

# Hyperbaric oxygen therapy of spinal cord injury

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## Abstract

Spinal cord injury (SCI) is a complex disease process that involves both primary and secondary mechanisms of injury and can leave patients with devastating functional impairment as well as psychological debilitation. While no curative treatment is available for spinal cord injury, current therapeutic approaches focus on reducing the secondary injury that follows SCI. Hyperbaric oxygen (HBO) therapy has shown promising neuroprotective effects in several experimental studies, but the limited number of clinical reports have shown mixed findings. This review will provide an overview of the potential mechanisms by which HBO therapy may exert neuroprotection, provide a summary of the clinical application of HBO therapy in patients with SCI, and discuss avenues for future studies.

**Key words:** spinal cord injury; hyperbaric oxygen therapy; preconditioning; apoptosis; inflammation; oxidative stress; angiogenesis; spinal cord edema; autophagy

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## INTRODUCTION

Spinal cord injury (SCI) can result from various etiologies, but a common feature is the potential for functional and psychological devastation following injury. Between 1993 and 2012, a total of 63,109 patients were afflicted by acute traumatic SCI in the United States based on the Nationwide Inpatient Sample (NIS) database.<sup>1</sup> In 2016, the prevalence of SCI in the United States was reported to be approximately 282,000 people with an incidence of 17,000 people per year.<sup>2</sup> The average age at injury has risen from 29 years in the 1970s to 42 years in 2016, and there is a four-fold higher incidence in males *versus* females.<sup>2</sup> The current length of hospital stay after SCI is 11 days, however, approximately 30% of affected patients are re-hospitalized for an average of 22 days, most commonly due to genitourinary disease.<sup>2</sup> While the total cost of caring for patients with SCI has been estimated to be over \$7 billion annually in the United States, this figure does not represent the immense suffering and life-changing deficits that can result from SCI.<sup>2</sup>

The pathophysiology of SCI involves both primary and secondary damage.<sup>3</sup> Primary damage involves the inciting mechanical injury, whereas secondary damage is delayed and involves a variety of pathophysiologic processes, including

apoptosis, oxidative stress, ischemia, edema, inflammation, and excitotoxicity.<sup>3-5</sup> While primary injury is often irreversible, prior studies have shown that the mechanisms of secondary injury can be targeted to improve neurological outcomes.<sup>3</sup> Methylprednisolone is the typical drug of choice to treat secondary injury after SCI, but its' side effects warrant investigation of safer treatment modalities.<sup>6</sup> One such option is hyperbaric oxygen (HBO) therapy, in which 100% oxygen is administered at a pressure between one and three times that of atmospheric pressure. Previous experimental and clinical studies have demonstrated the beneficial effects of HBO therapy, administered either before (preconditioning) or after SCI, on recovery of motor function, improvement of spinal cord histology, and attenuation of secondary injury mechanisms. This review seeks to outline the results of these experimental and clinical studies involving HBO therapy of SCI, either *via* preconditioning or post-SCI treatment, as well as to detail the proposed neuroprotective mechanisms and discuss avenues for further research.

## EXPERIMENTAL STUDIES: POTENTIAL MECHANISMS OF HBO NEUROPROTECTION

Given that ischemia is one of the most significant



mechanisms involved in secondary injury after SCI, a treatment modality that increases the oxygen tension of the injured spinal cord should theoretically help improve recovery.<sup>3,7</sup> The majority of oxygen in arterial blood is carried by hemoglobin and a smaller fraction is dissolved in plasma; oxygen that is administered at an increased pressure can increase the amount of dissolved oxygen, creating a larger pressure gradient that can drive oxygen into ischemic tissue.<sup>8-10</sup> Accordingly, in 1972, HBO applied to a SCI model in dogs showed significantly increased oxygen tension in the spinal cord and a correlation with significantly improved functional recovery.<sup>11</sup>

While oxygen availability plays a pivotal role in cell survival following SCI, further pathophysiologic pathways involved in the secondary injury following SCI have been proposed.<sup>3,12-14</sup> Experimental studies that have investigated the effect of HBO therapy on these pathways propose that the neuroprotection afforded by HBO therapy involves the following broad mechanisms: 1) decreasing apoptosis; 2) reducing oxidative stress; 3) diminishing inflammation; 4) promoting angiogenesis; 5) reducing spinal cord edema; 6) increasing autophagy. An overview of these potential neuroprotective mechanisms is provided in this review (Table 1).

