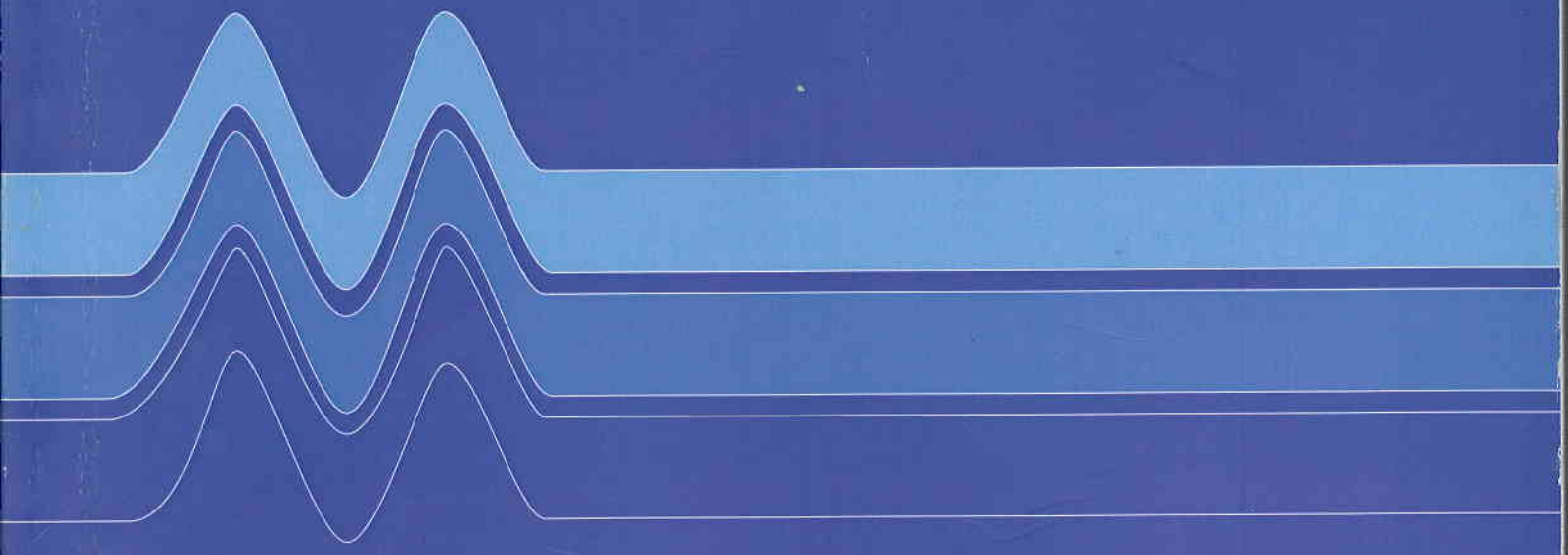
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EFFECT OF HYPERBARİC OXYGEN THERAPY ON TENSE REPAİR OF THE PERIPHERAL NERVES

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INTRODUCTION

Peripheral nerve injuries accompany 5% of all traumas (1). In some of these, nerve integrity is not compromised and nerves can heal spontaneously, but if a nerve is transected surgical intervention is required. There have been some advances in repair of peripheral nerves but full functional recovery cannot yet be achieved. Factors such as co-existing injuries, age, delay of repair, proximity of lesion and surgical problems like tension on the repair site have negative effects on healing (2,3). Even though there is not any objective criterion to define a tense repair, it can be defined as existence of gap at the repair site or difficulty of maintaining coaptation 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 not unusual complication as the damaged ends must be debrided to the healthy nerve tissue. Ischemia and hypoxia and edema resulted from these are thought to be responsible for poor healing of tense repairs (4).
Hyperbaric oxygen (HBO) has been shown to promote healing in many types of injury. HBO, also, is one of the new interventions that has been tried to facilitate peripheral nerve repair. The few studies that have been done about this topic involve various types of injuries and repairs. HBO was found to be effective for axon regeneration after transaction of a nerve (5). Similarly, it was shown to improve regeneration after crush injuries (6). In some of these studies, functional recovery was also tested and found to be faster with HBO (7). Likewise, hyperbaric oxygen was showed to improve nerve graft healing (8). However, effect of HBO on healing of peripheral nerves repaired under tension has not previously been studied.
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 is necessary to achieve coaptation, causing the capillaries to collapse. As a result, the injury site becomes hypoxic (1). Following hypoxia, edema develops at the injury site. To minimize the effect of such complications, grafting is preferred to primary end to end repair under tension and is accepted to have better outcomes. It appears, therefore, that hyperbaric oxygen is 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.

