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Hyperbaric Oxygen Therapy in Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Abstract

Traumatic brain injuries (TBI) are among the leading causes of death and chronic disability worldwide. TBI is a complex process encompassing primary injury to the brain tissue and cerebral vasculature induced by the initial impact, secondary injury, including cascade of subsequent neuroinflammatory processes, and regenerative responses with enhanced neurogenesis and angiogenesis. To date, there remains no approved pharmacological therapy that is able to prevent the secondary injury. Therefore, the development of safe and efficacious neuroprotective treatments currently represents the greatest unmet need in the management of TBI. Increasing number of experimental and clinical studies present convincing evidence that hyperbaric oxygen therapy (HBOT), as an adjunctive therapy, may be the suitable neurotherapeutic method for improving neurological outcome after TBI. Irrespective to treatment protocol HBOT appeared to alleviate the detrimental and neurotoxic effects of pathological sequel initiated by TBI and to stimulate endogenous reparative mechanisms. However, the exact mechanisms by which HBOT exerts its beneficial effects on recovery after brain injury are still deficient. In this review we will summarize up to date results of HBOT in experimental and clinical TBI and try to put more light on cellular and molecular mechanisms underlying beneficial effects of HBOT on functional recovery after brain injury.

Keywords: hyperbaric oxygen therapy, traumatic brain injury, neuroprotection, oxidant/antioxidant balance, oxidative stress, anti-apoptosis, anti-inflammation, neuronal plasticity, synaptogenesis, neurogenesis, angiogenesis

1. Introduction

Traumatic brain injuries (TBI) are one of the leading causes of death and chronic disability especially among the working population, and represent an important public health problem worldwide. Globally, about 10 million people are affected by TBI every year with projections that TBI will be one of the major causes of death and disability by the year 2020 [1]. Since TBI is a complex injury that encompasses a broad spectrum of symptoms and disabilities, the manifestations of head injury may be clinically very variable ranging from mild, to moderate or severe, depending on the extent and duration of damage to the brain. Many cognitive, physical and psychological skills can be affected, exerting a devastating impact on the patients and their family [2]. Over the years more than 30 phase III clinical trials failed emphasizing the urgent need for efficient treatment modalities and new directions in the future research to improve posttraumatic morbidity and mortality. Considering the complexity of TBI it is reasonable to assume that only combination of different treatment protocols could provide better prognosis for recovery to all forms of TBI [3]. In this view, hyperbaric oxygenation (HBO) or hyperbaric oxygen therapy (HBOT) appeared as an adjunctive therapy that may have the synergistic effect with other treatment protocols, suggesting that combining therapies with HBOT could provide better results than either alone [4]. According to definition given by the Undersea and Hyperbaric Medical Society (UHMS), hyperbaric medicine is a therapeutic approach in which a patient breathes 100% oxygen intermittently, while the pressure of the treatment chamber is higher than ambient (1 atmosphere absolute, 1 ATA = 101.3 kPa) [5–7]. In comparison to the normobaric conditions increased oxygen supply under hyperbaric conditions enables easier diffusion of oxygen into the injured tissue [8]. Accordingly, the HBOT can be used to obtain 100% saturation of hemoglobin and to significantly elevate the volume of physically dissolved oxygen fraction in blood plasma. This increased blood oxygen level then can penetrate to ischemic areas and perilesioned tissue more deeply than under normobaric conditions [9–11]. Thus, the HBOT has found its place, as the primary or adjuvant therapy in the treatment protocols for different clinical conditions [12, 13].

On the other hand, opinions about usage of HBOT as adjunctive therapy for the treatment of patients with brain injuries are still controversial [14–16]. In this way HBO is a very motivating therapeutic modality, which is known to produce oxidative stress by itself [17], but reduces oxidative stress when used in pathological conditions [18, 19]. The main concern in HBOT is oxidative stress and/or oxygen toxicity that can affect multiple organs. However, these side-effects are dependent on treatment parameters – pressure and duration of the treatment [20–23].

Substantial amount of evidences has been published indicating that HBOT can interfere with the processes that are following brain injury and moderate its consequences [14, 24–27]. Recent results of experimental and clinical studies and potential mechanisms of HBOT in TBI are reviewed by Wang et al. [28] and Hu et al. [7]. However, knowledge about the exact mechanisms by which HBOT exerts its beneficial effects is still deficient. Therefore, data presented in this chapter are meant to put more light on cellular and molecular mechanisms underlying neuroprotective effects of hyperbaric oxygenation after the brain injury.

2. Potential cellular and molecular mechanisms underlying HBOT

Increasing number of animal studies on HBOT in experimental TBI revealed a myriad of diverse mechanisms that may underlie neuroprotective effects of HBOT. Researchers suggested that many of these cellular and molecular mechanisms and signaling pathways work in parallel, or together, contributing to repair of the injured brain [6, 7, 23, 25, 28]. These mechanisms involve: (1) alleviation of secondary injury; (2) increasing of tissue oxygenation; (3) reducing of neurodegeneration; (4) decreasing of apoptosis; (5) regulation of oxidant/antioxidant status; (6) reduction of oxidative stress; (7) attenuation of reactive gliosis (microgliosis and astrogliosis) and glial scarring; (8) reducing of inflammation; (9) enhancement of neuronal plasticity; (10) promoting of synaptogenesis, neurogenesis and angiogenesis.

2.1. HBOT suppresses development of secondary brain damage

TBI involves primary and secondary injury. Primary injury occurs at the time of the impact and is the result of immediate mechanical damage of neural pathways followed by a permanent neuronal loss. The site of mechanical impact is called the “core”. Surrounding regions consist of neuronal tissue that have not been directly affected by trauma and are often addressed to as “penumbra area”. Neurons inside this zone are at risk due to a cascade of events, known as secondary injury that involves: impaired blood flow (limited or not at all), inflammation, development of edema, acidosis and hemorrhage, and the loss of most of their connections with the other neurons [11, 21, 22]. Secondary degeneration can also progress into the surrounding intact regions of the brain. Compromised blood flow and insufficient oxygen supply leads to tissue hypoxia and the resulting energy failure, which initiates a cascade of cellular events that culminate with neuronal cell apoptosis [23]. Thus, the consequence of secondary injury is degeneration of neurons that previously have not been exposed to trauma [29–31]. Most of the neurotherapeutic strategies are directed toward the containment of the secondary processes and the preservation and reactivation of the penumbra area and perilesioned region [30]. Cumulative evidence have proved that HBOT may reduce development of secondary brain damage and prevent neuronal apoptosis in animal models of TBI [32, 25], ischemic stroke [33–37], and hypoxia-ischemia [38–40], which was manifested by diminishing of brain infarction area and improvement of neurological deficits. Recently, Baratz-Goldstein et al. [41] demonstrated that both immediate (initiated 3 h post-injury) and delayed treatments with HBO (initiated 7 days post-injury) have a potential to prevent a neuronal loss in mouse model of moderate TBI.

2.2. HBOT reduces neuronal degeneration and prevents apoptosis after brain injury by regulation of oxidant/antioxidant status and reduction of oxidative stress

One of the main processes in this pathological cascade is oxidative stress that develops in the cells which have been exposed to trauma and in the cells at “penumbra area”. Reactive oxygen species (ROS) are one of the products of oxidative stress [2] that are responsible for cellular damaging and apoptosis. The first line of the defense against ROS are enzymes located in mitochondria, such as manganese superoxide dismutase (SOD2) [42]. In our previous study, we have shown that repetitive HBOT influenced the pattern of SOD2 expression both on gene and protein level in

cortical stab injury model (CSI) of TBI [43]. We applied HBO protocol of 60 min exposure to 100% oxygen at 2.5 ATA, once a day for 3 or 10 consecutive days. HBOT significantly increased mRNA levels of SOD2 at both time points compared to the corresponding lesioned group. Exposure to HBOT for 3 days down-regulated SOD2 protein levels in the injured cortex, while after 10 days of HBOT an up-regulation of SOD2 was observed. Using double-immunofluorescence staining we have demonstrated that HBOT attenuated SOD2 expression both in neuronal and astroglial cells surrounding the lesion site. Staining of the injured cortex with Fluoro-Jade®B (as a marker of degenerating neurons) revealed that HBOT significantly decreased the number of degenerating neurons in the injured cortex, and this effect was more pronounced after 10 consecutive HBOT. In according to this, we concluded that antioxidative and neuroprotective effect of HBOT is in part due to its influence on expression pattern of SOD2 [43].