### HBO therapy decreases apoptosis *via* various mediators

Cells that survive the primary injury from SCI are susceptible to secondary damage, particularly *via* apoptosis, which has been observed to persist for several weeks after SCI and thus is an attractive target for intervention.<sup>38</sup> Many studies have used terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) staining to show that HBO therapy significantly reduces apoptosis in injured spinal cord tissue, and most of the studies have proposed potential pathways by which HBO therapy attenuates apoptotic cell death after SCI.<sup>15,19,20,23,27,30,31,33</sup>

Inducible nitric oxide synthase (iNOS) has been shown to induce apoptosis by increasing nitric oxide after SCI, and in 2004, Yu et al.<sup>15,39</sup> suggested that HBO therapy decreases apoptosis after traumatic SCI by downregulating the hypoxia-induced expression of the iNOS gene; this was evidenced by significantly decreased numbers of iNOS-positive cells at 1 and 2 days post-SCI in the group that received a single session of HBO therapy administered 30 minutes after SCI compared to the SCI only group.

The inflammatory cytokines, interleukin (IL)-1 $\beta$  and tumor necrosis factor (TNF)- $\alpha$ , have been implicated in apoptosis after SCI.<sup>40-42</sup> In 2010, a rat model of SCI *via* spinal cord compression by an aneurysm clip at T<sub>8-9</sub> resulted in significantly increased levels of IL-1 $\beta$  and TNF- $\alpha$  at four hours post-SCI in animals that received normobaric air;

however, these cytokines were significantly reduced in the group that received 120 minutes of HBO therapy at 2.5 ATA (approximately 245 kPa) immediately after SCI, a finding that was also accompanied by decreased apoptosis by TUNEL staining, suggesting that the attenuation of apoptosis by HBO therapy may be related to downregulation of IL-1 $\beta$  and TNF- $\alpha$ .<sup>20</sup>

In 2014, Long et al.<sup>30</sup> studied the role of the pro-apoptotic protein adaptor molecule apoptosis-associated speck-like protein (ASC), in the neuroprotection provided by HBO therapy. It is known that activation of NACHT domain-, leucine-rich-repeat- and pyrin domain-containing protein 3 (NALP3) *via* oxidative stress leads to activation and binding of ASC, which subsequently recruits caspase-8 to form a ternary complex that triggers apoptosis.<sup>43,44</sup> Long et al.<sup>30</sup> showed that mRNA and protein expression of ASC increases after SCI but is significantly decreased by daily HBO therapy started immediately after SCI, suggesting that HBO prevents formation of the ternary complex by attenuating oxidative stress.

Endoplasmic reticulum (ER) stress is another mechanism by which oxidative stress plays a role in apoptosis after SCI.<sup>33</sup> CCAAT-enhancer-binding protein homologous protein (CHOP) is a transcription factor that has shown to be a key player in ER stress-induced apoptosis.<sup>45</sup> In addition, caspase-12 is an ER-associated caspase that also mediates apoptosis *via* ER stress.<sup>46</sup> A variety of mechanisms activate caspase-12, which in turn activates caspase-3, a cytoplasmic protein that is an executioner of apoptosis.<sup>47</sup> In the study by Liu et al.,<sup>33</sup> mRNA and protein levels of CHOP, caspase-12, and caspase-3 were all increased in SCI-only rats compared to controls; however, the upregulation of these three factors was mitigated in the group that received daily HBO treatments at 2.0 ATA starting 6 hours after SCI, suggesting that HBO therapy attenuates SCI-induced neuronal apoptosis by downregulating the ER-stress-induced apoptotic pathway.

In 2009, Wang et al.<sup>19</sup> studied the effects of HBO preconditioning (HBO-PC) on apoptosis after SCI. HBO-PC led to higher levels of mitochondrial Bcl-2, an anti-apoptotic protein, both prior to ischemia-reperfusion injury and during the reperfusion period; furthermore, pro-apoptotic cytochrome c release from mitochondria and levels of caspase-3 and caspase-9 were decreased during reperfusion. These effects were all reversed by L-nitroarginine-methyl-ester (L-NAME), a nonspecific nitric oxide synthase (NOS) inhibitor, indicating that nitric oxide appears to be an important factor in the anti-apoptotic effects of HBO-PC.<sup>19</sup> However, the study by Yu et al.<sup>15</sup> suggested that HBO therapy post-SCI reduced apoptosis by inhibiting hypoxia-induced iNOS, implying that nitric oxide is a mediator of apoptosis. It is known that nitric oxide has contrasting effects depending on

