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 (HBO_2T) involves placing a patient in a sealed chamber and elevating its pressure several-fold above ambient air pressure while the patient breathes 100% oxygen. HBO_2T induces a number of physiological actions, and which wounds are selected for HBO_2T depends on the specific actions of HBO_2T relative to the wound's healing requirements. Although nerve traumas are not yet indicated for HBO_2T, there are many animal and clinical examples showing the benefits of HBO_2T in inducing neurological recovery following nerve trauma. This review examines the general mechanisms required to induce wound healing and the actions of HBO_2T which meet these requirements. It then examines the requirements for inducing axon regeneration and how many are met by HBO_2T. Finally, we discuss anecdotal evidence that HBO_2T enhances the rate and extent of axon regeneration in both animal models and clinically. We conclude that HBO_2T 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 (HBO_2T) 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 pO_2) [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 pO_2 [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 pO_2, and drops to almost zero in hypoxic patients [15,16]. Thus, since enhanced oxygen presentation induces events required for wound healing, HBO_2T, which provides enhanced oxygen to tissues, is a reasonable place to start wound healing therapy [8,17,18]. However, before discussing HBO_2T 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 bactericidal 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 (HBO_2T) 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 HBO_2T treatments can vary depending on the type and severity of a wound. The importance and success of HBO_2T in wound healing is well established, making HBO_2T 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_2T became a real research and clinical tool.

In 1965, Dr. I. Boerema reported that HBO_2T assisted in cardiopulmonary surgery, the transposition of great vessels and for pulmonic stenosis [48,49]. Subsequently, W.H. Brummelkamp published findings that anerobic infections were inhibited by HBO_2T [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_2T 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_2T 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 benefits from hyperoxygenation through improved preservation of energy metabolism and the reduction of edema. Therefore, application of HBO_2T 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.

HBO_2T is critical to the treatment of chronic non-healing wounds due its inducing angiogenesis, which is promoted by the increased oxygen gradient caused by HBO_2T [58-60]. Decreased edema noted following HBO_2T allows better diffusion of oxygen and nutrients through tissues while also relieving pressure on surrounding vessels and structures [61]. In this light, HBO_2T has been used for treating venous and arterial insufficiencies, burn wounds, crush injuries, marginal flaps and skin grafts. Before initiating HBO_2T, 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].

HBO_2T 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, HBO_2T 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 HBO_2T 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 HBO_2T [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 HBO_2T 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 (HbO_2) 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 statue 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 (HbO_2). The delayed response phase represents an excess of incoming blood flow, which appears as an increase in HbO_2/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 cm^3 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 HBO_2T is likely of little benefit. Patients with intermediate responses may benefit from HBO_2T. An appropriate candidate for HBO_2T is a patient who has local ischemia but responds to the oxygen challenge [66]. The response to the HBO_2T by further transcutaneous oxygen should be tested after 14-20 HBO_2 treatments.

Systemic *vs.* Topical HBO_2T

It is critical to make a distinction between systemic HBO_2 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 HBO_2T 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 HBO_2T [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

HBO_2T 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 HBO_2T 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]. HBO_2T 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 HBO_2T

HBO_2T is very safe under appropriate supervision and use, although toxic effects of oxygen are observed at extremely high doses over prolonged periods. However, because HBO_2T increases the relative dose of oxygen, susceptible patients must be identified and HBO_2T 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 HBO_2T to patients with diabetes mellitus, because HBO_2T may affect glucose uptake and metabolism [86]. The vasoconstricting effects of HBO_2T therapy may impair subcutaneous absorption of insulin as well, rendering the patient hypoglycemic [87].

HBO_2T protocols

HBO_2T 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.

HBO_2T induces an eight- to ninefold increased vascular density over both normobaric oxygen and

air-breathing controls, and oxygen appears to require hyperbaric pressures to generate its therapeutic effects on chronically hypovascular irradiated tissue [89]. Therefore, researchers conducting wound healing studies continue to try to take advantage of the angiogenic properties of increasing oxygen gradients resulting from HBO_2T [79]. Prospective blinded randomized trials and well-executed laboratory studies continue to define further the role of HBO_2T in medical therapeutics.

Appropriate HBO_2T use and patient recovery requires proper supervision by a physician trained in its use, and who works closely with a surgeon. When ethically used for appropriate indications, it is a useful adjunct to surgical practice.

Local wound management, with appropriate debridement, irrigation, infection control and daily dressing changes, is required to aid in healing. Patient positioning 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_2T. The general approach to these problem wounds is therefore multidisciplinary.

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 so the metatarsal heads become prominent and promote further susceptibility to ulceration via pressure [91].

Early work with HBO_2T in foot wounds of patients with diabetes engendered much controversy, with many anecdotal reports [92]. There is now an increasing body of evidence to suggest efficacy for this condition [93], and HBO_2T is now widely used by wound care clinics.

Reperfusion injuries

The benefits of HBO_2T 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 HBO_2T that may increase oxygen free radicals to benefit the reperfusion injury seems paradoxical. HBO_2T 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 HBO_2T 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, HBO_2T enhances wound healing by its anti-inflammatory effects [121].

HBO_2T and peripheral axon regeneration

For HBO_2T 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 HBO_2T at 3 ATA for two hours. Non-HBO_2T-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, HBO_2T-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, HBO_2T should enhance the rate and extent of neurological recovery following nerve trauma.

Following a facial nerve crush, rabbits treated with HBO_2T 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, HBO_2T preserved nerve function mediated via preservation of neurotrophic and endothelial factor expression [123]. In other experiments, HBO_2T 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 HBO_2T at 0.5 and 2.5 ATA pO_2, which induced no difference in the total number of regenerating axons, although the axons regenerated 15% further than those of non-HBO_2T-treated animals [124]. However, the beneficial influence of HBO_2T showed no significant dose-dependence in the range of 0.5 and or 2.5 ATA pO_2, although at 2.5 ATA of pO_2 axon regeneration was moderately enhanced [124]. Significantly more extensive axon regeneration induced by HBO_2T has been reported. Following a nerve crush, or when a nerve was transected and the ends anastomosed, HBO_2T significantly increased the rate of axon regeneration [125].

For transected peripheral nerves, HBO_2T 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 HBO_2T while breathing 100% oxygen showed enhanced rates of regeneration [127-129]. Data from another series of experiments found that HBO_2T 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 HBO_2T 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 HBO_2T influenced axon regeneration may be related to the differences in the HBO_2T protocols use, which differed in pressure, as well as in the duration and number of sessions.

Clinical

Clinically HBO_2T 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 HBO_2T, 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 HBO_2T 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 HBO_2T while 54 served as controls. Neurological recovery was significantly better in the HBO_2T versus non-HBO_2T-treated patients, regardless of the type of nerve repair that had been performed [132]. The influence of HBO_2T on neurological recovery was equal if the nerve repair was performed at short or prolonged times post-nerve injury [132]. Interestingly, the influence of HBO_2T 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 HBO_2T 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].

HBO_2T and induction of CNS neurological recovery

Following brain injury, HBO_2T decreases cerebral edema, normalizes water content in the brain, decreases the severity of brain infarction and maintains blood-brain barrier integrity [139]. In addition, HBO_2T attenuates motor deficits, decreases the risks of sequelae and prevents recurrent cerebral circulatory disorders, thereby leading to improved neurological outcomes and clinical survival [140]. HBO_2T also accelerates the regression of atherosclerotic lesions, promotes antioxidant defenses and suppresses the proliferation of macrophages and foam cells in atherosclerotic lesions [141]. HBO_2T 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]. HBO_2T 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]. HBO_2T 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]. HBO_2T 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].

HBO_2T increases the number of bone marrow stem cells in systemic circulation which may provide new cells that can assist in promoting axon regeneration [142].

HBO_2T and recovery from spinal cord trauma

In the case of spinal cord lesions induced in rats, HBO_2T 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 HBO_2T showed improved neurological function in terms of locomotor function [149]. In another series of experiments on rats with spinal cord injury, combining HBO_2T 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, HBO_2T has been found to provide greater prevention against oxidative spinal cord injury than methylprednisolone [151].