In this chapter, using the cortical suction ablation (CSA) model of brain injury, described in our previous publications [44, 45], and the same HBOT protocol, we demonstrated that 10 repetitive HBOT altered activities of antioxidant enzymes and reduced lipid peroxidation, thereby preventing neuronal degeneration and apoptosis. Oxidant/antioxidant status in the injured cortex after HBOT is presented in **Figure 1**. HBOT significantly increased glutathione-peroxidase (GPX) activity in the injured cortex compared to all other groups. Injury markedly lowered the level of superoxide dismutase (SOD) activity, while HBOT returned SOD activity to almost control levels. The content of Malondialdehyde (MDA), which was used as an indicator of lipid peroxidation and reflects the membrane damage caused by ROS, was the highest in the tissue samples from injured cortex. HBOT initiated statistically significant reduction of MDA levels, pointing to preservation of membrane integrity. The similar trend of changes of MDA levels was determined in the serum indicating that serum concentrations of MDA may be used as a marker of degree of brain damage.

To evaluate the effect of HBOT on neurodegeneration/apoptosis we performed double-immunofluorescence staining: neurons undergoing degeneration were visualized with Fluoro-Jade®B, while NeuN (neuronal cell nuclei) was used as a marker of neuronal cell bodies. As it is shown in **Figure 2**, in the perilesioned cortex a huge number of neurons undergoing degeneration (**Figure 2A, E**) was significantly reduced after the HBOT (**Figure 2B, F**). Moreover, when the cortical sections from the injured (L) group were observed at higher magnification the formation of apoptotic bodies (**Figure 2G, I**, asterisks) in the neuronal nuclei was observed, indicating that they have entered into the process of apoptosis. On the contrary, in the sections from LHBO group the majority of neurons have healthy nuclei in which the nucleolus was clearly visible (**Figure 2H, J**, arrow heads). These results indicate that increased activities of antioxidant enzymes and reduction of lipid peroxidation underlies observed neuroprotective and anti-apoptotic effects of HBOT. Similarly, Li et al. [51], in the rat model of brain ischemia-reperfusion injury (IRI), have shown that HBO preconditioning lessened neuronal injury, reduced the level of MDA and increased the antioxidant activity of catalase (CAT) and SOD. They suggested that an up-regulation of the antioxidant enzyme activities after HBO preconditioning may play an important role in the generation of tolerance against IRI.

Maintaining proper mitochondrial function is essential for cellular function, since ROS are formed in mitochondria when energy metabolism is compromised. Niizuma and co-workers [52] demonstrated that mitochondrial dysfunction and oxidative stress may determine neuronal

Figure 1. Effects of HBOT and cortical injury on the activities of antioxidant enzymes and lipid peroxidation in the injured cortex. Glutathione-peroxidase (GPX) activity is measured using coupled enzyme method by measuring the decrease of NADPH at 340 nm [46] and is expressed as unites per milligram of protein (U/mg). One unit (U) catalyzes the oxidation by H_2O_2 of 1.0 μ mol of reduced glutathione to oxidized glutathione per minute. Total superoxide dismutase (SOD) activity is determined at room temperature according to the method of Misra and Fridovich [47], and is measured at 480 nm. One unit of SOD is defined as the amount of enzyme that inhibits the speed of oxidation of epinephrine for 50%. The results are expressed as U/mg of protein. Malondialdehyde (MDA) content is determined both in the injured cortex and serum. Thiobarbituric acid (TBA) reactive product MDA is used as an indicator of lipid peroxidation. MDA content is measured according to the method of Aruoma et al. [48] as described previously in Mladenovic et al. [49]. The absorbance of the organic phase is read at 532 nm. The values are expressed as nmol of MDA/mg of protein, using a standard curve of 1,1,3,3-tetramethoxypropane. Total protein was quantified according to Lowry's method [50] using bovine serum albumin as standard. Values are mean $\pm$ SD from $n \geq 4$ independent determinations performed in duplicate. Acronyms for the groups ($n = 8$ per group) are as follows: C – intact control, CHBO intact control subjected to the HBO protocol, S – sham surgery, SHBO sham control HBO group, L - injured group and LHBO - injured group subjected to the HBO protocol. Significant difference from corresponding group * $P < 0.05$ vs. L, # $P < 0.05$ vs. C, † $P < 0.05$ vs. CHBO, ‡ $P < 0.05$ vs. S, § $P < 0.05$ vs. SHBO.

death/survival after stroke and neurodegeneration. Therefore, a lot of studies have been conducting with purpose of finding out whether HBOT have a role in the preservation of mitochondrial function and integrity [25, 32, 53–55]. Palzur, Vlodavsky and their colleagues,

Figure 2. HBOT reduces neurodegeneration and prevents apoptosis in the injured cortex. Fluoro-jade®B staining (green) is performed in order to visualize neuronal cells undergoing degeneration and cell death, while NeuN (red) is used as a marker of neuronal cell bodies. Procedure for double-immunofluorescence staining was as described in Parabucki et al. [43]. Cortical sections were incubated with mouse anti-NeuN (1:200, Milipore, USA) and then with 0.0004% solution of Fluoro-Jade®B (FJB, Chemicon International, Temecula, CA, USA) dissolved in 0.1% acetic acid. The slides were examined with Carl Zeiss AxioVert microscope with AxioCam monochromatic camera (Zeiss, Goettingen, Germany), equipped with ApoTome software for optical sectioning. (A, C, E) A huge number of NeuN⁺ neurons in the perilesioned cortex were co-stained with FJB (A, yellow fluorescence) indicating that they are undergoing neurodegeneration. Strikingly, when the cortical sections from the injured (L) group were observed at higher magnification, the formation of apoptotic bodies (G, I, asterisks) in the neuronal nuclei was observed, suggesting that they have entered into the process of apoptosis. (B, D, F) after 10 repetitive HBOT the number of NeuN⁺/FJB⁺ (B) and FJB⁺ (F) neurons was negligible. Correspondingly, in the LHBO cortical sections the majority of neurons have healthy nuclei in which the nucleolus was clearly visible (H, J, arrow heads). Rectangles indicate where the high magnification images are taken from. Scale bars: (A-J) 50 μ m.

using cortical deformation model of TBI and the HBO protocol which consisted of two successive 45 min sessions at 2.8 ATA, have shown that increased concentrations of oxygen in the cells lead to preservation of mitochondrial integrity due to significant decrease of the loss of mitochondrial trans-membrane potential. Additionally, HBOT reduced the release of pro-apoptotic mediators Cytochrome C (Cyt C) and the Bcl-2-associated X protein (Bax) from mitochondria, and up-regulated the expression of anti-apoptotic protein Bcl-2 (B-cell lymphoma 2), consequently alleviating neuronal apoptosis in the injured brain tissue [56]. Zhou et al. [57] have emphasized that maintenance of mitochondrial function is one of the most important effects of HBOT.

TBI leads to the impairment of cerebral oxygen delivery and consumption [58]. So, the main problem is how to make cerebral hyperoxia, which is possible either under normobaric (NBH) or hyperbaric conditions. Clinical trials have shown that hyperbaric O₂ has better effect than NBH on oxidative cerebral metabolism due to its ability to produce a brain tissue PO₂ > or = 200 mm Hg, which represents a graduated effect [14].

