Hyperbaric oxygen therapy and promoting neurological recovery following nerve trauma

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ABSTRACT
There is a constant search for new techniques that induce more extensive and rapid wound healing. Hyperbaric oxygen therapy (HBO₂T) involves placing a patient in a sealed chamber and elevating its pressure several-fold above ambient air pressure while the patient breathes 100% oxygen. HBO₂T induces a number of physiological actions, and which wounds are selected for HBO₂T depends on the specific actions of HBO₂T relative to the wound’s healing requirements. Although nerve traumas are not yet indicated for HBO₂T, there are many animal and clinical examples showing the benefits of HBO₂T in inducing neurological recovery following nerve trauma. This review examines the general mechanisms required to induce wound healing and the actions of HBO₂T which meet these requirements. It then examines the requirements for inducing axon regeneration and how many are met by HBO₂T. Finally, we discuss anecdotal evidence that HBO₂T enhances the rate and extent of axon regeneration in both animal models and clinically. We conclude that HBO₂T triggers most of the mechanisms required to induce axon regeneration.

WOUNDS AND WOUND HEALING
Certain characteristics of wounds (ischemic appearance, a history of a lack of healing, physical examination yielding no pulse or a transcutaneous oxygen evaluation suggesting tissue hypoxia), identify a wound as hypoxic, or related to arterial disease. What factors allow one to identify and classify patients with arterial wounds? How does one manage those primarily caused by peripheral arterial occlusion or damage? When and how does one use transcutaneous oximetry to evaluate this subgroup of patients and the use of endovascular interventions such as arteriography, angioplasty and arterial stenting? When is the use of hyperbaric oxygen therapy (HBO₂T) appropriate, and when should it be used to provide arterial revascularization to manage wounds?

Collagen production is maximal at 250mm Hg and falls to almost zero in severe clinical hypoxia (Km=25 mm Hg pO₂) [1-4] due to the failure of collagen fibril cross-linking, which requires the hydroxylation of proline and lysine to synthesize mature collagen [5]. Cell motility decreases as available cell energy production via oxygen decreases to or below 10mm Hg pO₂ [6-8]. Re-epithelization, essential to wound healing, is oxygen-dependent [9-11]. Similarly, elimination of bacteria within a wound is oxygen-dependent due to oxidative killing of bacteria [12-14], and reaches its maximal effect at several hundred mm Hg pO₂, and drops to almost zero in hypoxic patients [15,16]. Thus, since enhanced oxygen presentation induces events required for wound healing, HBO₂T, which provides enhanced oxygen to tissues, is a reasonable place to start wound healing therapy [8,17,18]. However, before discussing HBO₂T itself, it is important to consider the potential influences of oxygen on wound healing.

Bacterial infection
Although many factors contribute to poor wound healing, the most common is wound infection caused by foreign debris and necrotic tissue [19,20]. Therefore, debridement of all necrotic tissue and debris, whether performed by surgical means, the use of enzymatic agents or wound dressings, is critical for achieving wound healing [21].

However, wound hypoxia predisposes tissue to bacterial infection and inhibited wound healing by hypoxia by blocking fibroblast proliferation, collagen production and capillary angiogenesis [22], and also because leukocytes’ oxidative phosphorylation bacterial activities are severely impeded without normal
tissue oxygen levels [23]. Therefore, re-establishing vascularization and providing enhanced oxygen should lead to increased antibacterial activity within a wound [12-14,23].

**Angiogenesis**

Angiogenesis is a dynamic, oxygen-stimulated, oxygen-dependent and growth factor-dependent process [11,24-26], and is directly sensitive to oxygen as a function of local lactate delivery [27]. Therefore, once tissue oxygen tension decreases to 10mm HG there is no angiogenesis, which leads to further oxygen deficiency, preventing tissue granulation and blocking tissue healing [11].

**GROWTH AND WOUND HEALING FACTORS**

At the moment of trauma involving vascular injury, tissue factors and intracellular calcium are released, activating factor VII and initiating the extrinsic coagulation cascade [28]. Concomitant reflex vasoconstriction occurs to aid in hemostasis [29]. Hemostasis is ultimately secured by the end product of the coagulation cascade, the fibrin plug. The fibrin fibers become a provisional wound matrix and are the lattice on which platelets aggregate. Activated platelets are the most abundant cells in the wound in the early post-injury period. Platelets release proinflammatory substances, such as tissue growth factor-beta (TGF-beta) and platelet-derived growth factor (PDGF) [30], as well as a host of other wound healing and regeneration-promoting factors [31-33].

Growth factors are peptides that act on inflammatory cells, fibroblasts and endothelial cells to direct the processes involved in wound healing [34] and in promoting axon regeneration [35]. They are observed in the earliest period post-injury because PDGF and basic fibroblast growth factor (bFGF) are produced by injured cells at the time of trauma. Subsequently, activated platelets release TGF-beta and PDGF that mediate chemotaxis of neutrophils, monocytes and fibroblasts into the wound [36]. Recruited monocytes within the wound bed differentiate into macrophages, which play a central role in all stages of wound healing and orchestrate the wound healing process. During the early and short inflammatory phase macrophages exert pro-inflammatory functions like antigen-presenting, phagocytosis and the production of inflammatory cytokines and growth factors that facilitate the wound healing process [37]. Wound healing and neurotrophic factors can also be administered directly to wound sites by the application of autologous platelet-rich fibrin [34,38].

**Patient nutritional status**

A patient’s good nutritional status is essential for wound healing to take place. Protein deficiency contributes to poor healing rates due to reduced collagen formation and wound dehiscence [39]. Proteins, co-factors, essential fatty acids and proper calorie intake must be optimized for the collagen deposition, angiogenesis, epithelization and ground substance to facilitate wound healing. Skin breakdown resulting in high exudate loss can result in a deficit of as much as 100 gm of protein per day [40].

There is a correlation between low serum albumin and body mass index (BMI) and the development of pressure ulcers [41]. Because fatty acids are critical constituents of the cell membrane and are the source of prostaglandins that mediate inflammation, deficiency of essential fatty acids causes impaired wound healing. Deficiency of vitamins C or K leads to scurvy and coagulopathy, respectively [42]. Minerals, including calcium, iron, copper, zinc and manganese, must be delivered to the wound milieu to act as co-factors for vital reactions in the synthesis of proteins needed in the healing process [43].

Topical application of essential fatty acids to the entire body, including potential wound sites, improves tissue hydration and elasticity and helps prevent skin breakdown in individuals with a poor nutritional status [39]. Such application results in a 36% reduction in the development of pressure ulcers [44]. The influences of the topical application of essential fatty acids may be especially effective for the severely malnourished.