HBO_2T 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]. HBO_2T 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 HBO_2T 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 HBO_2T 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 HBO_2T is beneficial for maintaining the integrity of motor neurons and their axonal functions.

For the damaged brain, HBO_2T 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].

HBO_2T 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, HBO_2T 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 HBO_2T in the treatment of patients with stroke, atherosclerosis, cerebral palsy, intracranial pressure, headache and brain and spinal cord injury indicate that HBO_2T is promising and warrants further testing for inducing neurological recovery following CNS trauma.

Why is HBO_2T not used more extensively to promote axon regeneration?

HBO_2T is not used more extensively to promote axon regeneration because most of the early published data showing that HBO_2T enhanced axon regeneration were considered anecdotal. This created a continued reluctance to test HBO_2T for its influences on axon regeneration, even though the more recent data supporting

the influences of HBO_2T significantly outweigh the weaker earlier data and data in which no HBO_2T influences were observed. Other reasons for skepticism remains that different HBO_2T 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 HBO_2T 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 HBO_2T is equally effective in enhancing the rate of axon regeneration as HBO_2T applied every eight hours over several days [134]. Finally, additional reluctance to accept the influences of HBO_2T on the rate and extent of axon regeneration is that most studies have not addressed the mechanisms by which HBO_2T might achieve its influences. For example, is HBO_2T 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 HBO_2T promotes axon regeneration.

The data published more recently on the influences of HBO_2T on axon regeneration is of better quality and more strongly supports the beneficial actions of HBO_2T in promoting axon regeneration than earlier data. This data could be even further strengthened by releasing unpublished data on the actions of HBO_2T in which the treatment involved the same types of injuries treated under identical HBO_2T protocols. These publications should make more clinicians inclined to order HBO_2T treatments for their patients soon after the patient presents, rather than using HBO_2T as a treatment of last resort.

Mechanisms by which HBO_2T could promote axon regeneration

Differences are seen in the published data from animal and clinical studies, which could result from the effective HBO_2T 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 HBO_2T 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 HBO_2T 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 HBO_2T 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 HBO_2T 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 HBO_2T 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. HBO_2T 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].

HBO_2T immediately following nerve grafting could help prevent reperfusion toxicity. The angiogenesis induced by HBO_2T could also provide the oxygen required to support axon regeneration that takes place in association with the nerve graft.

HBO_2T induces the synthesis and release of neurotrophic factors and cytokines required for axon regeneration

HBO_2T 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].

HBO_2T 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 reduces scar formation, HBO_2T leads to better axon regeneration [177, 182]. As discussed later, HBO_2T enhances the growth of fibroblasts and their production of autocrine growth factors [8]. Finally, HBO_2T combined with platelet-derived growth factor (PDGF) and tumor growth factor-beta 1 (TGF-beta 1) enhances the promotion of axon regeneration [183].

HBO_2T 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 HBO_2T-induced EPO and GNDF 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 HBO_2T enhances neuroprotection and axon regeneration by inducing EPO [184].

HBO_2T 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].

HBO_2T and brain contusions

HBO_2T 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 HBO_2T 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 HBO_2T 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 HBO_2T 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 HBO_2T 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 HBO_2T [195]. How-

ever, the evidence indicated that HBO_2T decreases the incidence of mortality, although it may depend on subgroup selection [194].

HBO_2T 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 HBO_2T 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 HBO_2T 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 HBO_2T 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 HBO_2 treatment does not improve neurological outcome, and may even aggravate the oxidative stress of ischemic tissue [209].

Because HBO_2 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 HBO_2T improves clinical outcomes when applied during the acute presentation of ischemic stroke, although it recommended further research to better define the role of HBO_2T in treating this condition [210]. Thus, although clinical application of HBO_2T 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 HBO_2T is almost never given soon after trauma, when animal studies show it is most beneficial. The delay in administering HBO_2T is generally to allow the performance of various patient analyses [205,206]. Therefore, the clinical application of HBO_2T 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 HBO_2T application prevents good assessment of true HBO_2T potential.

HBO_2-preconditioning (HBO_2-PC) is neuroprotective following surgical brain injury by reducing edema, which leads to improved neurological outcomes [214]. HBO_2-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 cytoplasm 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-1alpha (HIF-1alpha) and the synthesis of the cytokine erythropoietin (EPO), HBO_2-PC also provides neuroprotection/hypoxic tolerance to the cerebral cortex, hippocampus [184, 217-220] and spinal

cord [221]. By increasing antioxidant enzyme activity, HBO_2T suppresses the mitochondrial and neuronal apoptotic pathways [222,223], even protecting cultured retinal neurocytes 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 HBO_2-PC inhibits the neuronal apoptosis pathways [226].

HBO_2T reverses hydrogen peroxide gas embolism-triggered mental state deterioration [160]. When combined with nicardipine administration, HBO_2T accelerates neurologic recovery after cerebral ischemia [162]. HBO_2T accelerates neurologic recovery after spinal cord injury (SCI) by stopping the spread of hemorrhage, reversing hypoxia and reducing edema [140]. Finally, HBO_2T improves the clinical outcome of patients with atherosclerosis, cerebral palsy, intracranial pressure, headache, and brain and SCI, which indicate that HBO_2T warrants further testing for its ability to induce neurological recovery following CNS trauma.

Blocking production of oxygen free radicals

Part of HBO_2-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, HBO_2-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, HBO_2T 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 HBO_2T induces enhanced axon regeneration by reducing or blocking production of oxygen radicals which inhibit axon regeneration.

Reactive oxygen species (ROS) are involved in neurodegenerative processes like Parkinson's and Alzheimer's diseases, and are involved in traumatic brain injury. Glial cells produce several factors which induce neurodegeneration/neurite growth inhibition, among which is a riboflavin-(vitamin B2)-like compound [231]. Elimination of riboflavin abolishes neurite growth inhibiting effect and enhances the regenerative response of rat retinal explants [231]. Riboflavin-mediated cytotoxicity is related to its involvement in the production of free radicals through photoabsorption, because antioxidants/free radical scavengers like pyruvate or vitamin C and E overcome the neurite outgrowth inhibiting influence of riboflavin or the radical stress [229]. Thus, in the CNS, astrocytes protect retinal ganglion (RGC) cells against ROS-induced oxidative stress by releasing soluble neurotrophic factors that support RGC axonal regeneration. Alternatively, axon regeneration and retinal ganglion cell protection can be induced by blocking free radical production after tissue injury [232]. Finally, glial cell-derived neurotrophic factor (GDNF) assists axon regeneration by suppressing the production by scavenging of free radicals [233].

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, HBO_2T produces tissue hyperoxia that reverses ischemic neuropathy [238]. However, application of HBO_2T 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. HBO_2T 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 HBO_2T 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 HBO_2T-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 HBO_2T-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 HBO_2T Therefore, it is important to evaluate patients prior to their undergoing HBO_2T 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 HBO_2T, or HBO_2T may not be beneficial to their healing. Finally, inclusion of these patients would bias the data against showing a benefit of HBO_2T on axon regeneration.

Caution in also required when applying HBO_2T to patients with diabetes mellitus because HBO_2T may affect glucose uptake and metabolism [86]. The vasoconstricting effects of HBO_2T may also impair subcutaneous absorption of insulin, rendering the patient hypoglycemic [87].

Conclusion

HBO_2T 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 HBO_2T on axon regeneration, yet the preponderance of data support the conclusion that HBO_2T 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 HBO_2T inducing axon regeneration is that the different studies involved too many HBO_2T 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 HBO_2T. Convincing data will require more studies using consistent protocols. Another challenge for clinicians to appreciate the potential of ordering HBO_2T is the lack of good understanding of the mechanisms by which HBO_2T potentially induces axon regeneration and neurological recovery. One goal of this review has been to examine the mechanisms of action of HBO_2T and to show how these mechanisms are directly associated with promoting axon regeneration. Finally, it is important to consider that although currently HBO_2T is typically used as a stand-alone treatment, the influences of HBO_2T can be enhanced when combined with other techniques. Therefore, thorough studies are required to demonstrate whether HBO_2T induces enhanced axon regeneration and the mechanism by which this is accomplished. ■

REFERENCES

1. Das RH, van Osch GJ, Kreukniet M, Oostra J, Weinans H, Jahr H. Effects of individual control of pH and hypoxia in chondrocyte culture. J Orthop Res. 2010; 4:537-545.