MATERIALS AND METHODS

Animals: The study was approved by Istanbul Faculty of Medicine local animal ethics committee. Sixteen young female albino Wistar rats, weighing 250-300 grams were used. These animals were distributed randomly into two groups: HBO group- received surgical repair and HBO therapy; Control group received only the 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 xylisin (5 mg/kg) and ketamin HCL (50 mg/kg). Surgical procedure was performed by a surgeon in an animal laboratory. The animals were placed in 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 10 mm distal to the sciatic notch. A second marking suture was placed on the epineurium precisely 3 mm distal to first one and a "clean-cut" transection was performed. Thus, a 3 mm piece of nerve was excised. Afterwards these marking sutures were put 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 (HBO): 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 the treatments, the chamber was ventilated with 100% oxygen for ten minutes, and then compressed to 2.5 ATA in six minutes. After a sixty minute HBO treatment, the chamber was decompressed, again taking six minutes. HBO was administered 3 times a day for the first 72 hours, 2 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 HBO group animals.

Evaluation:

Walking track analysis: were done 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 the walkway which was 20 cm wide and 100 cm long and had a dark cabin at the end. On the track, graphic paper was placed 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 (9).

Electrophysiological study: All measurements were done in Istanbul University, Istanbul Faculty of Medicine Physiology Laboratory. On post-op 22 day, all animals were re-anesthetized 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 of the repair line. The proximal electrode was then connected to a stimulator (Bio Science 10550 Kymograph + Stimulator) and the distal electrode was connected to the recording system. The stimulating current was chosen to be sub-maximal and duration was 0.14 milliseconds. The compound field potentials were screened (National Instruments ETH-255 Bridge/Bio Amplifier) and were sent with a speed of 5000 specimen/second to the recording program (Lab Scribe). From these recordings, first and second wave latencies were determined.

Histo-pathological study: After the electrophysiological evaluations had been completed, the animals were sacrificed with high dose intracardiac xylisin and ketamin 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 procedure and were stained with Hematoxylen-Eosin stain. With a light microscope under 400X magnification, and axons were counted from five zones distal to the repair site and the averages were recorded.

Statistics: All data were collected as Microsoft Excel© folders and were analyzed with 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

Walking track analysis

Both HBO and Control groups went through walking track analysis on days 12,15,18,20 and 22 and SFI scores were calculated (Tables 1 and 2). On the SFI scale, 0 indicates normal function and -100 or less shows disability. All the values recorded were compared with Pillai's Trace test and SFI scores of HBO group were found to be significantly higher than SFI scores of Control group. (p: 0.026)

	Day 12	Day 15	Day 18	Day 20	Day 22
HBO-1	-128,26	-99,097	-105,479	-87,052	-95,795
HBO-2	-126,815	-94,771	-101,152	-85,228	-93,951
HBO-3	-125,515	-94,358	-91,071	-95,898	-92,979
HBO-4	-120,536	-96,286	-101,576	-81,339	-90,374
HBO-5	-126,588	-115,51	-91,675	-98,056	-89,007
HBO-6	-104,621	-88,323	-98,218	-104,547	-91,486
HBO-7	-101,263	-102,802	-97,88	-87,85	-86,187
HBO-8	-115,073	-99,112	-99,367	-91,164	-93,153

Table 1. SFI scores for HBO Group.