**WHAT IS HYPERBARIC OXYGEN THERAPY?**

A typical clinical hyperbaric oxygen therapy (HBO₂T) protocol involves placing a patient in a chamber once or twice a day and raising the air pressure to 1-3 atmospheres (ATA). Under these conditions the patient breathes 100% oxygen via a head hood for several cycles of about 45 minutes, with 20 minutes in between of breathing normal air [45]. However, sessions may last up to three hours and be repeated several times per day [46]. The number of HBO₂T treatments can vary depending on the type and severity of a wound. The importance and success of HBO₂T in wound healing is well established, making HBO₂T widely applied for this purpose, especially as an important adjunct to the management of problem wounds caused by chronic oxygen deficiency, where local oxygen tension is below that required for optimal healing.

When the normal reparative process of wound healing is interrupted, a chronic wound develops.
Definitions of wound, acute wound, chronic wound, healing and forms of healing, wound assessment, wound extent, wound burden and wound severity are well defined in the paper by G. S. Lazarus et al., 1994 [47]. Similarly, the cascade of cellular and molecular events involved in wound healing is discussed thoroughly elsewhere and will not be discussed in detail here.

A brief history of the development of hyperbaric medicine

In 1662, a British clergyman named Henshaw started recompression chamber history with an organ bellows-driven air control device that could create hyperbaric (above normal) and hypobaric (below normal) conditions within a large chamber. Although he had no scientific basis for his theories, Henshaw believed patients suffering from acute conditions would benefit from increased air pressure, while those suffering chronic ailments would profit from a lower-pressure environment. The primary drawback to this device was the lack of oxygen with which to fill the chamber because it was not discovered until 1774 by Joseph Priestley.

Physicist and chemist Robert Boyle contributed to understanding the use of pressurized gas by describing the behavior of an ideal gas as stated in Boyle’s law (1660) in the paper “New Experiments Physio-Mechanical, Touching the Spring of the air and its Effects.” Boyle described the effects of decompression illness in 1670 after using a vacuum pump to decompress a snake. Subsequently together with physicist Robert Hooke he designed an air pump to study the “elastic properties of air.” In the 1830s the study of hyperbaric medicine began, in which hyperbaric chambers were used containing 2 and 4 atmospheres of absolute pressure to increase blood circulation to the internal organs, improve cerebral blood flow and produce a feeling of well-being.

However, not until 1917 did the German company Dräger design a system in which oxygen under pressure started being used to treat individuals suffering from diving accidents. It was not until 1937 that physicians Albert Behnke and Louis Shaw started using hyperbaric oxygen to treat decompression sickness and when HBO$_2$T became a real research and clinical tool.

In 1965, Dr. I. Boerema reported that HBO$_2$T assisted in cardiopulmonary surgery, the transposition of great vessels and for pulmonic stenosis [48,49]. Subsequently, W.H. Brummelkamp published findings that anaerobic infections were inhibited by HBO$_2$T [50]. Boerema then published the article “Life Without Blood” reporting on fatally anemic pigs that were successfully treated with volume expansion and pressurized hyperoxygenation, which led to Boerema often being credited as the father of modern-day hyperbaric medicine [51].

Actions of HBO$_2$T benefiting wound healing

Delayed wound healing leads to increased complications for the patient and significantly increased medical expenses associated with prolonged hospitalization (A Consensus Conference, Ravenna, 2006). Elemental oxygen is required to maintain cellular respiration and allow normal cellular protein production. Therefore, HBO$_2$T is considered a method for augmenting oxygen availability to tissues. Oxygen is also an important mediator of wound healing and its availability influences wound healing rates. Tissue trauma leads to decreased oxygen delivery precisely to the damaged tissue which needs the oxygen most because of the tissue’s immediate increased demand for oxygen to perform the wound healing process [52]. Therefore, trauma-related blood flow reduction or elimination induces hypoxia, which stops cell energy production, stops wound healing [1,2,6,7] and interferes with many components of wound healing, causing the slowing of the healing process [52], especially during the critical inflammatory phase of wound healing [53]. The presence of oxygen, especially in increased concentrations, reduces wound edema [54,55] and significantly enhances the rate of wound healing [52]. Thus, there is a direct relationship between the available amount of oxygen in the wound and the rate of the healing processes [56], with part of this being directly related to the need for oxygen in the inflammatory phase of wound healing [53].

For the treatment of hypoxic and ischemic wounds, the most important effects of hyperbaric oxygenation are stimulating fibroblast proliferation and differentiation, increasing collagen formation and cross-linking, augmenting vascularization, stimulating leukocyte microbial killing, and inducing the release of various wound healing factors [11-14,22-26]. Ischemic soft tissues also benefit from hyperoxygenation through improved preservation of energy metabolism and the reduction of edema. Therefore, application of HBO$_2$T is an extremely reasonable adjunct to standard wound healing techniques [17]. In some cases, such as when neuroprotection is required against transient focal cerebral ischemia, higher pressure (3 ATA) is more effective [57]. However, for anoxic brain injuries, autism and other brain problems, the typical pressures used are 1.3-1.8 ATA.
HBO₂T is critical to the treatment of chronic non-healing wounds due to its inducing angiogenesis, which is promoted by the increased oxygen gradient caused by HBO₂T [58-60]. Decreased edema noted following HBO₂T allows better diffusion of oxygen and nutrients through tissues while also relieving pressure on surrounding vessels and structures [61]. In this light, HBO₂T has been used for treating venous and arterial insufficiencies, burn wounds, crush injuries, marginal flaps and skin grafts. Before initiating HBO₂T, it is important to optimize the patient’s overall medical status, facilitate nursing care of the patient and address local wound care and dressing [62].

HBO₂T is advocated for the treatment of severe trauma of the limbs in association with surgery because of its effects on peripheral oxygen transport, muscular ischemic necrosis, compartment syndrome and infection prevention [63,64]. Thus, HBO₂T is effective in improving wound healing and reducing repetitive surgery and is a useful adjunct in the management of severe crush injuries of the limbs [63,64].

Delivery of 100% oxygen under elevated pressure causes a systemic increase in blood oxygen concentration, while the elevated pressure leads to a significantly increased oxygen transfer from the blood to all body tissues. But with the specific aims of increasing oxygen transfer to tissues under stress are enhancing the rate of wound healing, reducing wound edema [54,55], reducing muscular ischemic necrosis and compartment syndrome, and preventing infection by killing bacteria within a wound in an oxygen-dependent manner [15,16,63], thus leading to recovery from trauma or stress [65]. An appropriate candidate for HBO₂T treatment is a patient who has local ischemia but responds to the oxygen challenge [66].

**Oxygen mapping of tissues and organs**

Important for promoting general wound care and healing is the determination of the oxygen-related wound pathophysiology to allow for an understand of the changing status of wound tissue during the wound healing process as a patient undergoes HBO₂T [67]. Transcutaneous oxygen measurements determine the local ramifications of macrovascular and microvascular disruption. It is an easy, reliable and non-invasive technique for measuring local oxygenation relative to healthy body regions and can be performed while non-healing wounds undergo HBO₂T to speed the healing process [67].