2. Qu CJ, Poytakangas T, Jauhiainen M, Auriola S, Lammi MJ. Glucosamine sulphate does not increase extracellular matrix production at low oxygen tension. Cell and Tissue Res. 2009; 337:103-111.

3. Guaccio A, Borselli C, Oliviero O, Netti PA. Oxygen consumption of chondrocytes in agarose and collagen gels: a comparative analysis. Biomaterials. 2008; 29:1484-1493.

4. Zeit, M, Ruiz-Torres A, Merker HJ. Collagen metabolism in granulating wounds of rat skin. Arch Dermatol Res. 1978; 263:207-214.

5. Wu J, Reinhardt DP, Batmunkh C et al. Functional diversity of lysyl hydroxylase 2 in collagen synthesis of human dermal fibroblasts. Exp Cell Res. 2006; 312:3485-3494.

6. Riches K, Morley ME, Turner NA et al. Chronic hypoxia inhibits MMP-2 activation and cellular invasion in human cardiac myofibroblasts. J Molec and Cell Cardiol. 2009; 47:391-399.

7. Milovanova TN, Bhopale VM, Sorokina EM et al. Hyperbaric oxygen stimulates vasculogenic stem cell growth and differentiation in vivo. J Applied Physiol. 2009; 106:711-728.

8. Kang TS, Gorti GK, Quan SY, Ho M, Koch RJ. Effect of hyperbaric oxygen on the growth factor profile of fibroblasts. Arch Facial Plast Surg. 2004; 6:31-35.

9. Ruocco V, Bimonte D, Luongo C, Florio M. Hyperbaric oxygen treatment of toxic epidermal necrolysis. Cutis. 1986; 38:267-271.

10. Melikian V, Laverson S, Zawacki B. Oxygen-derived free radical inhibition in the healing of experimental zone-of-stasis burns. J Trauma. 1987; 27:151-154.

11. Doillon CJ, Silver FH. Collagen-based wound dressing: effects of hyaluronic acid and fibronectin on wound healing. Biomaterials. 1986; 7:3-8.

12. Park MK, Myers RA, Marzella L. Oxygen tensions and infections: modulation of microbial growth, activity of antimicrobial agents, and immunologic responses. Clin Infect Dis. 1992; 14:720-740.

13. Bugge DM, Hegaret H, Wikfors GH, Allam B. Oxidative burst in hard clam (Mercenaria mercenaria) haemocytes. Fish Shellfish Immunol. 2006; 23:188-96.

14. Battistoni A, Ajello M, Ammendola S, Superti F, Rotilio G, Valenti P. Involvement of reactive oxygen species in bacterial killing within epithelial cells. Int J Immunopathol Pharmacol. 2004; 17:71-76.

15. Dwyer DJ, Kohanski MA, Collins JJ. Role of reactive oxygen species in antibiotic action and resistance. Curr Opinion in Microbiol. 2009; 12:482-489.

16. Cimsit M, Uzun G, Yildiz S. Hyperbaric oxygen therapy as an anti-infective agent. Expert Rev Anti Infect Ther. 2009; 7:1015-1026.

17. Conconi MT, Baiguera S, Guidolin D et al. Effects of hyperbaric oxygen on proliferative and apoptotic activities and reactive oxygen species generation in mouse fibroblast 3T3/J2 cell line. J Investig Med. 2003; 51:227-232.

18. Tompach PC, Lew D, Stoll JL. Cell response to hyperbaric oxygen treatment. Int J Oral Maxillofac Surg. 1997; 26:82-86.

19. Diehr, S, Hamp A, Jamieson B, Mendoza M. Do topical antibiotics improve wound healing? J Fam Pract. 2007; 56:140-144.

20. Leaper DJ. Silver dressings: their role in wound management. Int Wound J. 2006; 3:282-294.

21. Dinh TL, Veves A. Treatment of diabetic ulcers. Dermatol Ther. 2006; 19:348-355.

22. Thackham JA, McElwain DL, Long RJ. The use of hyperbaric oxygen therapy to treat chronic wounds: A review. Wound Repair Regen. 2008; 16:321-330.

23. Kabon B, Rozum R, Marschalek C et al. Supplemental postoperative oxygen and tissue oxygen tension in morbidly obese patients. Obes Surg. 20:885-894.

24. Doillon CJ, Dunn MG, Berg RA, Silver FH. Collagen deposition during wound repair. Scan Electron Microsc. 1985; 897-903.

25. Paternostro C, David E, Novo E, Parola M. Hypoxia, angiogenesis and liver fibrogenesis in the progression of chronic liver diseases. World J Gastroenterol. 16:281-288.

26. Dong H, Wang Q, Zhang Y, Jiang B, Xu X, Zhang Z. Angiogenesis induced by hVEGF165 gene controlled by hypoxic response elements in rabbit ischemia myocardium. Exp Biol Med (Maywood). 2009; 234:1417-1424.

27. Jeon O, Kang SW, Lim HW, Hyung Chung J, Kim BS. Long-term and zero-order release of basic fibroblast growth factor from heparin-conjugated poly(L-lactide-co-glycolide) nanospheres and fibrin gel. Biomaterials. 2006; 27:1598-1607.

28. Bardoel JW, Stadelmann WK, Tobin GR et al. Use of the rectus abdominis muscle for abdominal stoma sphincter construction: an anatomical feasibility study. Plast Reconstr Surg. 2000; 105:589-595.

29. McLachlan EM. Diversity of sympathetic vasoconstrictor pathways and their plasticity after spinal cord injury. Clin Auton Res. 2007; 17:6-12.

30. Kawase T, Okuda K, Wolff LF, Yoshie H. Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. J Periodontol. 2003; 74:858-864.

31. Rughetti A, Giusti I, D'Ascenzo S et al. Platelet gel-released supernatant modulates the angiogenic capability of human endothelial cells. Blood Transfus. 2008; 6:12-17.

32. Creaney L, Hamilton B. Growth factor delivery methods in the management of sports injuries: the state of play. British Journal of Sports Medicine. 2008; 42:314-320.

33. Wrotniak M, Bielecki T, Gazdzik TS. Current opinion about using the platelet-rich gel in orthopaedics and trauma surgery. Ortop Traumatol Rehabil. 2007; 9:227-238.

34. Berg C, Hammarstrom S, Herbertsson H et al. Platelet-induced growth of human fibroblasts is associated with an increased expression of 5-lipoxygenase. Thromb Haemost. 2006; 96:652-659.

35. Golz G, Uhlmann L, Ludecke D, Markgraf N, Nitsch R, Hendrix S. The cytokine/neurotrophin axis in peripheral axon outgrowth. Eur J Neurosci. 2006; 24:2721-2730.

36. Wei X, Zhang J, Zhuang X. [The correlation of PDGF and TNF-alpha and HA and nasal mucosa healing after ESS]. Lin Chuang Er Bi Yan Hou Ke Za Zhi. 2006; 20:771-773.

37. Delavary BM, van der Veer WM, van Egmond M, Niessen FB, Beelen RH. Macrophages in skin injury and repair. Immunobiology.

38. Soomekh DJ. Current concepts for the use of platelet-rich plasma in the foot and ankle. Clin Podiatr Med Surg. 28:155-170.

39. Patel GK. The role of nutrition in the management of lower extremity wounds. Int J Low Extrem Wounds. 2005; 4:12-22.

40. Russell L. The importance of patients' nutritional status in wound healing. Br J Nurs. 2001; 10:S42, S44-49.

41. Langkamp-Henken B, Hudgens J, Stechmiller JK, Herrlinger-Garcia KA. Mini nutritional assessment and screening scores are associated with nutritional indicators in elderly people with pressure ulcers. J Am Diet Assoc. 2005; 105:1590-1596.

42. Halligan TJ, Russell NG, Dunn WJ, Caldroney SJ, Skelton TB. Identification and treatment of scurvy: a case report. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005; 100:688-692.