Oxidative stress, and/or oxygen toxicity as unwanted side-effects of HBOT, as well as the fact that inhalation of pure oxygen at high pressures may lead to generation of ROS led researches to investigate which anti-oxidants can be used during HBO therapy. Studies have shown that hydrogen gas (H₂) could be useful for this purpose. It alleviates oxygen toxicity due to reduction of hydroxyl radical levels [59]. Even, adding of inert gases, such as argon or xenon during HBO treatments can potentially make further improvement of cerebral lesions [60].

2.3. HBOT attenuates reactive microgliosis, astrogliosis and glial scarring after brain injury

After the injury astrocytes become rapidly activated during the process of “reactive astrogliosis” and accumulate around the lesion site, acting as a barrier that impedes neuroregeneration and neurite outgrowth, and isolates intact CNS tissue from secondary lesions [61, 62]. Proportionally to the severity of injury, they undergo cell proliferation, hypertrophy, increased expression of glial fibrillary acidic protein (GFAP) and vimentin, and exhibit an enhancement of immune-modulating capacities [2, 44, 63–66]. In our recently published paper [66] we have shown that repetitive HBOT attenuates reactive astrogliosis, prevent glial scar formation, and down-regulates GFAP and vimentin gene, protein and tissue expression in the perilesioned cortex. Similarly, Baratz-Goldstein et al. [41] demonstrated that both immediate and delayed HBOT have a potential to reduce reactive astrogliosis in mice model of TBI. Here, we reported that HBOT reduces reactive microgliosis around the lesion site as well (**Figure 3B**). Besides decreasing the number of activated microglia, HBOT also alters the morphology of activated microglia to more ramified, resting form (**Figure 3B** upper rectangle, **D** inset, **F** inset).

HBO-induced suppression of microgliosis and astrogliosis was reported to give an account to beneficial effects of HBO treatment in different rat models of TBI [41, 67, 68], cerebral ischemia [69], neuropathic and inflammatory pain [70, 71]. In contrast, Lee et al. [72] reported that prolonged HBOT may increase degree of gliosis indicating that longer oxygen cycling might help overcoming detrimental effects of gliosis and providing its beneficial effects.

Figure 3. HBOT reduces reactive microgliosis in the perilesioned cortex and down-regulates ICAM-1 expression on microglial cells. (A) and (B) Effect of HBOT on reactive microgliosis in the injured cortex is determined using mouse anti-CD68 antibody (ED1, 1:100, Abcam, Cambridge, MA, USA) as a marker of activated microglia/macrophages. After using appropriate peroxidase linked secondary antibody (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), the products of immunoreactions were visualized with 3'3'-diaminobenzidine (DAB, Dako, Glostrup, Denmark). Immunohistochemical and immunofluorescence staining was performed as described in Lavrnja et al. [66]. (A) after cortical injury a huge number of activated microglia/macrophages are seen around and within the lesion site. (B) HBOT (60 min exposure to 100% oxygen at 2.5 ATA) initiated daily for 10 consecutive days attenuated reactive microgliosis and alters morphology of activated microglia to more ramified, resting form (B, upper rectangle). (C–H) expression of ICAM-1 (green) on microglial cells stained with Iba1 (red) in cortices of injured (L) and injured group subjected to HBO protocol (LHBO) is visualized with immunofluorescence double-labeling. Cortical sections were incubated with goat anti-ICAM1 (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and mouse anti-Iba1 (ionized calcium binding adaptor molecule 1, 1:500, Abcam, Cambridge, MA, USA) antibody, while nuclei were counterstained with DAPI (Invitrogen, Grand Island, NY, USA). All sections were photographed with Carl Zeiss Axiovert microscope with AxioCam monochromatic camera (Zeiss, Goettingen, Germany), equipped with ApoTome software for optical sectioning. (C, E, G) activated microglia characterized with round morphology showed up-regulation of ICAM-1 expression (G). (D, F, H) repetitive HBOT downregulated ICAM-1 expression on activated microglia within the lesion site. Microglial cells with ramified morphology (insets to D and F) do not express ICAM-1 (insets to D and H). Rectangles indicate where the high magnification images are taken from. Scale bars: (A, B) 50 μm (C–H) 5 μm .

2.4. HBOT prevents spreading of the neuroinflammation in the injured tissue

Inflammation is an important part of the pathophysiology of TBI and has a pivotal role in the extent of neuronal injury and repair. It is postulated that the initiation, progression and resolution of inflammation in TBI is multifaceted. These processes involve migration, recruitment and infiltration of leukocytes following blood-brain barrier (BBB) disruption and activation of resident immune cells of the CNS (microglia, astrocytes). Microglia and astrocytes then acquire immunological function and secrete inflammatory mediators such as pro- and anti-inflammatory cytokines, chemokines, adhesion molecules, complement factors, ROS and other factors [58, 73, 74]. The accumulation of neutrophils around the site of injury and their infiltration into the injured brain area is crucial for the initiation and progression of inflammation and the extent of secondary brain damage since they may release free oxygen and nitrogen radicals and pro-inflammatory cytokines [54, 58]. Neutrophils initially attach to vascular endothelium via binding to the endothelial intercellular adhesion molecules (ICAMs). In our previously mentioned paper [66] we have demonstrated injury-induced increase of gene and tissue expression of ICAM-1 (Intercellular Adhesion Molecule-1, CD54), an adhesion molecule that is important for trans-endothelial migration of neutrophils and propagation of inflammation [75, 76]. Using double-immunofluorescence staining we demonstrated its localization on various type of cells (astrocytes, vascular endothelium, neurons, activated microglia/macrophages and neutrophils), around the blood vessels, and in the proximity and within the lesion site. Ten successive treatments with HBO significantly decreased ICAM-1 mRNA expression returning it to control levels, while increased ICAM-1 immunoreactivity around the lesion site was diminished [66]. Herein, using double-immunofluorescence staining we have shown that HBOT reduced expression of ICAM-1 on activated microglia within the lesion site (**Figure 3H**). Furthermore, HBOT increased number of ramified/resting microglia in the perilesioned cortex (**Figure 3B**, upper rectangle). Interestingly, they do not express ICAM-1 (**Figure 3H**, inset). These data indicate that HBOT by reducing ICAM-1 expression and targeting the passage of immune cells through the BBB via inhibition of cell adhesion molecules may contribute to dampen the neuroinflammatory response to TBI. Several studies also have shown that HBOT reduces the expression of ICAM-1 and adhesion of neutrophils to the endothelium, which is correlated with improved neurological outcome [54, 77–80].

CD40 ligand (CD40L, also termed CD154, or GP39) and its counter receptor CD40 (a membrane protein that belongs to the tumor necrosis factor (TNF) receptor family) are well-known regulators of pro-inflammatory and immune responses in the CNS [81], and are members of CD40/CD40L/ICAM-1 deleterious cascade of events after TBI. Given that CD40/CD40L dyad fosters neuroinflammation, it is suggested that CD40/CD40L interaction may be involved in modulating the outcome from injuries of the brain [82–84]. Accordingly, strategies aimed at suppressing CD40/CD40L/ICAM-1 expression may attenuate inflammation and neuronal damage after TBI, which will ultimately be of benefit in recovery [85]. In our recent paper [66] we have for the first time shown that HBOT prevents injury-induced up-regulation of expression of CD40 and its ligand CD40L on microglia/macrophages, neutrophils, cortical neurons and reactive astrocytes. These results indicate that repetitive HBOT, by limiting expression of inflammatory mediators, supports formation of more permissive environment for repair and regeneration.