**Table 1: Functional, histological, and biochemical findings of experimental studies**

Author	Year	Functional outcomes	Histological findings	Biochemical findings
Yu et al. <sup>15</sup>	2004	Not assessed	Decreased apoptosis by TUNEL staining	Downregulation of iNOS & GDNF gene expression
Nie et al. <sup>16</sup>	2006	Improved motor recovery indicated by higher modified Tarlov scores	Increased neuron number & improved neuronal histology; reversed by AT	Increased activities of catalase, superoxide dismutase, & glutathione peroxidase; decreased MDA
Kahraman et al. <sup>17</sup>	2007	Not assessed	Not assessed	Increased superoxide dismutase & decreased TBARS compared to methylprednisolone
Li et al. <sup>18</sup>	2007	Not assessed	Not assessed	Increased protein & mRNA expression of heme oxygenase-1, increased cell viability, decreased DNA damage
Wang et al. <sup>19</sup>	2009	Increased recovery of neurological function indicated by a 15-point scale	Decreased apoptosis by TUNEL staining; fewer injured neurons indicated by Nissl staining	Increased antioxidant enzymes (Mn-SOD & catalase), pre-ischemic Bcl-2 expression, & NO; decreased cytochrome c release and caspases 3 & 9 during reperfusion. All reversed by L-NAME
Tai et al. <sup>20</sup>	2010	Decreased hindlimb motor deficits indicated by higher BBB scores	Decreased apoptosis by TUNEL staining; diminished spinal cord infarct	Decreased VEGF, GDNF, pro-inflammatory cytokines (IL-1 $\beta$ and TNF- $\alpha$ ), & MPO
Topuz et al. <sup>21</sup>	2010	Improved motor recovery indicated by greater performance on incline plane	Reduced level of hemorrhage, necrosis, and edema; increased	Decreased MDA; increased glutathione peroxidase, catalase, and superoxide dismutase
Dayan et al. <sup>22</sup>	2012	Improved motor recovery indicated by higher BBB scores	Not assessed	Decreased superoxide dismutase, glutathione peroxidase, NO, and NOS
Lu et al. <sup>23</sup>	2012	Improved motor recovery indicated by higher BBB scores; improved conduction along descending & ascending spinal cord tracts indicated by decreased MEP & SEP latencies	Decreased apoptosis by TUNEL staining; enhanced axonal growth indicated by increased HRP-positive and BDA-positive fibers near SCI site; increased number & size of reactive	Not assessed
Yang et al. <sup>24</sup>	2013	Improved motor recovery indicated by higher BBB scores	Not assessed	Increased VEGF expression; decreased expression of MMP-2, MMP-9, and IL-6; decreased edema indicated by significantly reduced spinal cord water content
Yang et al. <sup>25</sup>	2013	Improved motor recovery indicated by higher BBB scores	Not assessed	Significantly decreased mRNA & protein expression of HMGB1 & NF- $\kappa$ B
Tan et al. <sup>26</sup>	2014	Improved motor recovery indicated by higher BBB scores	Improved histological scores	Significantly decreased IL-1 $\beta$ , TNF- $\alpha$ , and mRNA & protein expression of NF- $\kappa$ B p65 protein
Xu et al. <sup>27</sup>	2014	Improved motor recovery indicated by higher BBB scores; improved electrophysiology indicated by reduced MEP latency	Decreased apoptosis by TUNEL staining; greater Nrf2 immunoreactivity in astrocytes versus neurons	Decreased edema indicated by reduced spinal cord water content; decreased enzymatic activity of caspases-3 & -9; increased Nrf2 mRNA/protein expression, DNA binding, and expression of downstream genes both <i>in vivo</i> and <i>in vitro</i> ; increased glutathione content
Huang et al. <sup>29</sup>		Not assessed	Increased neuronal HSP32 expression confirmed <i>via</i> immunostaining	Increased HSP32 expression; reversed by Zn-pp
Liu et al. <sup>28</sup>	2014	Improved motor recovery indicated by higher BBB scores	Significantly attenuated histological changes	Increased VEGF expression; downregulation of CX43 mRNA & protein
Long et al. <sup>30</sup>	2014	Improved motor recovery indicated by higher BBB scores	Decreased apoptosis by TUNEL staining; decreased expression of ASC	Decreased mRNA & protein expression of ASC; decreased caspase-3 expression
Wang et al. <sup>31</sup>	2014	Improved motor function indicated by higher BBB scores	Decreased apoptosis by TUNEL staining; greater number of NF-200 positive nerve fibers by IHC	Reduced mRNA & protein expression of AQP4 & AQP9

**Table 1: Continued**

Author	Year	Functional outcomes	Histological findings	Biochemical findings
Geng et al. <sup>32</sup>	2015	Improved motor recovery indicated by higher BBB scores	Greater myelin sparing and improved axonal regeneration	Decreased iNOS and CD 16/32 positive cells (M1 phenotype) and increased arginase-1 and CD206 positive cells (M2 phenotype); decreased Th1 cytokines (IFN- $\gamma$ and TNF- $\alpha$ ) and increased Th2 cytokines (IL-4 and IL-13)
Kang et al. <sup>34</sup>	2015	Improved motor recovery indicated by higher BBB scores	Not assessed	Significantly decreased mRNA & protein expression of TLR4; significantly decreased HMGB1 and NF- $\kappa$ B
Liang et al. <sup>35</sup>	2015	Improved motor recovery indicated by higher BBB scores	Not assessed	Decreased mRNA & protein expression of NALP3, caspase-1, and ASC; decreased IL-1 $\beta$ release
Liu et al. <sup>33</sup>	2015	Improved motor function indicated by higher BBB scores	Decreased apoptosis by TUNEL staining	Decreased mRNA & protein expression of CHOP, caspase-3 and caspase-12
Sun et al. <sup>36</sup>	2016	Improved motor recovery indicated by higher BBB scores	TEM: loose RER, increased free ribosomes, mitochondrial swelling, numerous autophagosomes	Significantly increased Beclin-1 & LC3-II, indicating increased autophagy
Wang et al. <sup>37</sup>	2016	Improved motor recovery indicated by higher BBB scores	Not assessed	Significantly decreased mRNA & protein levels of RAGE and MCP-1