43. Lansdown AB, Sampson B, Rowe A. Experimental observations in the rat on the influence of cadmium on skin wound repair. Int J Exp Pathol. 2001; 82:35-41.

44. Declair V. The usefulness of topical application of essential fatty acids (EFA) to prevent pressure ulcers. Ostomy Wound Manage. 1997; 43:48-52.

45. Daousi C, Benbow SJ, MacFarlane IA. Electrical spinal cord stimulation in the long-term treatment of chronic painful diabetic neuropathy. Diabet Med. 2005; 22:393-398.

46. Flegg JA, Byrne HM, McElwain DL. Mathematical model of hyperbaric oxygen therapy applied to chronic diabetic wounds. Bull Math Biol. 72:1867-1891.

47. Lazarus GS, Cooper DM, Knighton DR et al. Definitions and guidelines for assessment of wounds and evaluation of healing. Arch Dermatol. 1994; 130:489-493.

48. Boerema I. Hyperbaric oxygenation: The large chamber. Ann N Y Acad Sci. 1965; 117:883-890.

49. Boerema I. The use of hyperbaric oxygen. Am Heart J. 1965; 69:289-292.

50. Brummelkamp WH. [Treatment of infections due to anaerobic germs by inhalation of hyperbaric oxygen]. Ann Chir Thorac Cardiovasc. 1966; 5:607-610.

51. Boerema I, Meyne NG, Brummelkamp WH et al. [Life without blood.]. Ned Tijdschr Geneeskd. 1960; 104:949-954.

52. Gajendrareddy PK, Sen CK, Horan MP, Marucha PT. Hyperbaric oxygen therapy ameliorates stress-impaired dermal wound healing. Brain, Behavior, and Immunity. 2005; 19:217-222.

53. Eming SA, Brachvogel B, Odorisio T, Koch M. Regulation of angiogenesis: wound healing as a model. Progress in Histochem and Cytochem. 2007; 42:115-170.

54. Agostoni P, Caldara G, Bussotti M et al. Continuous positive airway pressure increases haemoglobin O2 saturation after acute but not prolonged altitude exposure. Europ Heart J. 2009. 31 :457-63

55. Gray A, Goodacre S, Newby DE, Masson M, Sampson F, Nicholl J. Noninvasive ventilation in acute cardiogenic pulmonary edema. New England J Med. 2008; 359:142-151.

56. Whitney JD. The influence of tissue oxygen and perfusion on wound healing. AACN Clin Issues in Crit Care Nursing. 1990; 1:578-584.

57. Eschenfelder CC, Krug R, Yusofi AF et al. Neuroprotection by oxygen in acute transient focal cerebral ischemia is dose dependent and shows superiority of hyperbaric oxygenation. Cerebrovasc Dis. 2008; 25:193-201.

58. Liu Y, Cox SR, Morita T, Kourembanas S. Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5' enhancer. Circ Res. 1995; 77:638-643.

59. Lee CC, Chen SC, Tsai SC et al. Hyperbaric oxygen induces VEGF expression through ERK, JNK and c-Jun/AP-1 activation in human umbilical vein endothelial cells. J Biomed Sci. 2006; 13:143-156.

60. Bayati S, Russell RC, Roth AC. Stimulation of angiogenesis to improve the viability of prefabricated flaps. Plast Reconstr Surg. 1998; 101:1290-1295.

61. Mychaskiw G, 2nd, Pan J, Shah S et al. Effects of hyperbaric oxygen on skin blood flow and tissue morphology following sciatic nerve constriction. Pain Physician. 2005; 8:157-161.

62. Davis JC, Dunn JM, Heimbach RD. Indications for hyperbaric oxygen therapy. Tex Med. 1980; 76:44-47.

63. Bouachou, G, Cronier P, Gouello JP, Toulemonde JL, Talha A, Alquier P. Hyperbaric oxygen therapy in the management of crush injuries: a randomized double-blind placebo-controlled clinical trial. J Trauma. 1996; 41:333-339.

64. Lebel D, Gortzak Y, Nyska M, Katz T, Atar D, Etzion Y. [Hyperbaric oxygen therapy for chronic diabetic wounds of the lower limbs--a review of the literature]. Harefuah. 2007; 146:223-227.

65. Milerad J. Effects of theophylline on ventilatory response to hypoxic challenge. Arch of Disease in Childhood. 1987; 62:1242-1246.

66. Grolman RE, Wilkerson DK, Taylor J, Allinson P, Zatina MA. Transcutaneous oxygen measurements predict a beneficial response to hyperbaric oxygen therapy in patients with nonhealing wounds and critical limb ischemia. Am Surg. 2011; 67; 1072-9; discussion 1080.

67. Boykin JV, Jr., Crossland MC, Cole LM. Wound healing management: enhancing patient outcomes and reducing costs. J Healthcare Resource Manag. 1997; 15:22, 24-26.

68. Langham MC, Magland JF, Epstein CL, Floyd TF, Wehrli FW. Accuracy and precision of MR blood oximetry based on the long paramagnetic cylinder approximation of large vessels. Magn Reson Med. 2009; 62:333-340.

69. Wolf U, Scholkmann F, Rosenberger R, Wolf M, Nelle M. Changes in hemodynamics and tissue oxygenation saturation in the brain and skeletal muscle induced by speech therapy - a near-infrared spectroscopy study. Sci World J. 2011; 11:1206-1215.

70. Akiyama T, Ohira T, Kawase T, Kato T. TMS orientation for NIRS-functional motor mapping. Brain Topogr. 2006; 19:1-9.

71. Omur-Ozbek P, Dietrich AM. Retronasal perception and flavour thresholds of iron and copper in drinking water. J Water Health 2011; 9:1-9

72. Omur-Ozbek P, Gallagher DL, Dietrich AM. Determining human exposure and sensory detection of odorous compounds released during showering. Environ Sci Technol. 2011; 45:468-473.

73. Cronje FJ. Oxygen therapy and wound healing - topical oxygen is not hyperbaric oxygen therapy. S Afr Med J. 2005; 95:840.

74. Gordillo GM, Schlanger R, Wallace WA, Bergdall V, Bartlett R, Sen CK. Protocols for topical and systemic oxygen treatments in wound healing. Methods Enzymol. 2004; 381:575-585.

75. Burghart K. The topical hyperbaric oxygen therapy debate. Ostomy Wound Manage. 2003; 49:8; author reply 8.

76. Lipsky BA, Berendt AR, Deery HG et al. Diagnosis and treatment of diabetic foot infections. Plast Reconstr Surg. 2006; 117:212S-238S.

77. Kalliainen LK, Gordillo GM, Schlanger R, Sen CK. Topical oxygen as an adjunct to wound healing: a clinical case series. Pathophysiol. 2003; 9:81-87.

78. Feldmeier JJ, Hopf HW, Warriner RA, 3rd, Fife CE, Gesell LB, Bennett M. UHMS position statement: topical oxygen for chronic wounds. Undersea Hyperb Med. 2005; 32:157-168.

79. Sander AL, Henrich D, Muth CM, Marzi I, Barker JH, Frank JM. In vivo effect of hyperbaric oxygen on wound angiogenesis and epithelization. Wound Repair and Regen. 2009; 17:179-184.

80. Friedman HI, Fitzmaurice M, Lefaivre JF, Vecchiolla T, Clarke D. An evidence-based appraisal of the use of hyperbaric oxygen on flaps and grafts. Plast Reconstr Surg. 2006; 117:175S-190S; discussion 191S-192S.

81. Kaid, CG, Khandelwal S. Hyperbaric oxygen: applications in infectious disease. Emerg Med Clinics of North America. 2008; 26:571-595.

82. Kuffler DP. The role of hyperbaric oxygen therapy in enhancing the rate of wound healing with a focus on axon regeneration. PR Health Sci J. 2011; 30: 35-42.

83. Rockswold SB, Rockswold GL, Defillo A. Hyperbaric oxygen in traumatic brain injury. Neurol Res. 2007; 29:162-172.

84. Allen BW, Demchenko IT, Piantadosi CA. Two faces of nitric oxide: implications for cellular mechanisms of oxygen toxicity. J Applied Physiol. 2009; 106:662-667.