Data of many studies has been shown that HBO suppress various mediators of inflammation [54, 67, 86, 87] indicating that the decreased brain edema, blood-brain barrier leakage, cell

apoptosis and improved neurological outcome are closely related to the inhibitory effect of HBOT on inflammation after TBI [7]. During the early stage of TBI, effect of HBOT in reducing inflammation was achieved by increasing anti-inflammatory cytokine interleukin-10 (IL-10) and transforming growth factor- β 1 (TGF- β 1) expression, decreasing of the RNA and protein levels of caspase-3, interleukin-8 (IL-8), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), macrophage inflammatory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1) and transforming growth-interacting factor (TGIF), as well as via reduction of the expression of matrix metalloproteinase-9 (MMP9) [28, 54, 67, 68, 88–90]. Recently, Geng et al. [91] demonstrated that HBOT suppressed protein expression of inflammasome components and reduced the levels of interleukin-1 β (IL-1 β), interleukin-18 (IL-18) and high-mobility group box 1 (HMGB1) protein in the injured brain tissues and serum. Based on these results authors assumed that HBOT may diminish the inflammatory response after TBI by inhibiting the activation of inflammasome signaling. Latest results of Meng et al. [86] showed that HBOT significantly increased the expression of nuclear factor (erythroid-derived 2)- related factor 2 (Nrf2) and heme oxygenase-1, and inhibited the expression of Toll-like receptor 4 and nuclear factor-kappa B in a rat TBI model [87]. Furthermore, HBOT decreases expression of nNOS, eNOS and iNOS (neuronal, endothelial and inducible nitric oxide synthases) mRNA in the cortex after acute traumatic cerebral injury [92].

2.5. HBOT improves neurofunctional recovery of the injured brain by enhancing neuronal plasticity and synaptogenesis

Growing number of studies have reported that, irrespective to diversity of protocols, HBO therapy applied after TBI improved neurological status including motor and cognitive function, as well as learning and memory abilities, indicating that the best prognosis is achieved by earlier and continuous HBO treatment [11, 41, 45, 57, 67, 88, 93]. On the other hand, Baratz-Goldstein et al. [41] demonstrated that delayed treatment with HBO (initiated 7 days post-injury) also lead to improvement in learning abilities in mice model of moderate TBI. Additionally, in their recent publication Lim et al. [90] suggested that HBO treatment may ameliorate TBI-induced depression-like behavior in rats.

In our previously published paper [45] we have demonstrated that HBOT improves recovery of locomotor performances and sensorimotor integration after cortical injury in rats by enhancing neuroplastic responses and promoting synaptogenesis. Using growth-associated protein 43 (GAP43) and synaptophysin (SYP) as markers of axonal sprouting and synaptogenesis, respectively, we were the first to demonstrate that HBOT induces over-expression of GAP43 and SYP in the neurons surrounding the injury site. Given that an increase in GAP43 and SYP expression occurs concomitantly with improvement of locomotor abilities, we suggested that mechanisms underlying HBOT action involve promoting of axonal sprouting and the formation of new functional synaptic circuits. This implies that axonal reorganization and synapse remodeling contribute to observed functional recovery. Recent results of Zhang et al. [93] that HBOT-induced increase of GAP43 and synaptophysin expression underlies observed enhancement of learning abilities in the controlled cortical impact (CCI) model of rat brain injury confirmed our assumptions. Furthermore, Chen and Chen [94] detected

HBOT-induced enhancement of SYP expression after hypoxia-ischemia and proposed that the induction of synaptic plasticity and reducing of the ultrastructural damage may underlie rehabilitation mechanisms of HBOT.

2.6. HBOT promotes neurogenesis and angiogenesis after TBI

Nowadays, stem cells are in the center of attention. Stimulation of neurogenesis after HBOT and influence of stem cells mobilization on motor and cognitive performances is demonstrated in numerous studies [67, 95–97]. Thus, Shandley et al. [97] showed that cognitive improvement observed after treatment with HBO in patients with mild to moderate TBI is correlated with stem cell mobilization. Based on these findings they hypothesized that stem cells, mobilized by HBOT treatment, are recruited to repair damaged neuronal tissue. Yang et al. [98] and Wang et al. [99] reported that HBOT promotes the migration and differentiation of endogenous neural stem cells (NSCs) in neonatal rats with hypoxic-ischemic (HI) brain damage. Authors have shown that after HBOT, an increase in newly generated neurons, oligodendrocytes and myelination was observed in the HI group treated with HBO compared to the untreated HI rats. Further, it was suggested that HBOT-stimulated proliferation of NSCs protects the learning and memory ability of the HI rats [100]. In our recent preliminary reports [101, 102] we also noticed that HBOT when applied after brain injury promoted endogenous NSCs to migrate to the site of injury and differentiate into mature neurons, contributing to improved neurofunctional recovery of the injured brain. Moreover, we demonstrated that HBOT alters morphology of neuronal precursors to more matured morphology [102].

Mu et al. [103] suggested that activation of several signaling pathways and transcription factors (Wnt, hypoxia-inducible factors - HIFs, and cAMP response element-binding - CREB) play an important role in HBOT-induced neurogenesis. Furthermore, it was assumed that endogenous neurogenesis, enhanced by application of delayed HBO in the late-chronic phase of stroke, is possibly mediated by ROS/HIF-1 α / β -catenin pathway [96].

Interestingly, combining of HBOT with bone marrow stem cells (BMSCs) transplantation showed synergistic effect and had favorable influence in improving rehabilitation after rat spinal cord injury [104]. The same combination of HBOT and BMSCs transplantation proved to be more effective for repair of cognitive and neurological functions after TBI than monotherapy [105]. Similarly, long course of HBO treatments (for 3 weeks) promote the mobilization and migration of BMSCs to ischemic brain, stimulate expression of trophic factors and neurogenesis, and help in neuronal repair after ischemic stroke [72].

Besides neurogenesis HBOT may improve the outcome of TBI by stimulating angiogenesis [67, 95, 106, 107]. Using brain perfusion imaging, Tal et al. [106] demonstrated that 60 daily HBOT sessions stimulate cerebral angiogenesis in post-TBI patients, which induced significant improvement in the global cognitive scores. These data strongly suggest that one of the ways in which HBOT can induce neuroplasticity is angiogenesis. Given that HBOT was initiated 6 months to 27 years after the injury, obtained results imply that HBOT may improve perfusion to the chronic damaged brain tissue even months to years after the injury. Recent results of the same group [107] showed that in addition to the increased cerebral blood flow

and volume, HBOT improved both white and gray microstructures pointing to regeneration of nerve fibers. These micro structural changes correlate with the significant improvement in the memory, executive functions, information processing speed and global cognitive scores.

3. Conclusions

HBOT has been used as a primary or adjunctive therapy over the last 50 years, both in experimental and clinical studies. However, despite the decades of extensive research the entire spectrum of HBOT action is still not completely understood, although many mechanisms of its action have been proposed. Therefore, in this systematic review we elaborate the cellular and molecular mechanisms of HBOT actions. Based on the presented data it may be concluded that improved tissue oxygenation and cellular metabolism, anti-inflammation, anti-apoptosis, as well as intensifying of neuroplastic responses, promoting of synaptogenesis, neurogenesis and angiogenesis may constitute the multiple and complementary mechanisms underlying HBOT-induced neuroprotection. In addition, reduction of lipid peroxidation and up-regulation of antioxidant enzymes are among the mechanisms involved in the action of HBO. In that way, HBOT diminishes imbalance between oxidants and anti-oxidants that occurs after brain injury, and contributes to the maintenance of pro-/antioxidant homeostasis. Furthermore, HBOT effectively attenuates reactive astrogliosis and microgliosis, prevents tissue-damaging effects of neutrophils and suppresses formation of glial scar. Accordingly, by alleviating glia-mediated inflammatory response and limiting production of inflammatory mediators HBOT fosters formation of more permissive environment for tissue repair, allowing the recovery of impaired brain functions. Overall, although results clearly suggest the validity of HBO therapy for the treatment of TBI, the underlying mechanism still needs to be studied in depth.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' contribution

SP performed surgery and wrote the paper; SD was responsible for tissue preparation, immunohistochemistry and immunofluorescence; DK carried out the enzyme and lipid peroxidation

assays; RJ contributed substantially to the study by literature search and writing of the manuscript; PB carried out the HBOT and with MDj provided critical revision of the manuscript. All authors read and approved the final manuscript.