Determining transcutaneous oxygen levels can be affected by local factors such as improper electrode placement, cellulitis, edema and increased skin thickness. Systematically, ventilation and cardiac output, limb macroperfusion and the patient’s hemoglobin can also influence the results of transcutaneous oxygen measurements. The vasoconstrictive effects of smoking also may interfere with transcutaneous oxygen levels.

Determining the increased hemoglobin oxygen saturation in the blood within the tissue of interest can be achieved using field-mapping quantitative magnetic resonance imaging (MRI). The technique involves MRI susceptometry-based oximetry measuring blood oxygen saturation in the tissue of interest versus that of the surrounding tissue [68]. Similar analysis can be performed using picosecond diode lasers, fast photodetectors, and time-correlated single photon-counting electronics which enable depth-resolved estimations of changes in the absorption, and as a consequence, assessment of changes in hemoglobin concentrations from oxyhemoglobin (HbO₂) to deoxyhemoglobin (Hb) in the tissue of interest [69]. The use of transcranial magnetic stimulation (TMS) motor mapping together with functional near-infrared spectroscopic imaging (NIRS imaging) can provide information about the relationship between neuronal activity and oxygenation responses [70]. Increased neuronal activity with increased oxygen indicates an improvement in the neurological status of the tissue. Increased oxygen consumption is manifested by deoxygenation, which consists of a significant increase in deoxygenated hemoglobin concentration (HbR) and a non-significant decreasing tendency in oxygenated hemoglobin concentration (HbO₂). The delayed response phase represents an excess of incoming blood flow, which appears as an increase in HbO₂/total Hb and a decrease in HbR following the early response [70].

The normal lower extremity transcutaneous oxygen measurement should be approximately 50mm Hg. Minor physiologic variations may occur in the same individual. A standard control on the trunk of the patient (usually the second intercostal space) is used in conjunction with the local wound and surrounding tissue transcutaneous oxygen mapping. Measurements should be obtained from at least four sites at equal distances around the ulcer.

Under normal conditions, 97.5% of oxygen is carried in the bloodstream bound to hemoglobin, with the remaining 2.5% dissolved in plasma. Oxygen is combined with hemoglobin in the bloodstream, with each gram of hemoglobin combined with 1.34 cm³ of oxygen, the maximum physiologic maximum carrying capacity. Under normal conditions at sea level, the
arterial hemoglobin saturation is 97%, and the venous hemoglobin saturation is 70%. The oxygen content can be calculated according to Henry’s law, which states that “at a constant temperature, the amount of a gas that will dissolve in a liquid is proportional to the partial pressure of the gas” [71,72].

A hyperoxic challenge (100% oxygen for 20 minutes) normally increases the transcutaneous oxygen reading to greater than 300mm Hg [65]. Generally, responses of less than 50mm Hg require a vascular workup, and HBO₂T is likely of little benefit. Patients with intermediate responses may benefit from HBO₂T. An appropriate candidate for HBO₂T is a patient who has local ischemia but responds to the oxygen challenge [66]. The response to the HBO₂T by further transcutaneous oxygen should be tested after 14-20 HBO₂ treatments.

**Systemic vs. Topical HBO₂T**

It is critical to make a distinction between systemic HBO₂ and topical oxygen, because they are entirely different [73-75]. Topical oxygen involves the use of an airtight chamber or polyethylene bag sealed around a limb or the trunk by a constriction/tourniquet device or tape. High oxygen flow (usually 10 liters per minute) is introduced into the bag and over the wound at a pressure only slightly above 1.0 atmosphere [76]. Systemic HBO₂T involves 100% oxygen presentation in an environment in which the entire body is introduced to pressures of up to 2.4 times atmospheric pressure [57]. An additional difference between the two techniques is that topical oxygen therapy does not provide the oxygen penetration of the tissue to which it is delivered at the same concentration as when it is inspired.

The use of topical oxygen has been explored because its delivery is less complex and less expensive than systemic HBO₂T [77]. Data on the influences of topical oxygenation come mostly from small and non-randomized studies on a variety of wound types [78]. It is not a recommended form of therapy by the UHMS due to its unproven efficacy.

**Other uses**

HBO₂T benefits the acceptance of ischemic split-thickness skin grafts when there is a risk of their not taking, flap survival and salvage, wound re-epithelization, recovery from techniques in plastic and reconstructive surgery [79], and repair of crush injuries and acute post-traumatic limb ischemia [80]. Additional areas in which HBO₂T is useful for healing include: traumatic brain injuries preorbital reconstruction, gas gangrene, compartment syndromes, acute traumatic ischemia, enhancement of healing in selected problem wounds (ulcers and diabetic ulcers), exceptional blood loss anemia, necrotizing soft tissue infections, thermal burns [81]. HBO₂T has been advocated, both as an adjunctive or primary form of treatment, for a variety of disorders, including, osteoradionecrosis, carbon monoxide poisoning and irradiation of non-healing wounds to improve ischemic wounds before skin grafting [82].

**Caution in using HBO₂T**

HBO₂T is very safe under appropriate supervision and use, although toxic effects of oxygen are observed at extremely high doses over prolonged periods. However, because HBO₂T increases the relative dose of oxygen, susceptible patients must be identified and HBO₂T protocols modified to prevent oxygen toxicity [83]. Damaging or toxic effects of oxygen therapy are likely related to the unbridled formation and release of reactive oxygen species such as superoxide, hydroxyl radical and hydrogen peroxide [84]. Superoxide dismutase, catalase, glutathione and glutathione reductase keep the formation of these radicals in check until the oxygen load overwhelms the enzymes, resulting in detrimental effects on cell membranes, proteins and enzymes [85]. Other antioxidants used by the body include Vitamins C and E, selenium and glutathione [85].

Caution is required when applying HBO₂T to patients with diabetes mellitus, because HBO₂T may affect glucose uptake and metabolism [86]. The vasoconstricting effects of HBO₂T therapy may impair subcutaneous absorption of insulin as well, rendering the patient hypoglycemic [87].

**HBO₂T protocols**

HBO₂T for wound healing, compromised skin graft and/or flaps, thermal burns, crush injury and/or compartment syndrome involves 2.0-2.5 atmospheres with 100% oxygen [88]. In some cases, such neuroprotection against transient focal cerebral ischemia, even higher pressures (3 atmospheres) are more effective [57]. Similarly, in cases such neuroprotection against transient focal cerebral ischemia, 3 atmospheres are more effective than lower pressures [57], while for anoxic brain injuries, autism and other brain research, atmospheres of 1.3-1.8 pressure are used.