85. Mendis E, Kim MM, Rajapakse N, Kim SK. Sulfated glucosamine inhibits oxidation of biomolecules in cells via a mechanism involving intracellular free radical scavenging. Europ. J Pharmacol. 2008; 579:74-85.

86. Berendt AR. Counterpoint: hyperbaric oxygen for diabetic foot wounds is not effective. Clin Infect Dis. 2006; 43:193-198.

87. Al-Waili NS, Butler GJ, Beale J et al. Influences of hyperbaric oxygen on blood pressure, heart rate and blood glucose levels in patients with diabetes mellitus and hypertension. Arch Med Res. 2006; 37:991-997.

88. Phillips JC. Understanding hyperbaric oxygen therapy and its use in the treatment of compromised skin grafts and flaps. Plast Surg Nurs. 2005; 25:72-80; quiz 81-72.

89. Ren P, Kang Z, Gu G et al. Hyperbaric oxygen preconditioning promotes angiogenesis in rat liver after partial hepatectomy. Life Sciences. 2008; 83:236-241.

90. Moutschen, MP, Scheen AJ, Lefebvre PJ. Impaired immune responses in diabetes mellitus: analysis of the factors and mechanisms involved. Relevance to the increased susceptibility of diabetic patients to specific infections. Diabete Metab. 1992; 18:187-201.

91. Wieman TJ, Mercke YK, Cerrito PB, Taber SW. Resection of the metatarsal head for diabetic foot ulcers. Am J Surg. 1998; 176:436-441.

92. Lyon KC. The case for evidence in wound care: investigating advanced treatment modalities in healing chronic diabetic lower extremity wounds. Journal of Wound, Ostomy and Continence Nursing. 2008; 35:585-590.

93. Ong M. Hyperbaric oxygen therapy in the management of diabetic lower limb wounds. Singapore Med Journal. 2008; 49:105-109.

94. Corbucci GG, Perrino C, Donato G et al. Transient and reversible deoxyribonucleic acid damage in human left ventricle under controlled ischemia and reperfusion. J Am Col Cardiol. 2004; 43:1992-1999.

95. Zamboni WA, Browder LK, Martinez J. Hyperbaric oxygen and wound healing. Clin Plast Surg. 2003; 30:67-75.

96. Moali C, Hulmes DJ. Extracellular and cell surface proteases in wound healing: new players are still emerging. Eur J Dermatol. 2009; 19:552-564

97. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. Clin Dermatol. 2007; 25:9-18.

98. Madden JW, Peacock EE, Jr. Studies on the biology of collagen during wound healing. I. Rate of collagen synthesis and deposition in cutaneous wounds of the rat. Surgery. 1968; 64:288-294.

99. DelBalso AM, Adrian JC. Collagen gel in osseous defects. A preliminary study. Oral Surg Oral Med Oral Pathol. 1976; 42:562-569.

100. Bishop A. Role of oxygen in wound healing. J Wound Care. 2008; 17:399-402.

101. Rodriguez, PG, Felix FN, Woodley DT, Shim EK. The role of oxygen in wound healing: a review of the literature. Dermatol Surgery. 2008; 34:1159-1169.

102. Thackham JA, McElwain DL, Long RJ. The use of hyperbaric oxygen therapy to treat chronic wounds: A review. Wound Repair Regen. 2008; 16:321-330.

103. Parsons KK, Maeda N, Yamauchi M, Banes AJ, Koller BH. Ascorbic acid-independent synthesis of collagen in mice. Am J Physiol Endocrinol Metab. 2006; 290:E1131-1139.

104. Roberts GP, Harding KG. Stimulation of glycosaminoglycan synthesis in cultured fibroblasts by hyperbaric oxygen. Brit J Dermatol. 1994; 131:630-633.

105. Mariggio MA, Cassano A, Vinella A et al. Enhancement of fibroblast proliferation, collagen biosynthesis and production of growth factors as a result of combining sodium hyaluronate and aminoacids. Int J Immunopathol Pharmacol. 2009; 22:485-492.

106. Norstrom A. Acute effects of prostaglandins on the biosynthesis of connective tissue constituents in the non-pregnant human cervix uteri. Acta Obstet Gynecol Scand. 1984; 63:169-173.

107. Alvarez RH, Kantarjian HM, Cortes JE. Biology of platelet-derived growth factor and its involvement in disease. Mayo Clin Proc. 2006; 81:1241-1257.

108. Arii S, Teramoto K, Kawamura T. Current progress in the understanding of and therapeutic strategies for ischemia and reperfusion injury of the liver. J Hepatobiliary Pancreat Surg. 2003; 10:189-194.

109. Halbertsma FJ, Vaneker M, Scheffer GJ, van der Hoeven JG. Cytokines and biotrauma in ventilator-induced lung injury: a critical review of the literature. Neth J Med. 2005; 63:382-392.

110. Colic M, Gazivoda D, Vucevic D et al. Regulatory T-cells in periapical lesions. J Dent Res. 2009; 88:997-1002.

111. Stadelmann WK, Digenis AG, Tobin GR. Physiology and healing dynamics of chronic cutaneous wounds. Am J Surg. 1998; 176:26S-38S.

112. Raud J. Vasodilatation and inhibition of mediator release represent two distinct mechanisms for prostaglandin modulation of acute mast cell-dependent inflammation. Br J Pharmacol. 1990; 99:449-454.

113. Komoriya K, Ohmori H, Azuma A et al. Prostaglandin I2 as a potentiator of acute inflammation in rats. Prostaglandins. 1978; 15:557-564.

114. Brain SD, Williams TJ. Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. Br J Pharmacol. 1985; 86:855-860.

115. Hirasawa N, Ohuchi K, Sugio K, Tsurufuji S, Watanabe M, Yoshino S. Vascular permeability responses and the role of prostaglandin E2 in an experimental allergic inflammation of air pouch type in rats. Br J Pharmacol. 1986; 87:751-756.

116. Omata M, Watanabe M, Hirasawa N, Tsurufuji S, Mue S, Ohuchi K. A role of peripheral leukocytes in vascular permeability and edema formation in air pouch type allergic inflammation in rats. J Pharmacobiodyn. 1991; 14:267-275.

117. Romanovsky AA, Almeida MC, Aronoff DM et al. Fever and hypothermia in systemic inflammation: recent discoveries and revisions. Front Biosci. 2005; 10:2193-2216.

118. Rittner HL, Machelska H, Stein C. Leukocytes in the regulation of pain and analgesia. J Leukoc Biol. 2005; 78:1215-1222.

119. Schaible HG, Ebersberger A, Von Banchet GS. Mechanisms of pain in arthritis. Ann N Y Acad Sci. 2002; 966:343-354.

120. Cukjati D, Rebersek S, Karba R, Miklavcic D. Modelling of chronic wound healing dynamics. Med Biol Eng Comput. 2000; 38:339-347.

121. Al-Waili NS, Butler GJ. Effects of hyperbaric oxygen on inflammatory response to wound and trauma: possible mechanism of action. ScientificWorldJournal. 2006; 6:425-441.

122. Vilela DS, Lazarini PR, Da Silva CF. Effects of hyperbaric oxygen therapy on facial nerve regeneration. Acta Otolaryngol. 2008; 128:1048-1052.

123. Muller A, Tal R, Donohue JF et al. The effect of hyperbaric oxygen therapy on erectile function recovery in a rat cavernous nerve injury model. J Sex Med. 2008; 5:562-570.

124. Bajrovic FF, Sketelj J, Jug M, Gril I, Mekjavic IB. The effect of hyperbaric oxygen treatment on early regeneration of sensory axons after nerve crush in the rat. J Peripher Nerv Syst. 2002; 7:141-148.

125. Santos PM. A functional model system of an hypoxic nerve injury and its evaluation. Laryngoscope. 2000; 110:845-853.

126. Eguiluz-Ordonez R, Sanchez CE, Venegas A, Figueroa-Granados V, Hernandez-Pando R. Effects of hyperbaric oxygen on peripheral nerves. Plast Reconstr Surg. 2006; 118:350-357; discussion 358-359.