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References

- [1] Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC. The impact of traumatic brain injuries: A global perspective. *NeuroRehabilitation*. 2007;**22**(5):341-353
- [2] Pekovic S, Subasic S, Nedeljkovic N, Bjelobaba I, Filipovic R, Milenkovic I, et al. Molecular basis of brain injury and repair. In: Ruzdijic S, Rakic LJ, editors. *Neurobiological Studies – From Genes to Behaviour*. Kerala, India: Research Signpost; 2006. pp. 143-165
- [3] Margulies S, Hicks R. Combination therapies for traumatic brain injury: Prospective considerations. *Journal of Neurotrauma*. 2009;**26**(6):925-939. DOI: 10.1089/neu.2008.0794
- [4] Michalski D, Küppers-Tiedt L, Weise C, Laignel F, Härtig W, Raviolo M, et al. Long-term functional and neurological outcome after simultaneous treatment with tissue-plasminogen activator and hyperbaric oxygen in early phase of embolic stroke in rats. *Brain Research*. 2009;**15**, **1303**:161-168. DOI: 10.1016/j.brainres.2009.09.038
- [5] Feldmeier JJ, editor. *Hyperbaric Oxygen Therapy: 2003 Committee Report*, Rev. Ed. Kensington (MD): Undersea and Hyperbaric Medical Society, Inc.; 2003. p. 137
- [6] Brkic DP, Pekovic MS, Krstic ZD, Jovanovic ST. Hyperbaric oxygenation as an adjuvant therapy for traumatic brain injury: A review of literature. *Periodicum Biologorum*. 2014;**116**(1):29-36

- [7] Hu Q, Manaenko A, Xu T, Guo Z, Tang J, Zhang J. Hyperbaric oxygen therapy for traumatic brain injury: Bench-to-bedside. *Medical Gas Research*. 2016;**6**(2):102-110. DOI: 10.4103/2045-9912.184720
- [8] Gjedde A. The pathways of oxygen in brain. I. Delivery and metabolism of oxygen. *Advances in Experimental Medicine and Biology*. 2005;**566**:269-275. DOI: 10.1007/0-387-26206-7_36
- [9] Daugherty WP, Levasseur JE, Sun D, Rockswold GL, Bullock MR. Effects of hyperbaric oxygen therapy on cerebral oxygenation and mitochondrial function following moderate lateral fluid-percussion injury in rats. *Journal of Neurosurgery*. 2004;**101**(3):499-504. DOI: 10.3171/jns.2004.101.3.0499
- [10] Calvert JW, Cahill J, Zhang JH. Hyperbaric oxygen and cerebral physiology. *Neurological Research*. 2007;**29**(2):132-141. DOI: 10.1179/016164107X174156
- [11] Wang GH, Zhang XG, Jiang ZL, Li X, Peng LL, Li YC, et al. Neuroprotective effects of hyperbaric oxygen treatment on traumatic brain injury in the rat. *Journal of Neurotrauma*. 2010;**27**(9):1733-1743. DOI: 10.1089/neu.2009.1175
- [12] Kindwall EP. Uses of hyperbaric oxygen therapy in the 1990s. *Cleveland Clinic Journal of Medicine*. 1992;**59**(5):517-528
- [13] Schaub E, Pellegrini M, Pugin D. Carbon monoxide poisoning: An update for 2009. *Revue Médicale Suisse*. 2009;**5**(213):1606-1609
- [14] Rockswold SB, Rockswold GL, Zaun DA, Zhang X, Cerra CE, Bergman TA, et al. A prospective, randomized clinical trial to compare the effect of hyperbaric to normobaric hyperoxia on cerebral metabolism, intracranial pressure, and oxygen toxicity in severe traumatic brain injury. *Journal of Neurosurgery*. 2010;**112**(5):1080-1094. DOI: 10.3171/2009.7.JNS09363
- [15] Rockswold SB, Rockswold GL, Zaun DA, Liu J. A prospective, randomized phase II clinical trial to evaluate the effect of combined hyperbaric and normobaric hyperoxia on cerebral metabolism, intracranial pressure, oxygen toxicity, and clinical outcome in severe traumatic brain injury. *Journal of Neurosurgery*. 2013;**118**(6):1317-1328. DOI: 10.3171/2013.2.JNS121468
- [16] Bennett MH, Trytko B, Jonker B. Hyperbaric oxygen therapy for the adjunctive treatment of traumatic brain injury. *Cochrane Database of Systematic Reviews*. 2012;**12**:CD004609. DOI: 10.1002/14651858.CD004609.pub3
- [17] Thom SR. Oxidative stress is fundamental to hyperbaric oxygen therapy. *Journal of Applied Physiology* (1985). 2009;**106**(3):988-995. DOI: 10.1152/japplphysiol.91004.2008
- [18] Ozden TA, Uzun H, Bohloli M, Toklu AS, Paksoy M, Simsek G, et al. The effects of hyperbaric oxygen treatment on oxidant and antioxidants levels during liver regeneration in rats. *The Tohoku Journal of Experimental Medicine*. 2004;**203**(4):253-265

- [19] Oter S, Korkmaz A, Topal T, Ozcan O, Sadir S, Ozler M, et al. Correlation between hyperbaric oxygen exposure pressures and oxidative parameters in rat lung, brain, and erythrocytes. *Clinical Biochemistry*. 2005;**38**(8):706-711. DOI: 10.1016/j.clinbiochem.2005.04.005
- [20] Benedetti S, Lamorgese A, Piersantelli M, Pagliarani S, Benvenuti F, Canestrari F. Oxidative stress and antioxidant status in patients undergoing prolonged exposure to hyperbaric oxygen. *Clinical Biochemistry*. 2004;**37**(4):312-317. DOI: 10.1016/j.clinbiochem.2003.12.001
- [21] Smerz RW. Incidence of oxygen toxicity during the treatment of dysbarism. *Undersea & Hyperbaric Medicine*. 2004;**31**(2):199-202
- [22] Rockswold SB, Rockswold GL, Defillo A. Hyperbaric oxygen in traumatic brain injury. *Neurological Research*. 2007;**29**(2):162-172. DOI: 10.1179/016164107X181798
- [23] Bitterman H. Bench-to-bedside review: Oxygen as a drug. *Critical Care*. 2009;**13**(1):205. DOI: 10.1186/cc7151
- [24] Niklas A, Brock D, Schober R, Schulz A, Schneider D. Continuous measurements of cerebral tissue oxygen pressure during hyperbaric oxygenation--HBO effects on brain edema and necrosis after severe brain trauma in rabbits. *Journal of the Neurological Sciences*. 2004;**219**(1-2):77-82. DOI: 10.1016/j.jns.2003.12.013
- [25] Palzur E, Vlodavsky E, Mulla H, Arieli R, Feinsod M, Soustiel JF. Hyperbaric oxygen therapy for reduction of secondary brain damage in head injury: An animal model of brain contusion. *Journal of Neurotrauma*. 2004;**21**(1):41-48. DOI: 10.1089/089771504772695931
- [26] Lin JW, Tsai JT, Lee LM, Lin CM, Hung CC, Hung KS, et al. Effect of hyperbaric oxygen on patients with traumatic brain injury. *Acta Neurochirurgica. Supplement*. 2008; **101**:145-149
- [27] Fischer BR, Speckmann EJ, Greiner C, Gorji A, Wölfer J, Wassmann H. Hyperbaric oxygen in neurosurgery. *Acta Neurochirurgica*. 2009;**151**(4):415-418. DOI: 10.1007/s00701-009-0228-2
- [28] Wang Y, Chen D, Chen G. Hyperbaric oxygen therapy applied research in traumatic brain injury: From mechanisms to clinical investigation. *Medical Gas Research*. 2014; **4**(4):18. DOI: 10.1186/2045-9912-4-18
- [29] Eriskat J, Plesnila N, Stoffel M, Baethmann A. Assessment of regional cortical blood flow following traumatic lesion of the brain. *Acta Neurochirurgica. Supplement*. 1997;**70**:94-95
- [30] Thomale UW, Schaser K, Kroppenstedt SN, Unterberg AW, Stover JF. Cortical hypoperfusion precedes hyperperfusion following controlled cortical impact injury. *Acta Neurochirurgica. Supplement*. 2002;**81**:229-231
- [31] Zauner A, Daugherty WP, Bullock MR, Warner DS. Brain oxygenation and energy metabolism: Part I-biological function and pathophysiology. *Neurosurgery*. 2002;**51**(2):289-301

