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Effect of VEGF and CX43 on the promotion of neurological recovery by hyperbaric oxygen treatment in spinal cord–injured rats

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Abstract

BACKGROUND CONTEXT: Spinal cord injury (SCI) is a serious health issue that may result in high health care costs, with additional social and psychological burdens. Hyperbaric oxygen (HBO) treatment has been found to be beneficial for neurological recovery; however, the underlying mechanisms are yet to be characterized.

PURPOSE: The aim of this study was to investigate the mechanisms of HBO treatment in SCI by measuring the expression levels of vascular endothelial growth factor (VEGF) and Connexin43 (CX43) in the injured spinal cord tissue.

STUDY DESIGN/SETTING: An experiment animal study of rats undergoing SCI and HBO treatment.

METHODS: The spinal cord injury model was established in rats, which were randomly divided into the following four groups: (1) the sham-operated group (SH), (2) the sham-operated and hyperbaric oxygen treatment group (SH+HBO), (3) the spinal cord injury group (SCI), and (4) the spinal cord injury and hyperbaric oxygen treatment group (SCI+HBO). For groups of SH+HBO and SCI+HBO, the animals received 1 hour of HBO at 2.0 ATA in 100% O2 twice per day for 3 days and then daily for the following days consecutively after surgery. After operation, neurological assessments were performed, the spinal cord tissue samples were harvested for histopathological evaluation, Western blot and real-time polymerase chain reaction analysis.

RESULTS: The Basso-Bettie-Bresnahan scores were significantly improved in the SCI+HBO group compared with the SCI group on the postoperative 7th and 14th days. The histology scores were significantly decreased by HBO treatment compared with that in the SCI group on the postoperative 3rd, 7th, and 14th days. Western blot analysis and real-time polymerase chain reaction revealed that the expression level of vascular endothelial growth factor (VEGF) in the SCI+HBO group was significantly increased compared with the SCI group. The protein expression level of CX43 and its mRNA level in the SCI+HBO group were significantly decreased on the postoperative 3rd and 7th days, whereas its expression was significantly increased by HBO treatment on the postoperative 14th day compared with the SCI group.

CONCLUSIONS: HBO treatment improved neurological recovery when applied after SCI. The expression level changes of VEGF and CX43 may contribute to the further

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understanding on the molecular mechanisms of HBO treatment on SCI.

Keywords: Connexin43; Hyperbaric oxygen treatment; Spinal cord injury; Vascular endothelial growth factor

Introduction

Spinal cord injury (SCI) is a devastating condition for the individual patient and costly to society as a whole by requiring substantial long-term health-care expenditures. Trauma to the spinal cord causes both primary and secondary injury. Primary injury to the spinal cord immediately disrupts cell membranes, destroys myelin and axons, and damages microvessels, thereby triggering devastating secondary injuries [1]. Secondary injury refers to a complex array of pathophysiological processes, including ischemia, edema, local inflammation, production of free radicals, and hyperoxidation [2,3]. The end result of SCI is that some of the neurons and glial cells die days or weeks after spinal cord injury as the result of the secondary injury, even though they survived from the initial injury [4,5].

Numerous spinal cord protection methods, including distal aortic perfusion, reattachment of critical intercostal arteries, hypothermia, and administration of various pharmacologic agents, have been suggested to minimize the devastating complication of SCI. However, applications of these techniques had only decreased but not eliminated postoperative spinal cord dysfunction, thus further investigations are necessary to improve the treatment of SCI [6,7]. Hyperbaric oxygen (HBO) therapy is a medical treatment that administers 100% oxygen at a controlled pressure (greater than sea level) for a prescribed period of time (60–90 minutes). Because of the capability of increasing tissue oxygenation, HBO treatment raises the tissues' tolerance to ischemia and corrects the metabolic disorders apparent in the ischemic tissue. Recent reports claimed that HBO treatment is beneficial for neurological recovery in acute and chronic SCI [8,9], but the underlying mechanism needs to be further characterized.

In the central nervous system (CNS), vascular endothelial growth factor (VEGF) plays a pivotal role not only in vascularization, but also in neurotrophic, neuronal proliferation, and the growth of coordinated vascular and neuronal networks [10]. Gap junctions are intercellular channels that are integral for the functioning of the glial network. They allow the passage of ions, metabolites, and second messengers between neighboring cells [11]. The primary structural unit of a gap junction is a membrane protein termed connexin. Connexin43 (CX43) is the predominant protein for the formation of gap junctions in the CNS and is expressed primarily on astrocytes, activated microglia, developing neurons, the smooth muscle, and endothelial cells of blood vessels. CX43 is an important mediator of CNS injury [12]. In this study, we attempted to explore the possible mechanism of HBO treatment in SCI by measuring the expression levels of VEGF and CX43 in the injured spinal cord tissue.

Materials and methods

Animal care

Healthy adult male Sprague-Dawley (SD) rats, weighing 250 to 300 g at the beginning of the study, were kept two per cage for at least 5 days after their arrival at our laboratory. The rats had access to food and water ad libitum and were housed within a room with a 12:12-hour light/dark cycle. This study was performed in accordance with the ethical guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Capital Medical University (Beijing, China).

SCI model

The SCI was performed aseptically under anesthesia using intraperitoneal injections of 10% chloral hydrate at a dose of 350 mg/kg. Following the method described by Basso et al. [13], the rats were positioned prone on the operating table, the back skin of rats was shaved and disinfected with povidone-iodine solution, and an incision was made longitudinally extending from the mid to low thoracic regions, followed by a laminectomy, including all T10, to expose the spinal cord. The MASCIS (Multicenter Animal Spinal Cord Injury Study) impactor was used to produce the SCI. The moderate SCI was created by dropping the10-g rod from a distance of 25 mm. The sign of successful model: the wagging tail reflex in rats, retraction of the lower limbs and body flutter, flaccid paralysis of both lower extremities. The fascia and skin were closed with sutures for each layer, and animals were allowed to recover from anesthetic and surgical procedures in intensive care. Postoperatively, the bladder was compressed by manual abdominal pressure twice a day until bladder function was restored. All rats received a single dose of subcutaneous injection of penicillin sodium 0.8 mg/g per day until the hematuria disappeared.

Experimental groups

Ninety-six rats were randomly assigned to one of the following four groups (each group n=24): (1) the sham-operated group (SH), (2) the sham-operated and hyperbaric oxygen treatment group (SH+HBO), (3) the spinal cord injury group (SCI), and (4) the spinal cord injury and hyperbaric oxygen treatment group (SCI+HBO). Rats in each group were randomly divided into four small groups in a time-dependent manner again (1 day, 3 days, 7 days, and 14 days after surgery, each group n=6). The rats in the SH group solely went through laminectomy, and did not accept SCI or HBO treatment.