HBO₂T induces an eight- to ninefold increased vascular density over both normobaric oxygen and
Foot wounds of diabetic patients

Foot wounds of patients with diabetes offer a particularly difficult problem because these patients often have an impaired immune system, predisposing them to infections [90]. Blood supply to the wounds is hindered because the red blood cells are sticky and non-pliable, which leads to capillary occlusion and distal ischemia. Neuropathies render the foot insensitive and impair motor function; this can lead to a flattened foot and pressure relief with special beds, orthosis or splints may be necessary to optimize the local wound milieu. Patients should be advised to stop smoking, since nicotine adversely affects the wound’s vascularity and increases potential complications of HBO

Reperfusion injuries

The benefits of HBO₂T on ischemic insults, ischemia reperfusion injuries, and crush injuries also have been subject to controversy. These injuries result from the reperfusion that follows an extended period of ischemia [94]. Oxygen free radicals rise, thromboxane A2 and adhesion molecules are activated, platelet aggregation occurs and vascular vasoconstriction activity is increased. The endothelium is damaged, which promotes vascular leakage, edema and thrombosis. Tissue necrosis ensues, and the activation of white blood cells is pivotal to the reperfusion injury. Using HBO₂T that may increase oxygen free radicals to benefit the reperfusion injury seems paradoxical. HBO₂T promotes hyperoxygenation and vasoconstriction to decrease edema and neurovascularization and inhibits neutrophil activation, preventing margination, rolling and accumulation of white cells [95]. Neutrophils therefore are not permitted to produce detrimental oxygen free radicals.

Mechanisms by which HBO₂T induces wound healing

Wound healing is a dynamic process requiring oxygen for optimal restoration of tissue integrity and re-establishment of function. Healing results from a cascade of processes, including blood coagulation, inflammation and its reduction, ground substance and matrix synthesis, angiogenesis, fibroplasia, epithelization, wound contraction and tissue remodeling [96]. These complex, overlapping processes are best organized into four phases of healing: hemostasis, the inflammatory phase, the proliferative phase and the maturation phase [97]. If any of these reparative processes is interrupted, a wound remains chronic.

Wound healing requires increased collagen production, fibroblast proliferation and enhanced vascularization. Arterial occlusion or vasoconstriction, hypotension, hypothermia and peripheral venous congestion delay wound healing by slowing the production of collagen. As tissue oxygen levels falls, collagen fibril cross-linking begins to fail because oxygen is required for the hydroxylation of proline and lysine to synthesize mature collagen [5].

Deposition of collagen

Collagen synthesis, deposition and reduced collagen degradation, which are critical for wound healing [4,98-102], and requires oxygen dependent prolyl-hydroxylase hydroxylation of proline [103]. Wound healing takes place where the rate of collagen synthesis is maximum and is also associated with the enhanced presence of hyaluronic acid (HA) and fibronectin (FN), which leads to an increased number of fibroblasts and an increased deposition of oriented collagen fibers [22,104,105].

Macrophage activation and recruitment

During the progression of the inflammatory phase, eicosanoids interact with other cells to increase the ratio of PGF2-alpha to PGE2 during late inflammation, which is a stimulus for fibroblasts to begin to synthesize collagen.
and ground substance [106]. Additionally, macrophages that are recruited to wound sites release growth factors at optimal levels to strongly induce the influx of fibroblasts, then keratinocytes and endothelial cells, into the wound [34,37]. The cellular population of the wound becomes predominantly mononuclear, with a declining number of neutrophils and macrophages, signaling the end of the inflammatory phase and the initiation of the proliferative phase [37]. At this time fibroblasts are driven by macrophage-derived bFGF, TGF-beta and PDGF to proliferate and synthesize glycosaminoglycans and proteoglycans, the building blocks of the new extracellular matrix of granulation tissue, and collagen [107], which fibroblasts assemble extracellularly into collagen fibers. These fibers are then cross-linked and organized into bundles, the major component of acute wound connective tissue. Finally, with decreasing hyaluronic acid concentration and rising chondroitin sulfate levels there is a slowing of the in-migration of fibroblast migration and proliferation, leading to the maturation phase of wound healing.

**T-cells**
After hemostasis has been obtained, polymorphonuclear (PMN) leukocytes enter the area of injury, drawn by chemotactic substances released by platelets and release cytokines [108,109]. Other leukocytes, specifically helper T-cells, are sources of the cytokine interleukin (IL)-2 that promote the proliferation of further T-cells to aid in the immunogenic response to injury [110]. T-cells are also important for stimulating and activating macrophages [109]. The macrophages are primarily responsible for wound debridement, but as discussed earlier, also secrete substances such as bFGF, a chemotactic and mitogenic factor for fibroblasts and endothelial cells, and interleukin (IL)-1, which stimulates the proliferation of multiple cells of inflammation and induces the replication of endothelial cells, promoting angiogenesis.

**Inflammation**
Inflammation following tissue trauma provides both benefits and hindrances to wound healing. The physiologic processes underlying wound inflammation begin immediately upon tissue injury. The inflammatory phase of wound healing is clinically characterized by signs of redness, heat, swelling, pain and loss of function [111] with the inflammation generated by metabolites and redness caused by vasodilation, primarily a result of prostacyclin (PGI2) [112]. The edema is potentiated by PGE2 and prostaglandin F2-alpha (PGF2-alpha), where PGI2 and PGE2 promote local blood flow, causing the localized warmth in the area of inflammation, but also allows for the entry of inflammatory cells into the wound, due to increased vascular permeability [113-116]. These cells then release cytokines responsible for fever production [117]. Pain is elicited by the effects of PGI2, PGE, and PGE2 on peripheral sensory nerve endings [118, 119]. Simultaneously, the coagulation cascade, the arachidonic acid pathways and the synthesis and release of growth factors and cytokines initiate and maintain the inflammatory phase and the sequence of cells involved in the process [120]. However, in the presence of an uncontrolled pathological inflammatory response in the wound bed, HBO2T enhances wound healing by its anti-inflammatory effects [121].

**HBO2T and peripheral axon regeneration**
For HBO2T to promote and enhance axon regeneration, it must trigger mechanisms associated with axon regeneration, such as enhanced metabolism of neurons and other associated cells, angiogenesis, increased oxygen presentation, collagen synthesis and deposition, macrophage recruitment, protein synthesis, Schwann cell proliferation, synthesis and release of neurotrophic factors, the induction of axotomized neurons to extend axons, axon elongation, increased axon diameters, axon myelination, and development of appropriate axon conduction velocities.