- [32] Palzur E, Zaaroor M, Vlodavsky E, Milman F, Soustiel JF. Neuroprotective effect of hyperbaric oxygen therapy in brain injury is mediated by preservation of mitochondrial membrane properties. *Brain Research*. 2008;**1221**:126-133
- [33] Zhou JG, Liu JC, Fang YQ. Effect of hyperbaric oxygen on the expression of proteins Bcl-2 and Bax in the gerbil hippocampus CA1 following forebrain ischemia reperfusion. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2000;**16**(4):298-301
- [34] Yin D, Zhou C, Kusaka I, Calvert JW, Parent AD, Nanda A, et al. Inhibition of apoptosis by hyperbaric oxygen in a rat focal cerebral ischemic model. *Journal of Cerebral Blood Flow and Metabolism*. 2003;**23**(7):855-864. DOI: 10.1097/01.WCB.0000073946.29308.55
- [35] Li Y, Zhou C, Calvert JW, Colohan AR, Zhang JH. Multiple effects of hyperbaric oxygen on the expression of HIF-1 alpha and apoptotic genes in a global ischemia-hypotension rat model. *Experimental Neurology*. 2005;**191**(1):198-210. DOI: 10.1016/j.expneurol.2004.08.036
- [36] Lou M, Ding MP, Wen SQ. Effect of hyperbaric oxygenation treatment on the apoptotic cell death pathway after transient focal cerebral ischemia. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2006;**22**(1):1-5
- [37] Peng Z, Xiao P, Guo H, Liu Q. Effect of early hyperbaric oxygen on neuronal apoptosis and learning and memory of cerebral ischemia-reperfusion injury in rats. *Zhong Nan Da Xue Xue Bao. Yi Xue Ban*. 2009;**34**(6):468-475
- [38] Calvert JW, Yin W, Patel M, Badr A, Mychaskiw G, Parent AD, et al. Hyperbaric oxygenation prevented brain injury induced by hypoxia-ischemia in a neonatal rat model. *Brain Research*. 2002;**951**(1):1-8
- [39] Calvert JW, Zhou C, Nanda A, Zhang JH. Effect of hyperbaric oxygen on apoptosis in neonatal hypoxia-ischemia rat model. *Journal of Applied Physiology* (1985). 2003;**95**(5):2072-2080. DOI: 10.1152/japplphysiol.00630.2003
- [40] Liu XH, Yan H, Xu M, Zhao YL, Li LM, Zhou XH, et al. Hyperbaric oxygenation reduces long-term brain injury and ameliorates behavioural function by suppression of apoptosis in a rat model of neonatal hypoxia-ischemia. *Neurochemistry International*. 2013;**62**(7):922-930. DOI: 10.1016/j.neuint.2013.03.004
- [41] Baratz-Goldstein R, Toussia-Cohen S, Elpaz A, Rubovitch V, Pick CG. Immediate and delayed hyperbaric oxygen therapy as a neuroprotective treatment for traumatic brain injury in mice. *Molecular and Cellular Neurosciences*. 2017;**83**:74-82. DOI: 10.1016/j.mcn.2017.06.004
- [42] Bidmon HJ, Kato K, Schleicher A, Witte OW, Zilles K. Transient increase of manganese-superoxide dismutase in remote brain areas after focal photothrombotic cortical lesion. *Stroke*. 1998;**29**(1):203-210
- [43] Parabucki AB, Bozić ID, Bjelobaba IM, Lavrnja IC, Brkić PD, Jovanović TS, et al. Hyperbaric oxygenation alters temporal expression pattern of superoxide dismutase 2 after cortical stab injury in rats. *Croatian Medical Journal*. 2012;**53**(6):586-597

- [44] Peković S, Filipović R, Subasić S, Lavrnja I, Stojkov D, Nedeljković N, et al. Down-regulation of glial scarring after brain injury: The effect of purine nucleoside analogue ribavirin. *Annals of the New York Academy of Sciences*. 2005;**1048**:296-310. DOI: 10.1196/annals.1342.027
- [45] Brkic P, Stojiljkovic M, Jovanovic T, Dacic S, Lavrnja I, Savic D, et al. Hyperbaric oxygenation improves locomotor ability by enhancing neuroplastic responses after cortical ablation in rats. *Brain Injury*. 2012;**26**(10):1273-1284. DOI: 10.3109/02699052.2012.667593
- [46] Wendel A. *Enzymatic Basis of Detoxication*. Vol. 1. NY: Academic Press; 1980. p. 333
- [47] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry*. 1972;**247**(10):3170-3175
- [48] Aruoma OI, Halliwell B, Laughton MJ, Quinlan GJ, Gutteridge JM. The mechanism of initiation of lipid peroxidation. Evidence against a requirement for an iron (II)-iron (III) complex. *Biochemical Journal*. 1989;**258**(2):617-620
- [49] Mladenović D, Krstić D, Colović M, Radosavljević T, Rasić-Marković A, Hrncić D, et al. Different sensitivity of various brain structures to thioacetamide-induced lipid peroxidation. *Medicinal Chemistry*. 2012;**8**(1):52-58
- [50] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*. 1951;**193**(1):265-275
- [51] Li J, Liu W, Ding S, Xu W, Guan Y, Zhang JH, et al. Hyperbaric oxygen preconditioning induces tolerance against brain ischemia-reperfusion injury by upregulation of antioxidant enzymes in rats. *Brain Research*. 2008;**1210**:223-229. DOI: 10.1016/j.brainres.2008.03.007
- [52] Niizuma K, Endo H, Chan PH. Oxidative stress and mitochondrial dysfunction as determinants of ischemic neuronal death and survival. *Journal of Neurochemistry*. 2009;**109**(1):133-138. DOI: 10.1111/j.1471-4159.2009.05897.x
- [53] Vlodavsky E, Palzur E, Feinsod M, Soustiel JF. Evaluation of the apoptosis-related proteins of the BCL-2 family in the traumatic penumbra area of the rat model of cerebral contusion, treated by hyperbaric oxygen therapy: A quantitative immunohistochemical study. *Acta Neuropathologica*. 2005;**110**(2):120-126. DOI: 10.1007/s00401-004-0946-8
- [54] Vlodavsky E, Palzur E, Soustiel JF. Hyperbaric oxygen therapy reduces neuroinflammation and expression of matrix metalloproteinase-9 in the rat model of traumatic brain injury. *Neuropathology and Applied Neurobiology*. 2006;**32**(1):40-50. DOI: 10.1111/j.1365-2990.2005.00698.x
- [55] Soustiel JF, Palzur E, Vlodavsky E, Veenman L, Gavish M. The effect of oxygenation level on cerebral post-traumatic apoptosis is modulated by the 18-kDa translocator protein (also known as peripheral-type benzodiazepine receptor) in a rat model of cortical contusion. *Neuropathology and Applied Neurobiology*. 2008;**34**(4):412-423. DOI: 10.1111/j.1365-2990.2007.00906.x