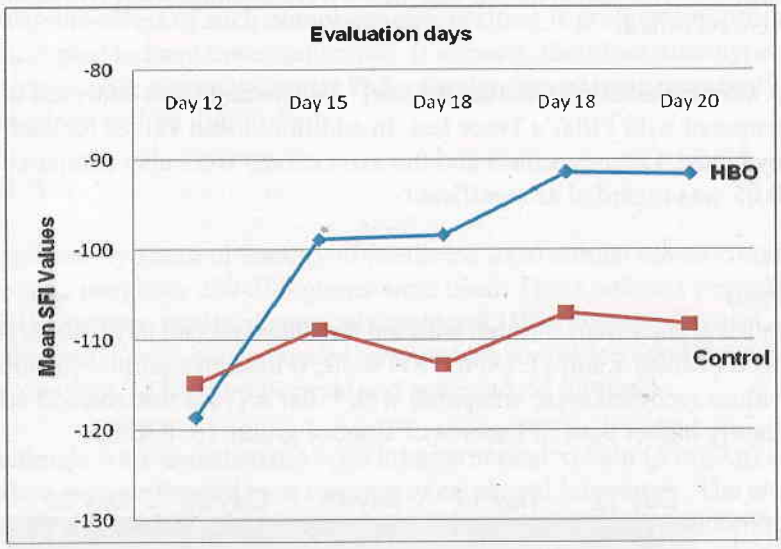
	Day 12	Day 15	Day 18	Day 20	Day 22
C-1	-113,689	-117,381	-116,686		
C-2	-121,291	-114,818	-122,728	-107,83	-113,278
C-3	-116,269	-104,121	-116,18	-100,669	-115,63
C-4	-105,072	-101,212	-104,55	-114,549	-112,237
C-5	-114,721	-105,115	-111,49	-102,401	-100,521
C-6	-123,892	-111,53	-106,567	-111,525	-109,06
C-7	-106,008	-109,416	-117,041	-101,695	-101,162
C-8	-118,19	-106,767	-107,03	-109,951	-105,78

Table 2. SFI scores for Control Group

Mean SFI scores for each evaluation day (Table 3) were also compared with Mann-Whitney U test. The results on postop day 12 showed no statistically significant difference (p□: 0.294). On postop days 15, 18, 20 and 22 the HBO group SFI values were significantly higher than the control group (Table 3 & Graphic 1).

		Day 12	**Day 15**	**Day 18**	**Day 20**	**Day 22**
HBO Group	**Mean**	-118,58	-98,78	-98,30	-91,39	-91,61
	SD	10,58	7,99	4,89	7,63	3,04
Control Group	**Mean**	-114,89	-108,79	-112,78	-106,94	-108,23
	SD	6,67	5,54	6,38	5,41	5,95
	P	*0,294*	*0,012*	*0,001*	*0,004*	*0,001*

Table 3. Mean SFI scores and SD values and p values for both groups.



Electrophysiological study

The mean latency values gathered from compound field potential curve are shown in Table 4. The values were compared with Mann-Whitney U test and a statistical difference was not found for both Latency 1 and 2. (p□: 0.219 and p□: 0.562)

	LATENCY 1		LATENCY 2	
	HBO	**Control**	**HBO**	**Control**
Mean	0,01175	0,01371	0,06238	0,06700
SD	0,003991	0,004112	0,011160	0,006976
	p=0,219		*p=0,562*	

Table 4. Mean latency and p values for HBO and Control groups

Histo-pathological study

Upon histopathological evaluation, the exterior parts of the nerve were observed to be healing more effectively with less edema than the central parts. Also, HBO group nerves were observed to be less edematous. The axon counts of all animals are given in Table 5. These values were compared with Mann-Whitney U test and HBO group axons were found to be significantly greater. (p: 0.008).

HBO	504	349	410	398	378	407	590	445	433,71 ± 83,62	*p*
Animal	1	2	3	4	5	6	7	8	Mean ±SD	***0.008***
Control		282	210	230	280	287	285	267	285,75 ± 70,31	

Table 5. Average axon numbers of the groups

DISCUSSION

Nerve has an important role in functional recovery after an injury. Use of microsurgical repair methods improves outcomes, but even with optimal surgical techniques, results may not be entirely satisfactory (3). Tension is one of the most important factors causing delay and improper healing, and hypoxia and edema are considered chiefly responsible (4). HBO is a treatment modality that has been shown to be beneficial in hypoxic wounds. It is also reported to enhance nerve regeneration and to promote functional recovery in peripheral nerve repair. Based on these potential benefits, studying the effects of HBO on tense repairs at early stages appears reasonable. In our study, in order to create a tense repair model, we excised a 3 mm piece of nerve which causes 3.3 +/- 1.09 g of tension and corresponds to mild tension in rat sciatic nerve (4, 10). After treating one group with HBO for three weeks, we found that functional recovery was faster and axonal regeneration was better with HBO and we propose it as an alternative to grafting when mild tension is detected.