**Animal models**
Of critical importance to re-establishing neurological function following nerve trauma is the maintenance of the integrity of the nerve and associated tissues at the trauma site. Constriction or pressure nerve trauma results in the production of oxygen-derived free radicals that lead to neurogenic inflammation ischemia and reperfusion. Working with the adult rat sciatic nerve constriction model, experimental animals underwent HBO2T at 3 ATA for two hours. Non-HBO2T-treated animals showed marked tissue edema at the constriction site as well as swollen mitochondria, loss of cellular integrity, multiple vacuole formations in nerve and muscle tissue, widened sarcomeres in muscle, and degenerative changes in the nerve myelin sheaths [61]. However, HBO2T-treated animals had minimal to no edema and had preserved cellular structure including mitochondrial integrity, no vacuole formation, and maintenance of normal, easily identifiable nerve structures [61]. These results indicate that by maintaining tissue integrity, HBO2T should enhance the rate and extent of neurological recovery following nerve trauma.
Following a facial nerve crush, rabbits treated with HBO₂T had an increase in the mean diameter of regenerating axons but showed no difference in number of myelinated axons compared to untreated animals [122]. Using the rat cavernous nerve (CN) crush injury model, HBO₂T preserved nerve function mediated via preservation of neurotrophic and endothelial factor expression [123]. In other experiments, HBO₂T has been shown to promote more extensive and faster axon regeneration than is seen in control animals, such as in the adult rat nerve crush model HBO₂T at 0.5 and 2.5 ATA pO₂, which induced no difference in the total number of regenerating axons, although the axons regenerated 15% further than those of non-HBO₂T-treated animals [124]. However, the beneficial influence of HBO₂T showed no significant dose-dependence in the range of 0.5 and or 2.5 ATA pO₂, although at 2.5 ATA of pO₂ axon regeneration was moderately enhanced [124]. Significantly more extensive axon regeneration induced by HBO₂T has been reported. Following a nerve crush, or when a nerve was transected and the ends anastomosed, HBO₂T significantly increased the rate of axon regeneration [125].

For transected peripheral nerves, HBO₂T induces longer nerve action potential propagation latency and greater signal amplitude, axons and blood vessel numbers and faster neurological recovery than was seen for non-treated control animals [126].

The standard clinical technique for repairing peripheral nerves with a gap is to graft lengths of sensory nerve into the gap so the grafts can serve as a pathway through which the transected axons can regenerate. Animals with nerve gaps bridged with nerve grafts given HBO₂T while breathing 100% oxygen showed enhanced rates of regeneration [127-129]. Data from another series of experiments found that HBO₂T induced significantly larger numbers of axons to regenerate, the axon had faster conduction velocities and greater signal amplitudes — both signs of the advanced axon regeneration and myelination — larger numbers of blood vessels, and the animals had more rapid and extensive neurological recovery [126]. However, other data indicate that HBO₂T does not induce more extensive axon regeneration and neurological recovery than is seen in control animals [130]. The differences in these data as to whether HBO₂T influenced axon regeneration may be related to the differences in the HBO₂T protocols use, which differed in pressure, as well as in the duration and number of sessions.

Clinical

Clinically HBO₂T induced axon regeneration in a patient who had suffered 34 months of paraplegia, associated with progressive muscle atrophy, indications of muscle denervation. The patient was treated at 2 ATA daily for one month and then once per week for one year. Within one month of initiating HBO₂T, muscle fibrillation decreased and muscle strength increased, both indications of axon reinnervation of the denervated muscle fibers [131]. It was hypothesized that the induction of muscle fiber reinnervation resulted from HBO₂T enhancing motor neuron oxygen metabolism, allowing them to maintain enhanced metabolic activity that is required to maintain extensive muscle fiber innervation.

In another clinical study, of 114 patients with transected nerves who had been anastomosed or received nerve grafts, 65 underwent HBO₂T while 54 served as controls. Neurological recovery was significantly better in the HBO₂T versus non-HBO₂T-treated patients, regardless of the type of nerve repair that had been performed [132]. The influence of HBO₂T on neurological recovery was equal if the nerve repair was performed at short or prolonged times post-nerve injury [132]. Interestingly, the influence of HBO₂T on the extent of neurological recovery was greater when initiated at increased time post-nerve trauma [132].

These data from animal and clinical studies show that HBO₂T significantly increases the rate, distance and number of axons that regenerate, leading to more extensive neurological recovery than is seen in controls [61,124-127,133-138].

HBO₂T and induction of CNS neurological recovery

Following brain injury, HBO₂T decreases cerebral edema, normalizes water content in the brain, decreases the severity of brain infarction and maintains blood-brain barrier integrity [139]. In addition, HBO₂T attenuates motor deficits, decreases the risks of sequelae and prevents recurrent cerebral circulatory disorders, thereby leading to improved neurological outcomes and clinical survival [140]. HBO₂T also accelerates the regression of atherosclerotic lesions, promotes antioxidant defenses and suppresses the proliferation of macrophages and foam cells in atherosclerotic lesions [141]. HBO₂T improves the function of damaged cells, by inducing the production of antioxidant enzymes and reducing antioxidative stress [142], improving immune function [142,143] inducing anti-inflammatory actions [142,144], attenuating the effects of hypoxia on the neonatal brain, enhancing gross motor function and fine motor control and
alleviating spasticity in children with cerebral palsy [139, 145]. HBO₂T reduces the infiltration of neutrophils into sites of traumatic brain injury (TBI), where they play a deleterious role in recovery due to their release of MMP-1 and MMP-9 [144]. HBO₂T inhibits neuronal death, improves blood flow in regions affected by chronic neurologic disease, as well as aerobic metabolism in brain injury, and accelerates the resolution of clinical symptoms [146]. HBO₂T increases the production of reactive oxygen intermediates (ROIs), responsible for producing cellular oxidative stress throughout the body and thereby providing antiviral protection [147].

Hyperbaric oxygen has also been reported to accelerate neurologic recovery after spinal cord injury by ameliorating mitochondrial dysfunction in the motor cortex and spinal cord, arresting the spread of hemorrhage, reversing hypoxia, and reducing edema [141]. For additional information see the recent review by Edwards [148]. HBO₂T increases the number of bone marrow stem cells in systemic circulation which may provide new cells that can assist in promoting axon regeneration [142].

**HBO₂T and recovery from spinal cord trauma**

In the case of spinal cord lesions induced in rats, HBO₂T accelerates neurologic recovery after spinal cord injury by ameliorating mitochondrial dysfunction in the motor cortex and spinal cord, arresting the spread of hemorrhage, reversing hypoxia and reducing edema [139]. Spinal cord-lesioned rats treated with GM1 ganglioside and HBO₂T showed improved neurological function in terms of locomotor function [149]. In another series of experiments on rats with spinal cord injury, combining HBO₂T with hypothermia reduced secondary tissue damage [150]. Methylprednisolone is typically administered immediately following spinal cord trauma to reduce tissue damage caused by oxidative stress. However, HBO₂T has been found to provide greater prevention against oxidative spinal cord injury than methylprednisolone [151].

**HBO₂T and motor neuron disease**

The Wobbler mouse is a model of human motor neuron disease involving the impairment of mitochondrial complex IV in the central nervous system, including motor cortex and spinal cord [152]. HBO₂T at 2 ATA, one hour/day for 30 days, has been shown to induce a 40% improvement in respiration by complex IV in mitochondria isolated from motor cortex neurons, and to delay the onset of motor deficits [152]. This suggests that HBO₂T significantly ameliorates mitochondrial dysfunction in the motor cortex and spinal cord and greatly delays the onset of the disease in an animal model of motor neuron disease.