Note: BBB: Basso, Beattie, Bresnahan Locomotor scale; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling; iNOS: inducible nitric oxide synthase; GDNF: glial derived neurotrophic factor; VEGF: vascular endothelial growth factor; IL: interleukin; TNF: tumor necrosis factor; MPO: myeloperoxidase; CHOP: CCAAT-enhancer-binding protein homologous protein; NF-200: neurofilament-200; IHC: immunohistochemistry; AQP: aquaporin; Mn-SOD: manganese superoxide dismutase; Bcl-2: b cell lymphoma 2; L-NAME: L-nitroarginine-methyl-ester; ASC: adaptor molecule apoptosis-associated speck-like protein; NALP3: NACHT domain-, leucine-rich-repeat- and pyrin domain-containing protein 3; MEP: motor-evoked potential; SEP: somatosensory-evoked potential; HRP: horseradish peroxidase; BDA: biotin dextran amine; SCI: spinal cord injury; Nrf2: nuclear factor erythroid 2-related factor 2; AT: 3-amino-1,2,4-triazole; MDA: malondialdehyde; NO: nitric oxide; NOS: nitric oxide synthase; TBARS: thiobarbituric acid reactive substances; HSP32: heat shock protein 32 (heme oxygenase-1); Zn-pp: zinc protoporphyrin IX; CX43: Connexin43; MMP: matrix metalloproteinase; IFN: interferon; TLR4: toll like receptor 4; HMGB1: high-mobility group protein B1; NF- $\kappa$ B: nuclear factor kappa B; RAGE: receptor for advanced glycation end products; MCP-1: monocyte chemoattractant protein-1; TEM: transmission electron microscope; RER: rough endoplasmic reticulum; LC3-II: light chain 3 type II.

the amount present: high concentrations in the micromolar range are seen in inflammation, whereas smaller amounts are important in the neuroprotection created by various preconditioning methods.<sup>48,49</sup> The study by Wang et al.<sup>19</sup> observed nitric oxide production in the nanomolar range after HBO-PC, indicating that small amounts of nitric oxide can induce mechanisms involved in the tolerance induced by HBO-PC. Another preconditioning study in 2012 also demonstrated decreased neuronal apoptosis after SCI, indicating the potential use of HBO-PC to provide neuroprotection prior to neurosurgical procedures involving the spinal cord.<sup>23</sup>

### HBO therapy decreases oxidative stress and lipid peroxidation

Free radical generation occurs early after SCI and plays a key role in secondary injury.<sup>12</sup> The central nervous system is particularly susceptible to the oxidative stress due to its high lipid content; accordingly, lipid peroxidation is a significant component of secondary injury after SCI and a popular target of pharmacological intervention.<sup>3</sup> Many studies have shown the ability of HBO therapy to attenuate oxidative stress, but few have studied the exact mechanism

by which HBO therapy affects lipid peroxidation.

Malondialdehyde (MDA) is a marker of lipid peroxidation after SCI.<sup>50</sup> In a 2006 study of spinal cord ischemia-reperfusion injury *via* aortic cross-clamping in rabbits, once daily HBO-PC for 5 days resulted in significantly lowered MDA levels after SCI in the HBO-PC group, with concomitant increases in catalase and superoxide dismutase.<sup>16</sup> These findings correlated with improved motor recovery indicated by higher modified Tarlov scores in the HBO-PC group, suggesting that HBO-PC protects against free-radical mediated lipid peroxidation and improves neurological outcomes by upregulating antioxidant enzymes.<sup>16</sup> Unlike the ischemia-reperfusion injury and HBO-PC paradigm used by Nie et al.,<sup>16</sup> the study by Topuz et al.<sup>21</sup> in 2010 involved a single 90-minute HBO treatment immediately after SCI secondary to clip compression of the spinal cord. HBO therapy increased activities of glutathione peroxidase, superoxide dismutase, and catalase, while significantly attenuating MDA levels compared to the SCI only group, further suggesting that HBO therapy can decrease lipid peroxidation by upregulating antioxidant enzymes; furthermore, these findings were accompanied by improved neurological recovery indicated by higher





scores on the inclined plane test, signifying that HBO therapy improves neurological outcomes by diminishing oxidative stress.<sup>21</sup> An earlier study in 2007 measured levels of thiobarbituric acid reactive substances (TBARS) to monitor lipid peroxidation in a rat SCI model involving clip compression at T<sub>8-10</sub> and showed that TBARS were significantly higher in the SCI only group *versus* sham-operated rats.<sup>17</sup> While methylprednisolone showed no significant effect on these levels when administered after SCI, HBO therapy at 2.8 ATA given twice daily over four days resulted in significantly decreased TBARS and significantly increased superoxide dismutase activity, further indicating the ability of HBO therapy to reduce lipid peroxidation and thus alleviate one of the major mechanisms of secondary injury.<sup>17</sup>