Generally, the animals treated with HBO were more active and appeared more comfortable during walking analysis. Until the 15th 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. This caused us to have improper footprints of this animal for the last two evaluations. During electrophysiological evaluation we noted that this animal's repair had failed and the nerve ends were apart. Therefore all the overall statistical analysis were done for only seven of the animals in the control group.

In a nerve study, there are several methods to evaluate functional recovery but gait analysis is considered to be the most accurate (9). Sciatic Functional Index which is commonly used to evaluate function, has been shown to be correlated to recovery more than other testing methods and is largely accepted as the gold standard of evaluating gait (11,12, 13). In our project, when all the SFI scores were compared, HBO group scores were significantly higher indicating that functional recovery was better in this group. Similarly, when each evaluation day's mean scores were compared, no difference was observed for the first evaluation day whereas HBO group scores were significantly higher for the remainder of evaluation days. Since conditions were the same for two groups with the exception of HBO as the only variable, the improvement can readily be attributed to the HBO therapy. This result is consistent with Zamboni et al's work in which they used SFI for gait analysis and found the scores to be higher with HBO 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 HBO 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 reorganization of them closest to 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, so the more the axons regenerate, the more the possibility that enough will reach to the distal (16, 17). Thus showing the regenerating axons through repair is also important. In our study, we found that the number of axons in the HBO group were significantly higher than in the Control group. This result suggests that HBO promotes axonal regeneration and is in line with other studies that show HBO increasing regeneration after a crush injury and grafting (6, 7, 9). On the contrary, in Bajrovic et al's study in which the sciatic nerve was crushed and made acellular, HBO didn't have any positive effect on axonal regeneration (18). This, we think, may be due 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 how it works. The most emphasized one is the antihypoxic effect of HBO. 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% axons can die due to apoptosis and it is triggered mostly by mediators released from mitochondria (19). Since mitochondrion is an oxygen dependant organelle, hypoxia may be causing these mediators release. So HBO, by raising oxygen tension in tissues, may break the hypoxia apoptosis cycle and help in resumption of viability and regeneration.

Another point that should be considered is HBO's enhancing effect on collagen synthesis (20). In a study where HBO's effect on tendon grafts was investigated, collagen synthesis and collagen density were found to be higher with HBO (21). Even though overproduction of collagen tissue causes scar tissue in the nerve in the course of 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 supporting tissue that must be strong, and that strength may be provided by a collagen network. In fact, some studies done with nerve conduits are based on this idea. 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 HBO, 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 HBO 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 cannot be established as statistical data.

The most challenging part of our study was the electrophysiological evaluation because of technical problems like constructing electrodes small enough to fit rat sciatic nerve or 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 difference between the electrophysiological results of two groups. Eguiluz-Ordonez, on the other hand, found significant difference in latencies of HBO and Control groups and this data was correlated to their other findings (5). However, all these recordings were not obtained from the nerve itself but from the muscle innervated by this nerve. Thus, studies like this are comparable to ours in terms of electrophysiology.

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 accepted as the technique of choice (4, 17, 23). This way tension is avoided but other injuries such as creation of a new wound, loss of sensation at the donor site may result (1, 17, 24). Along with these additional problems, an advantageous functional recovery could not be offered (1, 25). Nerve conduits are now seen as an alternative as the expected outcomes have not been reached with grafting (26). Nerve conduit is a kind of mediator that would work as the endoneural tube. Initially, muscle or vascular tissues were used as nerve conduits, but again many problems such as collapsing of the conduit or neuroma formation arose. Also, axonal growth would stop at some point since supporting tissue does not exist in these conduits (17). After conduits with biodegradable materials such as PLA Chitosan or collagen have been designed studies revealed satisfactory results (22, 26). However, these materials also have disadvantages; producing these at the desired size and placing the nerve ends in the

conduits are claimed to be challenging (17). High costs of these materials as well as potential neuroma formation are also problems that should be considered (1).

In clinical practice, tension after nerve injury is seen frequently because the injured ends are usually debrided and some retraction is inevitable (23). However, neither grafting nor newly developing methods are functionally perfect and problem-free. Since primary end to end repair is known to be better when possible, we think HBO allows for the use of primary repair even some tension is foreseen. Likewise it can be a good adjuvant when tension is detected after the repair. Clinical studies should be performed as it has been shown to promote healing in animal studies.

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