When HBO₂T was applied clinically to patients with amyotrophic lateral sclerosis, they were observed to show decreased motor fatigue and voluntary muscle contraction strength, as well as delays in the onset of motor weakness [153]. Although not directly related to axon regeneration, these data indicate that HBO₂T is beneficial for maintaining the integrity of motor neurons and their axonal functions.

For the damaged brain, HBO₂T inhibits neuronal death due to ischemia [139,154-156], arrests the progression of radiation-induced neurologic necrosis [139, 157,158], improves blood flow in regions affected by chronic neurologic disease as well as aerobic metabolism in brain injury, and accelerates the resolution of deleterious neurological symptoms [146,159]. Hyperbaric oxygen has also been reported to accelerate neurologic recovery after spinal cord injury by ameliorating mitochondrial dysfunction in the motor cortex and spinal cord, arresting the spread of hemorrhage, reversing hypoxia and reducing edema [139,140]. HBO₂T can even reverse hydrogen peroxide gas embolism-triggered mental state deterioration [160]. Clinical studies have shown that following fluid-percussion and cortical contusion brain injury, HBO₂T combined with moderate systemic hypothermia reduced the mortality rate and led to neurological improvements [161].

In addition, hyperbaric oxygen combined with nicardipine administration accelerates neurologic recovery after cerebral ischemia [162]. Finally, the positive results of HBO₂T in the treatment of patients with stroke, atherosclerosis, cerebral palsy, intracranial pressure, headache and brain and spinal cord injury indicate that HBO₂T is promising and warrants further testing for inducing neurological recovery following CNS trauma.

**Why is HBO₂T not used more extensively to promote axon regeneration?**

HBO₂T is not used more extensively to promote axon regeneration because most of the early published data showing that HBO₂T enhanced axon regeneration were considered anecdotal. This created a continued reluctance to test HBO₂T for its influences on axon regeneration, even though the more recent data supporting
the influences of HBO₂T significantly outweigh the weaker earlier data and data in which no HBO₂T influences were observed. Other reasons for skepticism remain that different HBO₂T studies have involved small sample sizes; many variables, such as pressure, time and duration of treatment; number of treatments; animal models; and types of injuries. Clearly, some of these issues could be easily resolved by performing larger-scale animal model studies in which consistent HBO₂T protocols are used. However, one difficulty in doing this is the dispute about what should be considered the “optimal protocol.”

As an example, data from one study found that a short initial period of HBO₂T is equally effective in enhancing the rate of axon regeneration as HBO₂T applied every eight hours over several days [134]. Finally, additional reluctance to accept the influences of HBO₂T on the rate and extent of axon regeneration is that most studies have not addressed the mechanisms by which HBO₂T might achieve its influences. For example, is HBO₂T exerting its influences directly on damaged axons or the cells associated with them, and if so, what are the mechanisms? Understanding the mechanisms would lend great strength to the argument that HBO₂T promotes axon regeneration.

The data published more recently on the influences of HBO₂T on axon regeneration is of better quality and more strongly supports the beneficial actions of HBO₂T in promoting axon regeneration than earlier data. This data could be even further strengthened by releasing unpublished data on the actions of HBO₂T in which the treatment involved the same types of injuries treated under identical HBO₂T protocols. These publications should make more clinicians inclined to order HBO₂T treatments for their patients soon after the patient presents, rather than using HBO₂T as a treatment of last resort.

**Mechanisms by which HBO₂T could promote axon regeneration**

Differences are seen in the published data from animal and clinical studies, which could result from the effective HBO₂T conditions for animal models being different from those that are effective clinically, or the fact that effective parameters applied in animal studies were not applied clinically. Therefore, the lack of a positive HBO₂T outcome should not be seen as a failure of the technique but failure to use the appropriate conditions. Following are discussions of the actions of HBO₂T from animal and clinical studies.

**Increased oxygen delivery**

Trauma reduces or eliminates blood flow and oxygen delivery, thus denying energy production by the cells precisely where oxygen is needed most to fill that tissue’s increased demand for oxygen to perform the wound healing process [52]. This need for oxygen is especially critical during the inflammatory phase of wound healing [53].

Inhalation of 100% oxygen under pressure increases the systemic concentration of oxygen in a patient’s blood, while the increased pressure leads to significantly increased oxygen transfer from the blood to all body tissues. Thus, the specific aim of HBO₂T is to increase oxygen transfer to tissues under stress, and thereby, in an oxygen-dependent manner, improve wound healing, reduce wound edema, reduce muscular ischemic necrosis, reduce compartment syndrome and prevent infection by killing bacteria within a wound [15].

Because HBO₂T increases the relative dose of oxygen, susceptible patients must be specially managed to avoid oxygen toxicity [83]. Damaging or toxic effects of oxygen therapy are likely related to the unbridled formation and release of reactive oxygen species, such as superoxide, hydroxyl radical and hydrogen peroxide [84]. Superoxide dismutase, catalase, glutathione and glutathione reductase keep the formation of these radicals in check [85]. Other antioxidants used by the body include vitamins C and E, selenium, and glutathione [85].

**Increased oxygen metabolism**

One mechanism by which HBO₂T induces the reconstruction of motor nerve terminals is by increasing oxygen metabolism of motor neurons [163] which do not otherwise have the metabolic capacity required to support axon sprouting and extension [164].

**Angiogenesis**

Angiogenesis is a dynamic, hypoxia-stimulated and growth factor-dependent process that is acutely sensitive to oxygen levels as a function of local lactate delivery [27]. Therefore, the absence of oxygen prevents angiogenesis, which leads to further oxygen deficiency and prevents tissue granulation and blocks tissue healing. HBO₂T increases nerve vascularization [136,165], which allows additional access of oxygen to neurons and cells associated with axon regeneration.

Many patients suffer trauma that destroys a length of the original nerve. To induce neurological recovery, the standard clinical approach is to bridge the nerve gap
with a length of sensory nerve. Although this technique leads to neurological recovery, it has significant limitations, among which are that good regeneration is generally limited to gaps <5 cm and for repairs performed fewer than eight months post-trauma. One explanation is the cells of the nerve graft suffer toxicity and death due to ischemia and reperfusion-induced generation of reactive oxygen species, which creates a toxic environment that inhibits extensive axon regeneration [166,167]. The idea that limited axon regeneration results from limited nerve graft revascularization comes from studies showing that the number of regenerating axons is significantly increased when a vascularized nerve graft is used [168,169].

HBO₂T immediately following nerve grafting could help prevent reperfusion toxicity. The angiogenesis induced by HBO₂T could also provide the oxygen required to support axon regeneration that takes place in association with the nerve graft.

HBO₂T induces the synthesis and release of neurotrophic factors and cytokines required for axon regeneration
HBO₂T induces the synthesis of a number of neurotrophic factors and other factors that are crucial for promoting axon regeneration and neurological recovery such as: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) [170], endothelial nitric oxide synthase factor [171-175], GDNF [176,177], basic fibroblast growth factor (bFGF) [8, 178], hepatocyte growth factor (HGF) expression [178], neuro-trophin-3 (NT-3) [176] and neurotrophin- (NT-4) [179].