Many studies have shown that HBO-PC leads to upregulation of antioxidant enzyme levels after SCI.<sup>16,18,19,27,29</sup> Nie et al.<sup>16</sup> showed that HBO-PC increased catalase and superoxide dismutase after reperfusion and correlated with improved motor recovery, and this was reversed by administration of the catalase inhibitor, 3-amino-1,2,4-triazole (AT), 1 hour before ischemia-reperfusion injury; furthermore, administration of dimethylthiourea (DMTU), a free radical scavenger, 1 hour prior to each daily HBO-PC caused a significant reduction in catalase and superoxide dismutase activities compared to the HBO + SCI group, indicating that the initial population of reactive oxygen species after HBO-PC may be responsible for activating the upregulation of antioxidant enzymes, which then reduce the oxidative stress associated with secondary injury post-SCI. Wang et al.<sup>19</sup> also showed that superoxide dismutase and catalase increased after HBO-PC in an ischemia-reperfusion SCI model in rats, and this led to decreased superoxide and hydrogen peroxide reactive oxygen species and correlated with improved motor recovery.

Heme oxygenase-1 is a heat shock protein (HSP32) that is increased within 24 hours after SCI.<sup>51,52</sup> The enzyme catalyzes heme breakdown to carbon monoxide, iron, and biliverdin, with bilirubin production resulting from the subsequent reduction of biliverdin.<sup>53</sup> Carbon monoxide, biliverdin, and bilirubin all have antioxidant properties, and heme oxygenase-1 has also been shown to protect against cell death by regulating iron.<sup>51,52,54</sup> Accordingly, heme oxygenase-1 knockout mice have increased susceptibility to oxidative injury.<sup>55</sup> In 2007, an *in vitro* study utilizing primary culture mice spinal neurons showed that a single HBO-PC session significantly increased cell viability beginning at 4 hours after treatment, reduced DNA damage, and enhanced protein and mRNA expression of heme oxygenase-1; however, administration of tin-mesoporphyrin IX

(SnMP), a specific heme oxygenase-1 inhibitor, 10 minutes prior to HBO-PC completely reversed the neuroprotection, indicating that heme oxygenase-1 plays an important role in the ischemic tolerance induced by HBO-PC.<sup>18</sup> Furthermore, HSPs are molecular chaperones that are important for repairing damaged proteins and have demonstrated to exert a beneficial effect after SCI by contributing to the attenuation of secondary injury.<sup>56</sup> In another *in vitro* study utilizing primary culture rat spinal neurons in 2014, a single 60-minute HBO-PC session 12 hours before injury significantly increased cell viability and increased levels of HSP32, which peaked at 12 hours post-HBO exposure; administration of zinc protoporphyrin IX (Zn-pp), a specific HSP32 inhibitor, immediately prior to HBO-PC reversed the effect of HBO-PC on cell viability, further suggesting that upregulation of HSP32 contributes to the neuroprotective effect of HBO-PC.<sup>29</sup>

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor involved in regulating the antioxidant response element (ARE), which governs the expression of antioxidant enzymes and is especially activated in astrocytes, thereby contributing to neuroprotection against oxidative stress after SCI.<sup>57-60</sup> A 2014 study showed that at 12 and 24 hours after a single session of HBO-PC, Nrf2 mRNA/protein expression, DNA binding activity, and expression of downstream genes involved in glutathione synthesis were all significantly increased in the spinal cord compared to controls; furthermore, glutathione content in the spinal cord was significantly increased by HBO-PC compared to the SCI only group up to 48 hours after ischemia-reperfusion injury.<sup>27</sup> Immunofluorescence staining of spinal cord tissue revealed greater Nrf2 immunoreactivity in astrocytes *versus* neurons, indicating that HBO-PC provides neuroprotection by upregulating Nrf2 specifically in astrocytes of the spinal cord.<sup>27</sup>

