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Hyperbaric oxygen treatment in the experimental spinal cord injury model

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Abstract

BACKGROUND CONTEXT: Spinal cord trauma is a major cause of mortality and morbidity. Although no known treatment for spinal cord injury exists, a limited number of effective treatment modalities and procedures are available that improve secondary injury. Hyperbaric oxygen (HBO) treatment has been used to assist in neurologic recovery after cranial injury or ischemic stroke.

PURPOSE: To report the findings on the effectiveness of HBO treatment on rats with experimental traumatic spinal cord injury. Improvement was evaluated through motor strength assessment and nitrite level assay testing.

STUDY DESIGN: We randomly distributed 40 rats among 5 groups of 8 rats each: sham incurable trauma, induced trauma, HBO treatment begun at the 1st hour, HBO treatment begun at the 6th hour, and HBO treatment begun at the 24th hour.

METHOD: The HBO treatment was administered to rats in three of the groups and conducted in two 90-minute sessions, under an absolute atmospheric pressure of 2.4 at 100% oxygen for 5 days. In the motor strength evaluations, all the rats were observed during the inclined plane test and clinical motor examination on the first, third, and fifth days. In addition, the nitrite levels of spinal cord tissues on the sixth day were also studied.

RESULTS: Results from the inclined plane levels and motor strength test from all the three groups undergoing HBO treatment were higher than those from Group 2. It was also determined that early HBO treatment resulted in higher recovery rates (groups 3 and 4). The highest levels were seen in the group in which the HBO treatments were started in the first hour (Group 3). It was noted that nitrite levels of rats in the group exposed to trauma increased, compared with the sham group, but increased levels also diminished after HBO treatments. Again, the greatest decrease in nitrite levels was evident in the group where the HBO treatment was started the earliest (Group 3).

CONCLUSIONS: Prompt HBO treatment after trauma significantly contributed to the clinical, histopathologic, and biochemical recovery of the rats.

Keywords: Hyperbaric oxygen treatment; Spinal cord injury; Rat; Nitrite; Experimental cord injury; Secondary mechanisms

Introduction

The spinal cord is unable to regenerate itself. Permanent damage occurs in patients who have experienced spinal cord injuries (SCIs) [1,2]. There are two mechanisms that increase the risk of further damage in SCIs [3–6]: primary mechanical injury and secondary ischemic injury. Mechanical harm occurs at the time of the event. These

FDA device/drug status: Approved (Ketamine, Xylazine, and Sefotaxim).

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injuries cause damage to the nerves, the spinal cord itself, and/or spinal vascular structures [7]. Secondary injury is the harm sustained by metabolic and biochemical factors occurring within hours after the primary injury [8,9]. Ischemia is one of the most significant factors leading to secondary injury with the chief problem in the early periods being inadequate perfusion [5,10]. Energy failure after the ischemia leads to a decrease in adenosine triphosphate levels, followed by the commencement of anaerobic respiration. Fehlings and Tator [1] state that ischemia after trauma constitutes the basis of secondary injury, but ischemia is a treatable and revocable process. The primary purpose of all experimental and clinical studies on traumatic spinal cord injury is to reduce secondary injury.

Although the total number of pathologic mechanisms caused by SCIs is not precisely known, processes, such as nitric oxide (NO) accumulation, resulting from increases in both calcium and free radicals are mentioned [7,11–16]. Lipid peroxidation is considered to be the principal cause among them [17]. These oxidative processes begin as a result of increases in hydrogen peroxide, superoxide ions, and NO, which cause oxidative damage to lipids, nucleic acids, and proteins, with the destructive potential of these free radicals increasing further as they raise endogenous antioxidants in the body [18]. Of these free radicals, NO is a primary molecule that plays a key role in many physiological processes such as vascular tonus regulation, thrombocyte functions, neuronal communication, and body defense [19]. Nitric oxide and its metabolites are reported to increase, especially in inflammatory and infectious conditions. It is understood that excessive NO production in cerebral ischemia and epilepsy leads to neurotoxicity [20]. Nitric oxide is primarily an unstable gas, which rapidly turns to nitrite, nitrate, and peroxynitrite compounds [20,21]. Because its half-life of metabolization is so short, tissue levels are difficult to determine. Levels of stable nitrite and nitrate end products may indirectly affect decisions regarding NO tissue levels. This method was used, for years, to determine NO tissue levels especially in experimental ischemia and reperfusion studies [20].

Hyperbaric oxygen (HBO) is one possible treatment and support approach after, or before, secondary injury and is administered in a closed pressure chamber, under pressure higher than one atmosphere allowing the patient to breathe pure oxygen through an oxygen mask, respiration cap, oxygen tent, or endotracheal tube. This is used to contribute to neurologic recovery after brain injury and cerebral ischemia [20,22–25]. The first experimental study in which HBO treatment was administered to spinal traumas was reported by Maeda [26]. Hyperbaric oxygen use for therapeutic purposes was seen for the first time in the study by Hartzog et al. [27].

Within the scope of our study, trauma and sham groups were set up to determine the rats' motor strength and nitrite levels in tissues after experimental rat spinal cord trauma. To reduce spinal cord ischemia, HBO treatment was administered at the 1st, 6th, and 24th hour after trauma. Differences among the groups were compared by the measurements taken from nitrite levels, motor strength evaluations, and histopathologic examinations of tissues, based on a tissue destruction scale.

Materials and methods

Surgical operations within the scope of the study were performed in research laboratories on experimental animals at the Aegean University Hospital and Celal Bayar University Hospital, with permission given by the University of Dokuz Eylul Ethics Committee of Experimental Animals, reference number 23/2009. In total, 40 Sprague-Dawley rats (8 rats in each of the 5 groups) were used for the study. In each group, the rats weighed between 200 and 250 g and were grouped randomly to form five groups (Table 1) as follows:

- Group 1: The Sham group—laminectomy only. No trauma or HBO treatment administered.
- Group 2: The trauma group—laminectomy and SCI.
- Group 3: Laminectomy, SCI, and HBO treatment administered in the first hour after trauma.
- Group 4: Laminectomy, SCI, and HBO treatment administered in the sixth hour after trauma.
- Group 5: Laminectomy and SCI, with HBO treatment administered in the 24th hour after trauma.

Anesthesia

All rats in the groups that underwent surgeries were injected with 2 mg/kg ketamine HCl (Ketalar; Parke-Davis, Eczacıbaşı, Istanbul) plus xylazine intraperitoneally for general anesthesia.

Surgery

Intramuscular Sefotaxim (Bilim Pharmaceuticals, Beyoğlu, Istanbul) of 40 mg/kg was prophylactically given, 30 minutes before the operation. Rats were positioned prone on a fixing table. Thoracic areas were first sterilized with PVD (Batticon solution; Adeka-Samsun) and shaved.

Table 1
Number of animals in each group

Groups	Number of rats
Group 1 (control group)	8
Group 2 (trauma group)	8
Group 3 (HBO treatment started at the first hour)	8
Group 4 (HBO treatment started at the sixth hour)	8
Group 5 (HBO treatment started at the 24th hour)	8

HBO, hyperbaric oxygen.