127. Haapaniemi T, Nishiura Y, Dahlin LB. Effects of hyperbaric oxygen treatment on axonal outgrowth in sciatic nerve grafts in rats. Scand J Plast Reconstr Surg Hand Surg. 2001; 35:7-11.

128. Nishiura Y, Haapaniemi T, Dahlin LB. Hyperbaric oxygen treatment has different effects on nerve regeneration in acellular nerve and muscle grafts. J Peripher Nerv Syst. 2001; 6:73-78.

129. Zamboni WA, Brown RE, Roth AC, Mathur A, Stephenson LL. Functional evaluation of peripheral-nerve repair and the effect of hyperbaric oxygen. J Reconstr Microsurg. 1995; 11:27-29; discussion 29-30.

130. Haapaniemi T, Nishiura Y, Dahlin LB. Functional evaluation after rat sciatic nerve injury followed by hyperbaric oxygen treatment. J Peripher Nerv Syst. 2002; 7:149-154.

131. Nakajima M, Kuwabara S, Uchino F, Hirayama K. [Enhanced regeneration of terminal axons after hyperbaric oxygen therapy in a patient resembling progressive post-poliomyelitis muscular atrophy]. Rinsho Shinkeigaku. 1994; 34:48-51.

132. Zhao DW. [Therapeutic effect of hyperbaric oxygen on recovery of surgically repaired peripheral nerve injury]. Zhonghua Wai Ke Za Zhi. 1991; 29:118-120, 143.

133. Bajrovic FF, Sketelj J, Jug M, Gril I, Mekjavic IB. The effect of hyperbaric oxygen treatment on early regeneration of sensory axons after nerve crush in the rat. J Peripher Nerv Syst. 2002; 7:141-148.

134. Haapaniemi T, Nylander G, Kanje M, Dahlin L. Hyperbaric oxygen treatment enhances regeneration of the rat sciatic nerve. Exp Neurol. 1998; 149:433-438.

135. Nishiura Y, Haapaniemi T, Dahlin LB. Hyperbaric oxygen treatment has different effects on nerve regeneration in acellular nerve and muscle grafts. J Peripher Nerv Syst. 2001; 6:73-78.

136. Eguiluz-Ordonez R, Sanchez CE, Venegas A, Figueroa-Granados V, Hernandez-Pando R. Effects of hyperbaric oxygen on peripheral nerves. Plast Reconstr Surg. 2006; 118:350-357; discussion 358-359.

137. Bradshaw PO, Nelson AG, Fanton JW, Yates T, Kagan-Hallet KS. Effect of hyperbaric oxygenation on peripheral nerve regeneration in adult male rabbits. Undersea Hyperb Med. 1996; 23:107-113.

138. Sanchez EC. Hyperbaric oxygenation in peripheral nerve repair and regeneration. Neurological Research. 2007; 29:184-198.

139. Al-Waili NS, Butler GJ, Beale J et al. Hyperbaric oxygen in the treatment of patients with cerebral stroke, brain trauma, and neurologic disease. Adv Ther. 2005; 22:659-678.

140. Al-Waili NS, Butler GJ, Beale J et al. Hyperbaric oxygen in the treatment of patients with cerebral stroke, brain trauma, and neurologic disease. Advances in Therapy. 2005; 22:659-678.

141. Al-Waili NS, Butler GJ, Beale J et al. Hyperbaric oxygen and malignancies: a potential role in radiotherapy, chemo-therapy, tumor surgery and phototherapy. Med Sci Monit. 2005; 11:RA279-289.

142. Rossignol DA. Hyperbaric oxygen therapy might improve certain pathophysiological findings in autism. Med Hypotheses. 2007; 68:1208-1227.

143. Al-Waili NS, Butler GJ, Petrillo RL, Carrey Z, Hamilton RW. Hyperbaric oxygen and lymphoid system function: a review supporting possible intervention in tissue transplantation. Technol Health Care. 2006; 14:489-498.

144. Vlodavsky E, Palzur E, Soustiel JF. Hyperbaric oxygen therapy reduces neuroinflammation and expression of matrix metalloproteinase-9 in the rat model of traumatic brain injury. Neuropathol Appl Neurobiol. 2006; 32:40-50.

145. Montgomery D, Goldberg J, Amar M et al. Effects of hyperbaric oxygen therapy on children with spastic diplegic cerebral palsy: a pilot project. Undersea Hyperb Med. 1999; 26:235-242.

146. Harch PG, Kriedt C, Van Meter KW, Sutherland RJ. Hyperbaric oxygen therapy improves spatial learning and memory in a rat model of chronic traumatic brain injury. Brain Res. 2007; 1174:120-129.

147. Baugh MA. HIV: reactive oxygen species, enveloped viruses and hyperbaric oxygen. Med Hypotheses. 2000; 55:232-238.

148. Edwards ML. Hyperbaric oxygen therapy. Part 2: application in disease. J Vet Emerg Crit Care (San Antonio). 20:289-297.

149. Marcon RM, Cristante AF, de Barros Filho TE, de Oliveira RP, dos Santos GB. Potentializing the effects of GM1 by hyperbaric oxygen therapy in acute experimental spinal cord lesion in rats. Spinal Cord. 48:808-813.

150. Topuz K, Colak A, Cemil B et al. Combined hyperbaric oxygen and hypothermia treatment on oxidative stress parameters after spinal cord injury: an experimental study. Arch Med Res. 41:506-512.

151. Kahraman S, Duz B, Kayali H et al. Effects of methyl-prednisolone and hyperbaric oxygen on oxidative status after experimental spinal cord injury: a comparative study in rats. Neurochem Res. 2007; 32:1547-1551.

152. Dave KR, Prado R, Busto R et al. Hyperbaric oxygen therapy protects against mitochondrial dysfunction and delays onset of motor neuron disease in Wobbler mice. Neuroscience. 2003; 120:113-120.

153. Steele J, Matos LA, Lopez EA et al. Phase I safety study of hyperbaric oxygen therapy for amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2004; 5:250-254.

154. Yin D, Zhou C, Kusaka I et al. Inhibition of apoptosis by hyperbaric oxygen in a rat focal cerebral ischemic model. J Cereb Blood Flow Metab. 2003; 23:855-864.

155. Wada K, Nishi D, Kitamura T et al. Hyperbaric oxygenation therapy enhances the protective effect of moderate hypothermia against forebrain ischemia in the gerbil hippocampus. Undersea Hyperb Med. 2006; 33:399-405.

156. Calvert JW, Yin W, Patel M et al. Hyperbaric oxygenation prevented brain injury induced by hypoxia-ischemia in a neonatal rat model. Brain Res. 2002; 951:1-8.

157. Chuba PJ, Aronin P, Bhambhani K et al. Hyperbaric oxygen therapy for radiation-induced brain injury in children. Cancer. 1997; 80:2005-2012.

158. Bui QC, Lieber M, Withers HR, Corson K, van Rijnsoever M, Elsaleh H. The efficacy of hyperbaric oxygen therapy in the treatment of radiation-induced late side effects. Int J Radiat Oncol Biol Phys. 2004; 60:871-878.

159. Yin D, Zhang JH. Delayed and multiple hyperbaric oxygen treatments expand therapeutic window in rat focal cerebral ischemic model. Neurocrit Care. 2005; 2:206-211.

160. Vander Heide SJ, Seamon JP. Resolution of delayed altered mental status associated with hydrogen peroxide ingestion following hyperbaric oxygen therapy. Academic Emergency Medicine. 2003; 10:998-1000.

161. Clifton GL. Hypothermia and hyperbaric oxygen as treatment modalities for severe head injury. New Horizons. 1995; 3:474-478.

162. Iwatsuki N, Takahashi M, Ono K, Tajima T. Hyperbaric oxygen combined with nicardipine administration accelerates neurologic recovery after cerebral ischemia in a canine model. Critical Care Med. 1994; 22:858-863.

163. Palzur E, Zaaroor M, Vlodavsky E, Milman F, Soustiel JF. Neuroprotective effect of hyperbaric oxygen therapy in brain injury is mediated by preservation of mitochondrial membrane properties. Brain Res. 2008; 1221:126-133.