### **HBO therapy reduces inflammation**

Many recent studies have investigated the effect of HBO on the inflammatory processes that follow SCI.<sup>20,24-26,31,32,34,35</sup> In 2010, a rat model involving clip compression injury of the spinal cord at T<sub>8-9</sub> showed decreased inflammatory cytokines, IL-1 $\beta$  and TNF- $\alpha$ , and decreased myeloperoxidase (MPO), an indicator of neutrophil infiltration, after a single HBO treatment immediately after SCI; additionally, increased level of the anti-inflammatory cytokine IL-10 was observed in the HBO therapy group, indicating that HBO not only reduces inflammatory mediators, but also promotes an anti-inflammatory phenotype.<sup>20</sup>

Macrophages and microglia are the primary mediators of inflammation in the secondary injury following SCI.<sup>3</sup> The macrophage response following after SCI is polar-



ized, consisting mostly of the “classically activated” M1 macrophages that promote the production of inflammatory cytokines and free radicals, and very low expression of the “alternatively activated,” anti-inflammatory M2 macrophages.<sup>61,62</sup> The effect of multiple HBO treatments on macrophage polarization after clip compression of the spinal cord in a rat model was examined in 2015 and showed that HBO significantly decreases the M1 phenotype (iNOS and CD16/32 positive cells) and its corresponding Th1 cytokines, interferon (IFN)- $\beta$  and TNF- $\alpha$ , and significantly increases the M2 phenotype (arginase-1 and CD206 positive cells) and its corresponding Th2 cytokines, IL-4 and IL-13; these findings were accompanied by improved axonal growth, significantly greater myelin sparing, and greater functional recovery, indicating that HBO therapy provides neuroprotection by promoting the anti-inflammatory M2 phenotype and downregulating the inflammatory M1 phenotype.<sup>32</sup>

As previously mentioned, decreased neutrophil infiltration, monitored by decreased MPO, has been observed after HBO therapy of SCI.<sup>20,37</sup> The study by Wang et al.<sup>37</sup> further examined the effect of HBO therapy on the inflammatory cell response after SCI by monitoring monocyte chemoattractant protein-1 (MCP-1), a chemokine involved in recruiting monocytes and lymphocytes to sites of inflammation and increases following SCI.<sup>63</sup> Inhibiting MCP-1 has shown to decrease mononuclear cell infiltration of injured spinal cord tissue, thereby decreasing inflammatory damage.<sup>64</sup> In 2016, the upregulation of MCP-1 after SCI was significantly decreased by HBO therapy and correlated with improved neurological outcomes in a rat model, indicating that downregulation of MCP-1 is involved in the neuroprotective effects of HBO therapy after SCI.<sup>37</sup>

The effect of HBO therapy on NALP3 was previously discussed in the context of apoptosis, but in 2015, the role of the NALP3 inflammasome in the ability of HBO therapy to attenuate inflammation after SCI was investigated. The NALP3 inflammasome is a multimeric protein complex consisting of NALP3, ASC, and caspase-1, that mediates IL-1 $\beta$  production and release, which in turn induces further inflammatory cytokine release.<sup>65</sup> Daily HBO therapy significantly decreased mRNA and protein expression of NALP3 at 1, 3, and 7 days post-SCI, whereas mRNA and protein levels of ASC and caspase-1 were significantly decreased at 3 and 7 days post-SCI; furthermore, IL-1 $\beta$  levels were also significantly reduced at 3 and 7 days post-SCI.<sup>35</sup> Given that reactive oxygen species can trigger NALP3 inflammasome signaling in retinal pigment epithelial cells, Liang et al.<sup>35,66</sup> proposed that the decreased expression of NALP3, ASC, caspase-1, and IL-1 $\beta$  after HBO therapy resulted from the previously evidenced ability of HBO

therapy to decrease ROS generation.

Matrix metalloproteinases (MMPs) are zinc-containing endopeptidases that are synthesized in an inactive, zymogen, form and are involved in extracellular matrix degradation.<sup>67</sup> MMP-2 and MMP-9 stimulate the production of pro-inflammatory cytokines, thereby contributing to secondary injury post-SCI.<sup>68-71</sup> In 2013, HBO therapy post-SCI resulted in significantly reduced MMP-2 and MMP-9 and correlated with decreased level of the inflammatory cytokine IL-6, indicating that the downregulation of MMPs plays a role in the neuroprotection provided by HBO.<sup>24</sup>

High-mobility group protein B1 (HMGB1) is a mediator of inflammation that is activated by inflammatory cytokines such as TNF- $\alpha$ , and has been shown to increase after SCI.<sup>72,73</sup> HMGB1 has been shown to stimulate toll like receptors (TLRs), receptor for advanced glycation end products (RAGE), and nuclear factor  $\kappa$ B (NF- $\kappa$ B), which in turn activates other inflammatory cytokines, including IL-1 $\beta$  and TNF- $\alpha$ , thereby contributing to inflammatory damage after SCI.<sup>74-77</sup> HBO therapy has the ability to reduce inflammation after SCI by significantly downregulating HMGB1 and its subsequent signaling cascades, including NF- $\kappa$ B and its p65 component, TLR4, TLR2, RAGE, IL-1 $\beta$  and TNF- $\alpha$ .<sup>25,26,34</sup>