- [56] Liu Z, Jiao QF, You C, Che YJ, Effect SFZ. Of hyperbaric oxygen on cytochrome C, Bcl-2 and Bax expression after experimental traumatic brain injury in rats. *Chinese Journal of Traumatology*. 2006;**9**(3):168-174
- [57] Zhou Z, Daugherty WP, Sun D, Levasseur JE, Altememi N, Hamm RJ, et al. Protection of mitochondrial function and improvement in cognitive recovery in rats treated with hyperbaric oxygen following lateral fluid-percussion injury. *Journal of Neurosurgery*. 2007;**106**(4):687-694. DOI: 10.3171/jns.2007.106.4.687
- [58] Werner C, Engelhard K. Pathophysiology of traumatic brain injury. *British Journal of Anaesthesia*. 2007;**99**(1):4-9. DOI: 10.1093/bja/aem131
- [59] Yu J, Yu Q, Liu Y, Zhang R, Xue L. Hydrogen gas alleviates oxygen toxicity by reducing hydroxyl radical levels in PC12 cells. *PLoS One*. 2017;**12**(3):e0173645. DOI: 10.1371/journal.pone.0173645
- [60] Chhor V, Canini F, De Rudnicki S, Dahmani S, Gressens P, Constantin P. Hyperbaric oxygen therapy and inert gases in cerebral ischemia and traumatic brain injury. *Annales Françaises d'Anesthésie et de Réanimation*. 2013;**32**(12):863-871. DOI: 10.1016/j.annfar.2013.09.005
- [61] Silver J, Miller JH. Regeneration beyond the glial scar. *Nature Reviews. Neuroscience*. 2004;**5**(2):146-156. DOI: 10.1038/nrn1326
- [62] Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in Neurosciences*. 2009;**32**(12):638-647. DOI: 10.1016/j.tins.2009.08.002
- [63] Calingasan NY, Erdely HA, Altar CA. Identification of CD40 ligand in Alzheimer's disease and in animal models of Alzheimer's disease and brain injury. *Neurobiology of Aging*. 2002;**23**(1):31-39
- [64] Pekny M, Wilhelmsson U, Bogestål YR, Pekna M. The role of astrocytes and complement system in neural plasticity. *International Review of Neurobiology*. 2007;**82**:95-111. DOI: 10.1016/S0074-7742(07)82005-8
- [65] Sofroniew MV, Vinters HV. Astrocytes: Biology and pathology. *Acta Neuropathologica*. 2010;**119**(1):7-35. DOI: 10.1007/s00401-009-0619-8
- [66] Lavrnja I, Parabucki A, Brkic P, Jovanovic T, Dacic S, Savic D, et al. Repetitive hyperbaric oxygenation attenuates reactive astrogliosis and suppresses expression of inflammatory mediators in the rat model of brain injury. *Mediators of Inflammation*. 2015;**2015**:498405
- [67] Lin KC, Niu KC, Tsai KJ, Kuo JR, Wang LC, Chio CC, et al. Attenuating inflammation but stimulating both angiogenesis and neurogenesis using hyperbaric oxygen in rats with traumatic brain injury. *Journal of Trauma and Acute Care Surgery*. 2012;**72**(3):650-659. DOI: 10.1097/TA.0b013e31823c575f
- [68] Lim SW, Wang CC, Wang YH, Chio CC, Niu KC, Kuo JR. Microglial activation induced by traumatic brain injury is suppressed by postinjury treatment with hyperbaric oxygen therapy. *The Journal of Surgical Research*. 2013;**184**(2):1076-1084. DOI: 10.1016/j.jss.2013.04.070

- [69] Günther A, Küppers-Tiedt L, Schneider PM, Kunert I, Berrouschot J, Schneider D, et al. Reduced infarct volume and differential effects on glial cell activation after hyperbaric oxygen treatment in rat permanent focal cerebral ischaemia. *The European Journal of Neuroscience*. 2005;**21**(11):3189-3194. DOI: 10.1111/j.1460-9568.2005.04151.x
- [70] Hui J, Zhang ZJ, Zhang X, Shen Y, Gao YJ. Repetitive hyperbaric oxygen treatment attenuates complete Freund's adjuvant-induced pain and reduces glia-mediated neuro-inflammation in the spinal cord. *The Journal of Pain*. 2013;**14**(7):747-758. DOI: 10.1016/j.jpain.2013.02.003
- [71] Zhao BS, Meng LX, Ding YY, Cao YY. Hyperbaric oxygen treatment produces an antinociceptive response phase and inhibits astrocyte activation and inflammatory response in a rat model of neuropathic pain. *Journal of Molecular Neuroscience*. 2014;**53**(2):251-261. DOI: 10.1007/s12031-013-0213-3
- [72] Lee YS, Chio CC, Chang CP, Wang LC, Chiang PM, Niu KC, et al. Long course hyperbaric oxygen stimulates neurogenesis and attenuates inflammation after ischemic stroke. *Mediators of Inflammation*. 2013;**2013**:512978. DOI: 10.1155/2013/512978
- [73] Morganti-Kossmann MC, Satgunaseelan L, Bye N, Kossmann T. Modulation of immune response by head injury. *Injury*. 2007;**38**(12):1392-1400. DOI: 10.1016/j.injury.2007.10.005
- [74] Hellewell SC, Morganti-Kossmann MC. Guilty molecules, guilty minds? The conflicting roles of the innate immune response to traumatic brain injury. *Mediators of Inflammation*. 2012;**2012**:356494. DOI: 10.1155/2012/356494
- [75] Benveniste EN, Nguyen VT, Wesemann DR. Molecular regulation of CD40 gene expression in macrophages and microglia. *Brain, Behavior, and Immunity*. 2004;**18**(1):7-12
- [76] Yilmaz G, Granger DN. Leukocyte recruitment and ischemic brain injury. *Neuromolecular Medicine*. 2010;**12**(2):193-204. DOI: 10.1007/s12017-009-8074-1
- [77] Buras JA, Stahl GL, Svoboda KK, Reenstra WR. Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: The role of NOS. *American Journal of Physiology. Cell Physiology*. 2000;**278**(2):C292-C302
- [78] Atochin DN, Fisher D, Demchenko IT, Thom SR. Neutrophil sequestration and the effect of hyperbaric oxygen in a rat model of temporary middle cerebral artery occlusion. *Undersea & Hyperbaric Medicine*. 2000;**27**(4):185-190
- [79] Miljkovic-Lolic M, Silbergleit R, Fiskum G, Rosenthal RE. Neuroprotective effects of hyperbaric oxygen treatment in experimental focal cerebral ischemia are associated with reduced brain leukocyte myeloperoxidase activity. *Brain Research*. 2003;**971**(1):90-94
- [80] Helms AK, Whelan HT, Torbey MT. Hyperbaric oxygen therapy of cerebral ischemia. *Cerebrovascular Diseases*. 2005;**20**(6):417-426. DOI: 10.1159/000088979
- [81] Chen K, Huang J, Gong W, Zhang L, Yu P, Wang JM. CD40/CD40L dyad in the inflammatory and immune responses in the central nervous system. *Cellular & Molecular Immunology*. 2006;**3**(3):163-169