164. Nakajima M, Kuwabara S, Uchino F, Hirayama K. [Enhanced regeneration of terminal axons after hyperbaric oxygen therapy in a patient resembling progressive postpoliomyelitis muscular atrophy]. Rinsho Shinkeigaku Clinical Neurology. 1994; 34:48-51.

165. Zamboni WA, Brown RE, Roth AC, Mathur A, Stephenson LL. Functional evaluation of peripheral-nerve repair and the effect of hyperbaric oxygen. J Reconstr Microsurg. 1995; 11:27-29; discussion 29-30.

166. Budic I, Pavlovic D, Cvetkovic T et al. The effects of different anesthesia techniques on free radical production after tourniquet-induced ischemia-reperfusion injury at children's age. Vojnosanit Pregl. 67:659-664.

167. Kawamura N, Schmelzer JD, Wang Y, Schmeichel AM, Low PA. The therapeutic window of hypothermic neuroprotection in experimental ischemic neuropathy: protection in ischemic phase and potential deterioration in later reperfusion phase. Exp Neurol. 2005; 195:305-312.

168. Prpa B, Huddleston PM, An KN, Wood MB. Revascularization of nerve grafts: a qualitative and quantitative study of the soft-tissue bed contributions to blood flow in canine nerve grafts. J Hand Surg Am. 2002; 27:1041-1047.

169. Colen KL, Choi M, Chiu DT. Nerve grafts and conduits. Plast Reconstr Surg. 2009; 124:e386-394.

170. Ostrowski RP, Graupner G, Titova E et al. The hyperbaric oxygen preconditioning-induced brain protection is mediated by a reduction of early apoptosis after transient global cerebral ischemia. Neurobiol of Disease. 2008; 29:1-13.

171. Wood MD, Macewan MR, French AR et al. Fibrin matrices with affinity-based delivery systems and neurotrophic factors promote functional nerve regeneration. Biotechnol and Bioengineering. 106:970-979.

172. Gordon T. The physiology of neural injury and regeneration: The role of neurotrophic factors. J Commun Disorders. 43:265-273.

173. Gordon T, Chan KM, Sulaiman OA, Udina E, Amirjani N, Brushart TM. Accelerating axon growth to overcome limitations in functional recovery after peripheral nerve injury. J Neurosurg 2009; 65: A132-44.

174. Chu TH, Li SY, Guo A, Wong WM, Yuan Q, Wu W. Implantation of neurotrophic factor-treated sensory nerve graft enhances survival and axonal regeneration of motoneurons after spinal root avulsion. Journal of Neuropathology and Exp Neurol. 2009; 68:94-101.

175. Michalski B, Bain JR, M Fahnestock. Long-term changes in neurotrophic factor expression in distal nerve stump following denervation and reinnervation with motor or sensory nerve. J Neurochem. 2008; 105:1244-1252.

176. Yu Y, Matsuyama Y, Yanase M et al. Effects of hyperbaric oxygen on GDNF expression and apoptosis in spinal cord injury. Neuroreport. 2004; 15:2369-2373.

177. Tai PA, Chang CK, Niu KC, Lin MT, Chiu WT, Lin CM. Attenuating experimental spinal cord injury by hyperbaric oxygen: stimulating production of vasculoendothelial and glial cell line-derived neurotrophic growth factors and interleukin-10. J Neurotrauma. 27:1121-1127.

178. Asano T, Kaneko E, Shinozaki S et al. Hyperbaric oxygen induces basic fibroblast growth factor and hepatocyte growth factor expression, and enhances blood perfusion and muscle regeneration in mouse ischemic hind limbs. Circ J. 2007; 71:405-411.

179. Thornton MR, Shawcross SG, Mantovani C, Kingham PJ, Birchall MA, Terenghi G. Neurotrophins 3 and 4 differentially regulate NCAM, L1 and N-cadherin expression during peripheral nerve regeneration. Biotechnol and Applied Biochem. 2008; 49:165-174.

180. Fu C, Hong G, Wang F. Favorable effect of local VEGF gene injection on axonal regeneration in the rat sciatic nerve. J Huazhong Univ Sci Technolog Med Sci. 2007; 27:186-189.

181. Hobson MI, Green CJ, Terenghi G. VEGF enhances intraneural angiogenesis and improves nerve regeneration after axotomy. J Anat. 2000; 197 Pt 4:591-605.

182. Atkins S, Loescher AR, Boissonade FM et al. Interleukin-10 reduces scarring and enhances regeneration at a site of sciatic nerve repair. J Periph Nerv System. 2007; 12:269-276.

183. Zhao LL, Davidson JD, Wee SC, Roth SI, Mustoe TA. Effect of hyperbaric oxygen and growth factors on rabbit ear ischemic ulcers. Archives of Surg. 1994; 129:1043-1049.

184. Gu GJ, Li YP, Peng ZY et al. Mechanism of ischemic tolerance induced by hyperbaric oxygen preconditioning XJ involves upregulation of hypoxia-inducible factor-1alpha and erythropoietin in rats. J Applied Physiol. 2008; 104:1185-1191.

185. Lykissas MG, Korompilias AV, Vekris MD, Mitsionis GI, Sakellariou E, Beris AE. The role of erythropoietin in central and peripheral nerve injury. Clin Neurol Neurosurg. 2007; 109:639-644.

186. McLeod M, Hong M, Mukhida K, Sadi D, Ulalia R, Mendez I. Erythropoietin and GDNF enhance ventral mesencephalic fiber outgrowth and capillary proliferation following neural transplantation in a rodent model of Parkinson's disease. Europ J Neurosci. 2006; 24:361-370.

187. Lohmeyer JA, Essmann E, Richerson SJ et al. Use of Erythropoietin as adjuvant therapy in nerve reconstruction. Langenbecks Arch Surgery. 2008; 393:317-323.

188. Gorio A, Gokmen N, Erbayraktar S et al. Recombinant human erythropoietin counteracts secondary injury and markedly enhances neurological recovery from experimental spinal cord trauma. Proceedings of the Nat Acad Sci United States of Am. 2002; 99:9450-9455.

189. Vitellaro-Zuccarello L, Mazzetti S, Madaschi L, Bosisio P, Gorio A, De Biasi S. Erythropoietin-mediated preservation of the white matter in rat spinal cord injury. Neurosci. 2007; 144:865-877.

190. Vitellaro-Zuccarello L, Mazzetti S, Madaschi L et al. Chronic erythropoietin-mediated effects on the expression of astrocyte markers in a rat model of contusive spinal cord injury. Neurosci. 2008; 151:452-466.

191. Pan HC, Chin CS, Yang DY et al. Human amniotic fluid mesenchymal stem cells in combination with hyperbaric oxygen augment peripheral nerve regeneration. Neurochem Res. 2009; 34:1304-1316.

192. Gorio A, Madaschi L, Di Stefano B et al. Methylprednisolone neutralizes the beneficial effects of erythropoietin in experimental spinal cord injury. Proc Nat Acad Sciences United States of Am. 2005; 102:16379-16384.

193. Voigt C, Forschler A, Jaeger M, Meixensberger J, Kuppers-Tiedt L, Schuhmann MU. Protective effect of hyperbaric oxygen therapy on experimental brain contusions. Acta Neurochir Suppl. 2008; 102:441-445.

194. McDonagh M, Helfand M, Carson S, Russman BS. Hyperbaric oxygen therapy for traumatic brain injury: a systematic review of the evidence. Archives of Physical Med and Rehabil. 2004; 85:1198-1204.

195. Bennett MH, Trytko B, Jonker B. Hyperbaric oxygen therapy for the adjunctive treatment of traumatic brain injury. Cochrane Database Syst Rev. 2004; CD004609.

196. Wu J, Yang H, Qiu Z, Zhang Q, Ding T, Geng D. Effect of combined treatment with methylprednisolone and nogo-a monoclonal antibody after rat spinal cord injury. J Int Med Res. 38:570-582.

197. Sribnic, EA, Samantaray S, Das A et al. Postinjury estrogen treatment of chronic spinal cord injury improves locomotor function in rats. J Neurosci Res. 88:1738-1750.