Gap junctions are specialized types of intercellular channels that allow for the passage of ions, molecules, and second messengers between adjacent cells.<sup>78</sup> Connexins are the major structural units of gap junctions, and connexin43 (CX43) composes gap junctions within the central nervous system.<sup>79</sup> Previous studies have shown that CX43 is upregulated in the injured spinal cord after SCI, allowing dissemination of injury mediators from damaged cells to surrounding healthy cells that survived the primary insult.<sup>80,81</sup> In 2014, Liu et al.<sup>28</sup> showed that daily HBO therapy after SCI significantly reduces mRNA and protein expression of CX43, thereby contributing to reduced inflammation by preventing the spread of inflammatory mediators from injured cells to healthy cells, as reported in other studies that have examined the role of CX43 and SCI.<sup>82,83</sup>

### **HBO therapy promotes angiogenesis**

Vascular endothelial growth factor (VEGF) is important for angiogenesis in the CNS *via* stimulation of endothelial cell proliferation and migration, but VEGF is also important in promoting neuronal proliferation and neuroprotection.<sup>84</sup> Tai et al.<sup>20</sup> first reported that a single HBO treatment initiated immediately after SCI results in increased VEGF-positive cells at 4–7 days post-SCI. Since then, two other studies involving daily HBO treatments after SCI have also showed significantly increased VEGF compared to the SCI-only group.<sup>24,28</sup> VEGF has previously been shown to increase



after SCI to restore blood supply by increasing vascular density, thereby promoting neuronal protection and functional recovery; thus, the improved neurological recovery seen with HBO therapy of SCI may be, in part, due to increased expression of VEGF.<sup>85</sup>

### HBO therapy reduces spinal cord edema

Recent studies have shown that HBO therapy significantly reduces spinal cord edema post-SCI.<sup>24,27,31</sup> MMP-2 and MMP-9 were previously discussed in the context of inflammation, but these enzymes also cause increased permeability of the blood-spinal cord barrier *via* degradation of type IV collagen, leading to spinal cord edema.<sup>68,69</sup> In 2013, twice daily HBO therapy after SCI in a rat model resulted in significantly decreased MMP-2 at 3 days post-SCI and MMP-9 levels at 2, 3, and 5 days post-SCI; spinal cord water content was also significantly decreased at 2 and 3 days post-SCI, suggesting the ability of HBO therapy to decrease spinal cord edema by downregulating metalloproteinases after SCI.<sup>24</sup>

In 2014, protein water channels called aquaporins were studied after HBO therapy of SCI.<sup>31</sup> Rats treated with HBO beginning at 4 hours after SCI and continuing for a total of four times daily over three consecutive days were observed to have significantly reduced mRNA and protein expression of aquaporin 4 (AQP4) and aquaporin 9 (AQP9).<sup>31</sup> Previous work has demonstrated the positive correlation between increased expression of AQP4 and increased spinal cord water content after SCI, and further studies showed that neurological outcome is greatly enhanced post-SCI in AQP4-null mice.<sup>86,87</sup> Accordingly, a component of the decreased spinal cord edema after HBO therapy of SCI involves the downregulation of AQP4, which leads to reduced water entry into the spinal cord.<sup>31</sup>

### HBO therapy increases autophagy

Autophagy is a mechanism of intracellular degradation by which autophagosomes deliver cellular components that require degrading to lysosomes to maintain cellular homeostasis.<sup>88</sup> Important factors for monitoring autophagy are beclin-1, a direct stimulator of autophagy, and light chain 3 type II (LC3-II), a protein that is directly proportional to the number of autophagic vacuoles.<sup>89,90</sup> The first and only study on the effect of HBO therapy on autophagy after SCI showed that Beclin-1 and LC3-II significantly increase in injured spinal cord neurons and glial cells at three days after SCI in rats receiving daily HBO therapy.<sup>36</sup> Further work is needed to elucidate the exact mechanism, but this first report by Sun et al.<sup>36</sup> indicates that HBO therapy upregulates autophagy after SCI to promote repair and protection.