- [82] Calingasan NY, Erdely HA, Altar CA. Identification of CD40 ligand in Alzheimer's disease and in animal models of Alzheimer's disease and brain injury. *Neurobiology of Aging*. 2002;**23**(1):31-39
- [83] Garlich CD, Kozina S, Fateh-Moghadam S, Handschu R, Tomandl B, Stumpf C, et al. Upregulation of CD40-CD40 ligand (CD154) in patients with acute cerebral ischemia. *Stroke*. 2003;**34**(6):1412-1418. DOI: 10.1161/01.STR.0000074032.64049.47
- [84] Ishikawa M, Vowinkel T, Stokes KY, Arumugam TV, Yilmaz G, Nanda A, et al. CD40/CD40 ligand signaling in mouse cerebral microvasculature after focal ischemia/reperfusion. *Circulation*. 2005;**111**(13):1690-1696. DOI: 10.1161/01.CIR.0000160349.42665.0C
- [85] Zhang B, Wu T, Chen M, Zhou Y, Yi D, Guo R. The CD40/CD40L system: A new therapeutic target for disease. *Immunology Letters*. 2013;**153**(1-2):58-61. DOI: 10.1016/j.imlet.2013.07.005
- [86] Meng XE, Zhang Y, Li N, Fan DF, Yang C, Li H, et al. Effects of hyperbaric oxygen on the Nrf2 signaling pathway in secondary injury following traumatic brain injury. *Genetics and Molecular Research*. 2016a;**15**(1). DOI: 10.4238/gmr.15016933
- [87] Meng XE, Zhang Y, Li N, Fan DF, Yang C, Li H, et al. Hyperbaric oxygen alleviates secondary brain injury after trauma through inhibition of TLR4/NF- κ B Signaling pathway. *Medical Science Monitor*. 2016b;**22**:284-288
- [88] Chen X, Duan XS, Xu LJ, Zhao JJ, She ZF, Chen WW, et al. Interleukin-10 mediates the neuroprotection of hyperbaric oxygen therapy against traumatic brain injury in mice. *Neuroscience*. 2014;**266**:235-243. DOI: 10.1016/j.neuroscience.2013.11.036
- [89] Wee HY, Lim SW, Chio CC, Niu KC, Wang CC, Kuo JR. Hyperbaric oxygen effects on neuronal apoptosis associations in a traumatic brain injury rat model. *The Journal of Surgical Research*. 2015;**197**(2):382-389. DOI: 10.1016/j.jss.2015.04.052
- [90] Lim SW, Sung KC, Shiue YL, Wang CC, Chio CC, Kuo JR. Hyperbaric oxygen effects on depression-like behaviour and Neuroinflammation in traumatic brain injury rats. *World Neurosurgery*. 2017;**100**:128-137. DOI: 10.1016/j.wneu.2016.12.118
- [91] Geng F, Ma Y, Xing T, Zhuang X, Zhu J, Yao L. Effects of hyperbaric oxygen therapy on Inflammasome signalling after traumatic brain injury. *Neuroimmunomodulation*. 2016;**23**(2):122-129. DOI: 10.1159/000445689
- [92] Zhou JG, Fang YQ, Liu CY, Zhou YQ, Ji YF, Liu JC. Effect of hyperbaric oxygen on the expression of nitric oxide synthase mRNA in cortex after acute traumatic cerebral injury. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2012;**28**(1):38-41
- [93] Zhang X, Wang X, Sun X, Sun X, Zhang Y, Zhang H. Differences in cognitive function of rats with traumatic brain injuries following hyperbaric oxygen therapy. *Medical Science Monitor*. 2016;**22**:2608-2615
- [94] Chen J, Chen YH. Effects of hyperbaric oxygen on synaptic ultrastructure and synaptophysin expression in hippocampus of neonatal rats with hypoxic-ischemic brain damage. *Zhonghua Er Ke Za Zhi*. 2010;**48**(3):199-203

- [95] Zhang T, Yang QW, Wang SN, Wang JZ, Wang Q, Wang Y, et al. Hyperbaric oxygen therapy improves neurogenesis and brain blood supply in piriform cortex in rats with vascular dementia. *Brain Injury*. 2010;**24**(11):1350-1357. DOI: 10.3109/02699052.2010.504525
- [96] Hu Q, Liang X, Chen D, Chen Y, Doycheva D, Tang J, et al. Delayed hyperbaric oxygen therapy promotes neurogenesis through reactive oxygen species/hypoxia-inducible factor-1 α / β -catenin pathway in middle cerebral artery occlusion rats. *Stroke*. 2014; **45**(6):1807-1814. DOI: 10.1161/STROKEAHA.114.005116
- [97] Shandley S, Wolf EG, Schubert-Kappan CM, Baugh LM, Richards MF, Prye J. Increased circulating stem cells and better cognitive performance in traumatic brain injury subjects following hyperbaric oxygen therapy. *Undersea & Hyperbaric Medicine*. 2017; **44**(3):257-269
- [98] Yang YJ, Wang XL, Yu XH, Wang X, Xie M, Liu CT. Hyperbaric oxygen induces endogenous neural stem cells to proliferate and differentiate in hypoxic-ischemic brain damage in neonatal rats. *Undersea & Hyperbaric Medicine*. 2008;**35**(2):113-129
- [99] Wang XL, Yang YJ, Xie M, Yu XH, Wang QH. Hyperbaric oxygen promotes the migration and differentiation of endogenous neural stem cells in neonatal rats with hypoxic-ischemic brain damage. *Zhongguo Dang Dai Er Ke Za Zhi*. 2009;**11**(9):749-752
- [100] Wei L, Wang J, Cao Y, Ren Q, Zhao L, Li X, et al. Hyperbaric oxygenation promotes neural stem cell proliferation and protects the learning and memory ability in neonatal hypoxic-ischemic brain damage. *International Journal of Clinical and Experimental Pathology*. 2015;**8**(2):1752-1759. DOI: PMC4396307
- [101] Brkic P, Dacic S, Jeremic R, Jovanovic T, Pekovic S. The effect of hyperbaric oxygenation on neurogenesis after brain injury in adult rats. Alessandro Marroni, Maja Medić, Mariana Sedlar and Minjon Zarić Milanović, editors. IX International Congress on Hyperbaric Medicine; May 11th-14th; Belgrade, Serbia. Belgrade: Centre for Hyperbaric Medicine; 2017. p. 26
- [102] Jeremic R, Dacic S, Brkic P, Djelic M, Jovanovic T, Pekovic S. Hyperbaric oxygenation enhances neurogenesis after injury to adult rat brain. Selma Kanazir and Ivana Bjelobaba, editors. 7th Congress of Serbian Neuroscience with international participation; October 25th-27th; Belgrade, Serbia. Belgrade: Serbian Neuroscience Society, Serbian Ministry of Education, Science and Technological development, Institute for Biological Research "Sinisa Stankovic", University of Belgrade; 2017. p. 84. ISBN: 978-86-917255-1-8
- [103] Mu J, Krafft PR, Zhang JH. Hyperbaric oxygen therapy promotes neurogenesis: Where do we stand? *Medical Gas Research*. 2011;**1**(1):14. DOI: 10.1186/2045-9912-1-14
- [104] Geng CK, Cao HH, Ying X, Yu HL. Effect of mesenchymal stem cells transplantation combining with hyperbaric oxygen therapy on rehabilitation of rat spinal cord injury. *Asian Pacific Journal of Tropical Medicine*. 2015;**8**(6):468-473. DOI: 10.1016/j.apjtm.2015.05.001

- [105] Zhou HX, Liu ZG, Liu XJ, Chen QX. Umbilical cord-derived mesenchymal stem cell transplantation combined with hyperbaric oxygen treatment for repair of traumatic brain injury. *Neural Regeneration Research*. 2016;**11**(1):107-113. DOI: 10.4103/1673-5374.175054
- [106] Tal S, Hadanny A, Berkovitz N, Sasson E, Ben-Jacob E, Efrati S. Hyperbaric oxygen may induce angiogenesis in patients suffering from prolonged post-concussion syndrome due to traumatic brain injury. *Restorative Neurology and Neuroscience*. 2015;**33**(6):943-951. DOI: 10.3233/RNN-15058
- [107] Tal S, Hadanny A, Sasson E, Suzin G, Efrati S. Hyperbaric oxygen therapy can induce angiogenesis and regeneration of nerve Fibers in traumatic brain injury patients. *Frontiers in Human Neuroscience*. 2017;**11**:508. DOI: 10.3389/fnhum.2017.00508