HBO₂T also induces axon regeneration by increasing nerve vascularization [79,171,172,176] by promoting the synthesis of endothelial nitric oxide synthase factor, TNF-alpha, MMP-9 and TIMP-1 [79], IL-10 [170,177] and VEGF [8,170,177,180,181]. By stimulating the production of interleukin-10 (IL-10), which induces scar formation, HBO₂T leads to better axon regeneration [177,182]. As discussed later, HBO₂T enhances the growth of fibroblasts and their production of autocrine growth factors [8]. Finally, HBO₂T combined with platelet-derived growth factor (PDGF) and tumor growth factor-beta 1 (TGF-beta 1) enhances the promotion of axon regeneration [183].

HBO₂T causes the up-regulation of genes for factors such as HIF-1alpha and its downstream target gene for the cytokine erythropoietin (EPO) [184], which are important for inducing axon regeneration. EPO controls the production of red cells, which are required to provide oxygen to tissues, and is present in the central and peripheral nervous system. EPO has direct and indirect effects on nerve cells by enhancing antioxidant enzyme production, antagonizing glutamate’s cytotoxic action, metabolizing free radicals, normalizing cerebral blood flow, affecting neurotransmitters release and stimulating neoangiogenesis [185]. Functional neurological recovery is also enhanced when HBO₂T-induced EPO and GDNF are combined [186].

Recombinant human EPO (rhEPO) has angiogenic and neuroprotective effects and increases peripheral nerve regeneration [187]. A single administration of recombinant human EPO (rhEPO) provides a significant anti-inflammatory function, preserves white matter, significantly enhances neurological recovery following contusive spinal cord injury (SCI) and has antiapoptotic and neuroprotective functions [188-190]. Thus HBO₂T enhances neuroprotection and axon regeneration by inducing EPO [184].

HBO₂T promotes axon regeneration by:
(1) inducing the accumulation of anti-inflammatory cytokines; and
(2) the accumulation of invading macrophages, which in turn release chemokines associated with improved motor function [191];
(3) decreasing neuronal apoptosis rates; and
(4) reducing microglial infiltration [192].

HBO₂T and brain contusions
HBO₂T improves the oxygen supply to the injured brain, reduces trauma-induced brain tissue loss [193], and improves behavioral and neurobiological outcomes [146]. Therefore, it has been proposed that HBO₂T should be a standard part of the post-CNS trauma intensive care regimen to reduce patient death and disability. A search of one database of patients following brainstem contusion found that HBO₂T induced a higher incidence of younger (versus older) patients regaining consciousness, which was accompanied by a significant decrease in patient mortality [194]. Another database review of three studies of patients who had suffered traumatic brain injury and were treated with HBO₂T found a significantly reduced risk of death and a trend toward, but no significant increase in, the chance of a favorable neurological outcome [195]. Although the last data suggested the routine application of HBO₂T could not be justified, the small patient number and quality of the data reported indicate the use of caution in interpreting the data and in determining which patients might be expected to benefit most from HBO₂T [195]. How-
ever, the evidence indicated that HBO2T decreases the incidence of mortality, although it may depend on subgroup selection [194].

**HBO2T reduces inflammation**

Inflammation plays a major pathological role in spinal cord injury (SCI) and it is considered critical to treat SCI patients with the anti-inflammatory drugs, such as methylprednisolone (MPSL) [196]. However, some clinical data suggest that MPSL is only modestly beneficial in SCI [197,198]. For example, MPSL reduces spinal cord lesion-enhanced NGF mRNA levels in the trauma epicenter and caudal section of the spinal cord, EPO increases NGF gene expression, which might facilitate axons regeneration toward the NGF-rich sites, and contributes to the enhancement of the nerve regenerative process [199]. Further, when MPSL is administered simultaneously with EPO, MPSL reduces all the physiological benefits of EPO [192]. Therefore, suppression of proinflammatory cytokines alone does not prevent secondary injury and suggests that glucocorticoids should not be co-administered in clinical trials evaluating the use of EPO for treatment of SCI; rather, it is better to rely on the trauma-induced EPO and exogenously administered EPO to exert their beneficial influences.

Inflammation activates the complement system, which in turn enhances inflammation and aggravates secondary injury, thus inhibiting axon regeneration [200]. The application of HBO2T can induce axon regeneration by reducing inflammation [201], whereby it reduces the toxicity of the cellular environment through which axons must regenerate.

**Neuroprotection**

Acute ischemic stroke (AIS) results in focal deprivation of blood-borne factors, including oxygen, and HBO2T applied following acute ischemic stroke increase the oxygen supply to the ischemic tissue, thus reducing the extent of irreversible tissue damage [202,203]. In animal models this is translated into generally improved motor neurologic outcomes following HBO2T applied less than six hours of transient (10-minute) ischemia [203-207], which is associated with a 2.6-fold (78% vs. 30%) increased survival following complete global ischemia versus controls as a result of reducing cerebral edema, normalizing brain water content, decreasing the extent of brain infarcts and maintaining the integrity and permeability of the blood-brain barrier [140,208]. However, increasing the duration of the HBO2 treatment does not improve neurological outcome, and may even aggravate the oxidative stress of ischemic tissue [209].

Because HBO2 treatment decreases patient mortality rate by six months post-stroke versus controls, it is used routinely in some clinics to treat stroke immediately following the incident [210]. However, by one year post-treatment, different neurological function scales show conflicting results, with some indicating significant neurological recovery and others none. This led one study to conclude that there was no sufficient evidence that HBO2T improves clinical outcomes when applied during the acute presentation of ischemic stroke, although it recommended further research to better define the role of HBO2T in treating this condition [210]. Thus, although clinical application of HBO2T is beneficial following an ischemic stroke [211], its benefits are typically not as robust as those seen in animal studies [205,212,213]. This is in part explained by: (1) the pressure applied clinically being less than what is effective in animal models [206]; and (2) clinically HBO2T is almost never given soon after trauma, when animal studies show it is most beneficial. The delay in administering HBO2T is generally to allow the performance of various patient analyses [205,206]. Therefore, the clinical application of HBO2T should be at a higher pressure and as soon as possible after a patient is admitted to the emergency room. The existence of so many variables in HBO2T application prevents good assessment of true HBO2T potential.