## FUTURE RESEARCH

While the benefits and potential neuroprotective mechanisms of HBO therapy in the treatment of SCI have been demonstrated in many experimental studies, very few clinical studies have been performed (**Table 2**). The most recent clinical study involved a total of 34 patients that suffered cervical hyperextension injury without bone damage.<sup>91</sup> In this retrospective study, the 13 patients that received 85 minutes of HBO therapy at 2.0 ATA once daily for ten days were found to have significantly higher improvement rates indicated by the Neurological Cervical Spine Scale (NCSS) versus the non-HBO group of 21 patients.<sup>91</sup> In the first clinical study of HBO therapy of SCI in 1977, 13 patients with compressive SCI that experienced persistent neurological deficits after surgery received between ten and 15 HBO treatments given at 1.5 ATA for 40 minutes each; 6 of these patients had significant motor improvement, but the other 7 patients did not display significant neurological change.<sup>92</sup> A year later, two studies involving complete and partial spinal cord lesions showed that HBO therapy at 2.5 ATA administered within 14 hours at most resulted in varying degrees of neurological improvement.<sup>93,94</sup> Although the study by Gamache et al.<sup>95</sup> in 1981 did not show a significant change in recovery rate in patients treated with HBO therapy *versus* conventional therapy, a later report by Yeo<sup>96</sup> showed that 15 of 27 patients with upper motor neuron SCI had meaningful functional recovery after 2–3 HBO treatments within 20 hours of injury.

Unlike experimental studies, these clinical reports demonstrate mixed results regarding the utility of HBO therapy after SCI. Future studies should address some of the underlying factors that may account for the discrepancies between experimental and clinical outcomes.

First, the optimal onset, duration, frequency, and pressure of HBO therapy after SCI require further investigation. Although many treatment paradigms have been studied in experimental models (**Table 1**), no single study has focused on comparing the efficacy these various paradigms in the context of SCI. This is an important area of research given that the heterogeneity of treatment paradigms employed by clinical studies is a factor that complicates the interpretation of outcomes and presents an obstacle in comparing multiple studies.

Second, while each experimental study utilized a single method of homogeneous, reproducible SCI (**Table 1**), spinal cord injuries in clinical practice are heterogeneous and thus may respond differently to HBO therapy. A single experimental study comparing the effect of HBO therapy on multiple injury models could shed light on the possible need for different HBO paradigms for certain types of injuries,



**Table 2: Characteristics and findings of clinical studies**

Author	Year	Injury type	HBO paradigm	Outcome
Holbach et al. <sup>92</sup>	1977	Compression injury	1.5 ATA for 40 minutes, 10–15 treatments	Significant motor improvement in six patients
Jones et al. <sup>94</sup>	1978	Complete or partial spinal cord transection between C6-12	2.5 ATA for 120 minutes, two treatments, onset within 12 hours	Neurological improvement in five patients and functional improvement in two patients
Yeo et al. <sup>93</sup>	1978	Complete or partial spinal cord lesion	2.5 ATA for 90 minutes, two treatments, onset within 14 hours	Faster and greater recovery in five patients
Gamache et al. <sup>95</sup>	1981	Complete or partial spinal cord lesion	Two groups: one received 2.5 ATA and the other received 2.0 ATA. See reference for timing details	No significant change in recovery rate compared to patients receiving conventional therapy
Yeo <sup>96</sup>	1984	Upper motor neuron SCI	2.5 ATA for 90 minutes, two to three treatments, average onset within nine hours of SCI	Meaningful functional recovery in 15 patients
Asamoto et al. <sup>91</sup>	2000	Cervical hyperextension without bone damage	2.0 ATA for 85 minutes once daily for ten days; onset within 24 hours post-SCI	Significantly higher improvement rate indicated by NCSS

Note: HBO: Hyperbaric oxygen; SCI: spinal cord injury; ATA: atmospheres absolute; NCSS: Neurological Cervical Spine Scale.

and such a study may also help explain the conflicting results observed in the clinical reports of HBO therapy post-SCI to this date.<sup>91-96</sup>

Third, many clinical studies have been performed to evaluate the effect of HBO-PC on brain injury, but few studies have focused on the clinical application of HBO-PC to protect against SCI, especially in the context of spinal cord protection during neurosurgical procedures.<sup>97</sup> Given that most experimental studies have focused on post-SCI HBO therapy rather than-PC (**Table 1**), further experimental research of HBO-PC prior to SCI should be performed before conducting clinical studies.

Finally, given that previous clinical studies have involved small patient samples, future studies with larger sets of patients are required to adequately gauge the effectiveness of HBO therapy for SCI in clinical practice.

## CONCLUSION

HBO therapy has been shown to exert neuroprotective effects when administered before or after SCI. Experimental studies have revealed various mechanisms that contribute to these neuroprotective effects, including improved spinal cord oxygen tension, decreased apoptosis, reduced inflammation, attenuation of oxidative stress, and improved angiogenesis and autophagy. However, even though HBO therapy of SCI is a promising treatment based on results of experimental studies, only a few clinical reports have been performed and have shown conflicting outcomes, thereby warranting further studies to elucidate the optimal HBO treatment paradigm and to

determine if the treatment paradigm should depend on the specific type of SCI.

### Author contributions

JHH planned the manuscript. NPP wrote the manuscript under the guidance of JHH. NPP performed literature search and analysis. JHH edited and finalized the manuscript. JHH and NPP both contributed to revisions of the manuscript.

### Conflicts of interest

None declared.

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