

<table>
<thead>
<tr>
<th>Latency 1</th>
<th>Latency 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBO₂</td>
<td>Control</td>
</tr>
<tr>
<td>Mean</td>
<td>0.01175</td>
</tr>
<tr>
<td>SD</td>
<td>0.003991</td>
</tr>
<tr>
<td>$p$</td>
<td>0.219</td>
</tr>
</tbody>
</table>
 Histopathological study

Upon histopathological evaluation, the exterior parts of the nerve were observed to be healing more effectively with less edema than the central parts. HBO2 Group nerves were observed to be less edematous (Figures 1 and 2, facing page). These values were compared with the Mann-Whitney U-test; the number of HBO2 Group axons was found to be significantly greater (p<0.008) than in the Control group (Table 4, above).

DISCUSSION

Nerves have an important role in functional recovery following injury. Use of microsurgical repair methods improves outcomes, but even with optimal surgical techniques, results may not be entirely satisfactory [3]. Tension, via the hypoxia and edema it causes, is one of the most significant causes of delayed and insufficient healing following nerve repair [4]. HBO2 is a treatment modality that has been shown to be beneficial in healing hypoxic wounds. It is also reported to enhance nerve healing following nerve repair [4]. HBO2 is a treatment with the work of Zamboni et al., in which they used SFI for gait analysis and found the scores to be higher with HBO2 after transecting and repairing the nerve [5]. Haapeniemi also used gait analysis for testing functional recovery after transaction and crush injuries [14]. He did not find a significant difference between HBO2-treated and control groups, but he used only "toe spread" for analysis, so this is not directly comparable to our study.

Another important indicator of repair is the number of regenerating axons and their organization pattern, compared with the original layout [15]. At the injured end, not all the axons which are seen to be sprouting and elongating through the injury zone reach the distal end. However, the more the axons regenerate, the greater the possibility that enough will reach to the distal end [16,17], thus showing the regeneration of axons following repair is important. In our study, we found that the number of axons in the HBO2 group were significantly higher than in the Control group. This result suggests that HBO2 promotes axonal regeneration and is in line with other studies that show HBO2 increasing regeneration after a crush injury and nerve grafting [6,7,10]. On the contrary, in Bajrovic et al.’s study in which the sciatic nerve was crushed and made acellular, HBO2 did not have any positive effect on axonal regeneration [18]. This, we think, may be due to different timing and duration of the treatment.

These data support the hypothesis that hyperbaric oxygen treatment enhances tense nerve repair, and there are several explanations about its mechanism. The most emphasized explanation is the oxygenating effect of HBO2. After an injury, circulation deteriorates and hypoxia develops [3]. Apoptosis is an important problem in nerve degeneration; after a sensory nerve cut, up to 50% of the axons can die due to apoptosis, which is triggered mostly by mediators released from mitochondria [19]. Since the mitochondrion is an oxygen-dependent organelle, hypoxia may be causing these me-

<table>
<thead>
<tr>
<th>TABLE 4. Average axon numbers of the groups in selected areas</th>
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<tbody>
<tr>
<td>Animal</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>HBO2</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>
FIGURE 1 – Representative histologic features from transverse sections of sciatic nerves in hyperbaric oxygen–treated group (hematoxylin-eosin stain; original magnification, X400).

FIGURE 2 – Representative histologic features from transverse sections of sciatic nerves in control group (hematoxylin-eosin stain; original magnification, X400).
The other two effects of HBO₂, angiogenesis and agents to release. Therefore, HBO₂, by raising oxygen tension in tissues, may break the hypoxia apoptosis cycle and help in resuming viability and regeneration.

Another point that should be considered is HBO₂'s enhancing effect on collagen synthesis [20]. In a study where HBO₂'s effect on tendon grafts was investigated, collagen synthesis and collagen density were found to be higher with HBO₂ [21]. Even though overproduction of collagen tissue causes scar tissue in the nerve over time, some fibroblast activity may still be necessary [16]. Collagen is not directly involved in nerve repair; however, endoneural tubes in which axon growth takes place rest on a strongly supportive tissue, whose strength may be provided by a collagen network. In previous studies, nerve conduits that were built from collagen or by adding collagen to another basic material were analyzed for their effect on regeneration [17,22]. They were found to be effective on regeneration, showing that collagen has an indirect place in nerve repair.

The other two effects of HBO₂, angiogenesis and edema reduction, are also thought to be important in healing [20]. Eguiluz-Ordonez et al. showed a significant increase of blood vessels in their HBO₂ group after a nerve transection injury [5]. In our study, we observed more blood vessels and less edema, especially at the center of the nerve, but these observations cannot be established as statistical data.

There are some limitations of the study. First, electrophysiological evaluation could have been performed more accurately. Because of technical problems like constructing electrodes small enough to fit rat sciatic nerves and getting clear recordings – as it was hard to stabilize the nerve on the hand-made electrode – this may have contributed to the lack of statistical differences between the electrophysiological results of the two groups. Eguiluz-Ordonez, on the other hand, found significant difference in latencies of HBO₂ and Control groups, and this data correlated to their other findings [5]. Second, special nerve stains could have shown the axonal sprouts and myelin levels; however, since our aim was to show the effect of two different treatment approaches on the nerve healing, it was not necessary to use any additional lab techniques. Finally, the follow-up period could have been extended to see the long-term effects of the hyperbaric oxygen treatment on nerve healing. However, our experiment was designed to see early effects of the hyperbaric treatment.

Primary end-to-end repair is the first choice when there is a nerve injury [23]. However, if tension is anticipated during repair, other methods are preferred at the initial surgery even if primary repair is technically possible. Nerve grafting with autograft is currently accepted as the technique of choice [4,17,23]. In addition to nerve grafting, vein grafting and similar techniques, surgeons may try extremity positioning to avoid tension at the injury site. For this, the surgeon places the injured extremity in a position that will eliminate or decrease tension during repair. A very good example of this is the repair of a median nerve cut, where the wrist is bent during repair for a tension-free procedure. However, when the extremity is put back into anatomical position to secure the repair, tension on the nerve can be detected easily. In cases like this, rather than grafting or keeping the extremity in awkward positions during surgery, hyperbaric oxygen might be a good adjuvant therapy.

In clinical practice, tension after nerve injury is seen frequently because the injured ends are usually debrided and some retraction is inevitable [23]. We believe that clinical studies using HBO₂ treatment following tense nerve repair should be performed, as it has been shown to promote healing in animal studies.

ACKNOWLEDGMENT
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REFERENCES