HBO2-preconditioning (HBO2-PC) is neuroprotective following surgical brain injury by reducing edema, which leads to improved neurological outcomes [214]. HBO2-PC at 3.5 ATA for one hour/day for five days provides neuroprotection against transient (eight-minute) forebrain ischemia, possibly through protein synthesis relevant to neurotrophin receptors and the inflammatory-immune system [215], and by decreasing cytoplasmic cytochrome C levels, caspase enzyme activity, up-regulating Mn-superoxide dismutase, catalase, the ratio of Bcl-2 and Bax expression, and by suppressing the mitochondrial apoptosis pathway, where nitric oxide is involved in neuroprotection and inhibiting oxidative injury [216]. By increasing the concentration of hypoxia-inducible factor-lalpha (HIF-lalpha) and the synthesis of the cytokine erythropoietin (EPO), HBO2-PC also provides neuroprotection/hypoxic tolerance to the cerebral cortex, hippocampus [184, 217-220] and spinal
cord [221]. By increasing antioxidant enzyme activity, HBO₂T suppresses the mitochondrial and neuronal apoptotic pathways [222,223], even protecting cultured retinal neurons from glutamate-induced cytotoxicity [224, 225]. In the case of retinal ganglion cells (RGCs), which die if the optic nerve is cut near the neurons, by suppressing caspase-3 and caspase-9 activity HBO₂-PC inhibits the neuronal apoptotic pathways [226].

HBO₂T reverses hydrogen peroxide gas embolism-triggered mental state deterioration [160]. When combined with nicardipine administration, HBO₂T accelerates neurologic recovery after cerebral ischemia [162]. HBO₂T accelerates neurologic recovery after spinal cord injury (SCI) by stopping the spread of hemorrhage, reversing hypoxia and reducing edema [140]. Finally, HBO₂T improves the clinical outcome of patients with atherosclerosis, cerebral palsy, intracranial pressure, headache, and brain and SCI, which indicate that HBO₂T warrants further testing for its ability to induce neurological recovery following CNS trauma.

**Blocking production of oxygen free radicals**

Part of HBO₂-induced neuroprotection is related to its induction of a synthesis of the brain-derived neurotrophic factor (BDNF) and its downstream influences involving suppression of p38 mitogen-activated protein kinase (p38) activation [215]. Thus, HBO₂-PC provides neuroprotection against global cerebral ischemia by reducing early apoptosis and inhibiting the conversion of early to late apoptosis by increasing the level of brain BDNF and suppressing p38 phosphorylation [170]. In the rat, HBO₂T reduces the extent of spinal cord damage following injury by stimulating the production of glial cell line-derived neurotrophic nerve growth factor (GDNF) [176], vascular endothelial growth factor (VEGF) [171-175] and interleukin-10 (IL-10) [227].

Neurotrauma leads to extensive production of oxygen-derived free radicals (ODFR), which interfere with axon regeneration. Antioxidants/free-radical scavengers like pyruvate or vitamin C and E [228] are major neuroprotective mechanisms that overcome the neurite growth inhibiting influence of oxidative stress [229]. Although, a sciatic nerve induces the appearance of vitamin E after about 30 days, this is too late to provide neuroprotection or assist in promoting axon regeneration [230]. Administration of exogenous vitamin E soon after a peripheral nerve crush enhances the rate of axon regeneration, apparently by reducing oxygen free-radical production [230]. These data suggest that HBO₂T induces enhanced axon regeneration by reducing or blocking production of oxygen radicals which inhibit axon regeneration.

**Anti-apoptosis actions**

EPO is also neuroprotective, increases the extent of peripheral axon regeneration and neurological recovery after trauma and exerts an anti-apoptotic action after central and peripheral nervous system injury by reducing the trauma-induced inflammatory responses [185,234-236]. Finally, EPO induces neurite outgrowth from retinal ganglion cells by inducing Stat3 phosphorylation and apparently up-regulating Bcl-X(L), a Bcl-2 homologue capable of promoting RGC regeneration [217].

**Nerve regeneration in diabetes**

Inducing axon regeneration in diabetics is especially challenging but is essential for reversal of neuropathy and recovery of nerve function due to acute compression and entrapment. Endoneural hypoxia caused by hyperglycemia-induced reductions in blood flow is developed in the course of diabetes, and the resulting ischemia reduces the extent of axon regeneration [237]. By raising the oxygen tension in ischemic tissues, HBO₂T produces tissue hyperoxia that reverses ischemic neuropathy [238]. However, application of HBO₂T to a diabetic model does not provide any benefit in the early stages of diabetic nerve regeneration [237] or following a nerve crush [239], which requires further examination.
Nerve regeneration in diabetes is essential for reversal of neuropathy and recovery of nerves from injury due to acute nerve compression and entrapment. Endoneural hypoxia due to hyperglycemia-induced blood flow reductions is observed early in the course of diabetes, and the resultant ischemia plays a role in the diminished neural regeneration. HBO₂T is capable of producing tissue hyperoxia by raising oxygen tensions in ischemic tissues and is beneficial in the reversal of experimental ischemic neuropathy. In work with a diabetes model, no benefits of HBO₂T were found in the early stages of diabetic nerve regeneration [237]. Similarly, no difference in axon regeneration was seen in the rate of regeneration following a nerve crush for HBO₂T-treated and control animals [240]. In the case of a sciatic nerve transection and intubation in a silicon tube with a gap between the nerve ends, no differences were seen between HBO₂T-treated animals and controls [241]. However, no difference would be expected in the absence of a matrix within the bridging tube.

Patient selection

Patient selection is critical to a successful outcome of HBO₂T. Therefore, it is important to evaluate patients prior to their undergoing HBO₂T to select out those with a compromised vascular system, arterial sclerosis or cardiac complications, such as congestive heart failure, diabetics with low blood sugar, people with seizures and those who are malnourished. These people might be at risk while undergoing HBO₂T, or HBO₂T may not be beneficial to their healing. Finally, inclusion of these patients would bias the data against showing a benefit of HBO₂T on axon regeneration.

Caution is also required when applying HBO₂T to patients with diabetes mellitus because HBO₂T may affect glucose uptake and metabolism [86]. The vasoconstricting effects of HBO₂T may also impair subcutaneous absorption of insulin, rendering the patient hypoglycemic [87].

Conclusion

HBO₂T is an extremely valuable technique for improving the outcome of patients with various types of wounds and several neurological diseases. Few clinical trials have thoroughly examined the influences of HBO₂T on axon regeneration, yet the preponderance of data support the conclusion that HBO₂T is beneficial in increasing the rate and extent of axon regeneration and neurological recovery, compared to controls. A difficulty for many to accept the data on HBO₂T inducing axon regeneration is that the different studies involved too many HBO₂T variables as well as different animal models, nerves and types of injuries. Therefore, it is not clear which variables are the major contributors to the effectiveness of HBO₂T. Convincing data will require more studies using consistent protocols. Another challenge for clinicians to appreciate the potential of ordering HBO₂T is the lack of good understanding of the mechanisms by which HBO₂T potentially induces axon regeneration and neurological recovery. One goal of this review has been to examine the mechanisms of action of HBO₂T and to show how these mechanisms are directly associated with promoting axon regeneration. Finally, it is important to consider that although currently HBO₂T is typically used as a stand-alone treatment, the influences of HBO₂T can be enhanced when combined with other techniques. Therefore, thorough studies are required to demonstrate whether HBO₂T induces enhanced axon regeneration and the mechanism by which this is accomplished.

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