198. Munts AG, van der Plas AA, Ferrari MD, Teepe-Twiss IM, Marinus J, van Hilten JJ. Efficacy and safety of a single intrathecal methylprednisolone bolus in chronic complex regional pain syndrome. Eur J Pain. 14:523-528.

199. Fumagalli F, Madaschi L, Brenna P et al. Single exposure to erythropoietin modulates Nerve Growth Factor expression in the spinal cord following traumatic injury: comparison with methylprednisolone. Eur J Pharmacol. 2008; 578:19-27.

200. Guo Q, S Li, Liang Y, Zhang Y et al. Effects of C3 deficiency on inflammation and regeneration following spinal cord injury in mice. Neurosci Lett. 485:32-36.

201. Matchett GA, Martin RD, Zhang JH. Hyperbaric oxygen therapy and cerebral ischemia: neuroprotective mechanisms. Neurol Res. 2009; 31:114-121.

202. Poli S, Veltkamp R. Oxygen therapy in acute ischemic stroke - experimental efficacy and molecular mechanisms. Curr Mol Med. 2009; 9:227-241.

203. Veltkamp R, Warner DS, Domoki F et al. Hyperbaric oxygen decreases infarct size and behavioral deficit after transient focal cerebral ischemia in rats. Brain Res. 2000; 853:68-73.

204. Konda A, Baba S, Iwaki T et al. Hyperbaric oxygenation prevents delayed neuronal death following transient ischaemia in the gerbil hippocampus. Neuropath and Applied Neurobiol. 1996; 22:350-360.

205. Helms AK, Whelan HT, Torbey MT. Hyperbaric oxygen therapy of cerebral ischemia. Cerebrovascular Diseases. 2005; 20:417-426.

206. Veltkamp R, Siebing DA, Heiland S et al. Hyperbaric oxygen induces rapid protection against focal cerebral ischemia. Brain Res. 2005; 1037:134-138.

207. Chang CF, Niu KC, Hoffer BJ, Wang Y, Borlongan CV. Hyperbaric oxygen therapy for treatment of postischemic stroke in adult rats. Exp Neurol. 2000; 166:298-306.

208. Takahashi M, Iwatsuki N, Ono K, Tajima T, Akama M, Koga Y. Hyperbaric oxygen therapy accelerates neurologic recovery after 15-minute complete global cerebral ischemia in dogs. Critical Care Med. 1992; 20:1588-1594.

209. Xue L, Yu Q, Zhang H, Liu Y, Wang C, Wang Y. Effect of large dose hyperbaric oxygenation therapy on prognosis and oxidative stress of acute permanent cerebral ischemic stroke in rats. Neurol Res. 2008; 30:389-393.

210. Bennett MH, Wasiak J, Schnabel A, Kranke P, French C. Hyperbaric oxygen therapy for acute ischaemic stroke. Cochrane Database Syst Rev. 2005; CD004954.

211. Veltkamp R, Siebing DA, Sun L et al. Hyperbaric oxygen reduces blood-brain barrier damage and edema after transient focal cerebral ischemia. Stroke. 2005; 36:1679-1683.

212. Rusyniak DE, Kirk MA, May JD et al. Hyperbaric oxygen therapy in acute ischemic stroke: results of the Hyperbaric Oxygen in Acute Ischemic Stroke Trial Pilot Study. Stroke. 2003; 34:571-574.

213. Anderson DC, Bottini AG, Jagiella WM et al. A pilot study of hyperbaric oxygen in the treatment of human stroke. Stroke. 1991; 22:1137-1142.

214. Jadhav V, Ostrowski RP, Tong W, Matus B, Chang C, Zhang JH. Hyperbaric oxygen preconditioning reduces postoperative brain edema and improves neurological outcomes after surgical brain injury. Acta Neurochir Suppl. 106:217-220.

215. Yamashita S, Hirata T, Mizukami Y et al. Repeated preconditioning with hyperbaric oxygen induces neuroprotection against forebrain ischemia via suppression of p38 mitogen activated protein kinase. Brain Res. 2009; 1301:171-179.

216. Wang L, Li W, Kang Z et al. Hyperbaric oxygen preconditioning attenuates early apoptosis after spinal cord ischemia in rats. J Neurotrauma. 2009; 26:55-66.

217. Kretz A, Happold CJ, Marticke JK, Isenmann S. Erythropoietin promotes regeneration of adult CNS neurons via Jak2/Stat3 and PI3K/AKT pathway activation. Molec and Cell Neurosci. 2005; 29:569-579.

218. Peng Z, Ren P, Kang Z et al. Up-regulated HIF-1alpha is involved in the hypoxic tolerance induced by hyperbaric oxygen preconditioning. Brain Res. 2008; 1212:71-78.

219. Li JS, Zhang W, Kang ZM et al. Hyperbaric oxygen preconditioning reduces ischemia-reperfusion injury by inhibition of apoptosis via mitochondrial pathway in rat brain. Neurosci. 2009; 159:1309-1315.

220. Xiong L, Zhu Z, Dong H, Hu W, Hou L, Chen S. Hyperbaric oxygen preconditioning induces neuroprotection against ischemia in transient not permanent middle cerebral artery occlusion rat model. Chinese Med J. 2000; 113:836-839.

221. Dong H, Xiong L, Zhu Z, Chen S, Hou L, Sakabe T. Preconditioning with hyperbaric oxygen and hyperoxia induces tolerance against spinal cord ischemia in rabbits. Anesthesiol. 2002; 96:907-912.

222. Li J, Liu W, Ding S et al. Hyperbaric oxygen preconditioning induces tolerance against brain ischemia-reperfusion injury by upregulation of antioxidant enzymes in rats. Brain Res. 2008; 1210:223-229.

223. Li Z, Liu W, Kang Z et al. Mechanism of hyperbaric oxygen preconditioning in neonatal hypoxia-ischemia rat model. Brain Res. 2008; 1196:151-156.

224. Yang ZJ, Xie Y, Bosco GM, Chen C, Camporesi EM. Hyperbaric oxygenation alleviates MCAO-induced brain injury and reduces hydroxyl radical formation and glutamate release. Eur J Applied Physiol and Occupatioal Physiol. 108:513-522.

225. Zhong Y, Yao H, Deng L, Cheng Y, Zhou X. Promotion of neurite outgrowth and protective effect of erythropoietin on the retinal neurons of rats. Graefes Arch for Clin and Exp Ophthal. 2007; 245:1859-1867.

226. Wang R, Xu J, Xie J et al. Hyperbaric oxygen preconditioning promotes survival of retinal ganglion cells in a rat model of optic nerve crush. J Neurotrauma. 27:763-770.

227. Tai PA, Chang CK, Niu KC, Lin MT, Chiu WT. Lin, CM. Attenuating experimental spinal cord injury by hyperbaric oxygen: stimulating production of vasculoendothelial and glial cell line-derived neurotrophic growth factors and interleukin-10. J Neurotrauma. 2010; 27:1121-1127.

228. Arato E, Kurthy M, Sinay L et al. Effect of vitamin E on reperfusion injuries during reconstructive vascular operations on lower limbs. Clinical Hemorheology and Microcirc. 44:125-136.

229. Lucius R, Mentlein R, Sievers J. Riboflavin-mediated axonal degeneration of postnatal retinal ganglion cells in vitro is related to the formation of free radicals. Free Radical Biol and Med. 1998; 24:798-808.

230. Al Moutaery K, Arshaduddin M, Tariq M, Al Deeb S. Functional recovery and vitamin E level following sciatic nerve crush injury in normal and diabetic rats. Intl J Neurosc. 1998; 96:245-254.

231. Cameron NE, Cotter MA. Metabolic and vascular factors in the pathogenesis of diabetic neuropathy. Diabetes. 1997; 46 Suppl 2:S31-37.

232. Lucius R, Sievers J. Postnatal retinal ganglion cells in vitro: protection against reactive oxygen species (ROS)-induced axonal degeneration by cocultured astrocytes. Brain Res. 1996; 743:56-62.

233. Cheng H, Fu YS, Guo JW. Ability of GDNF to diminish free radical production leads to protection against kainate-induced excitotoxicity in hippocampus. Hippocampus. 2004; 14:77-86.

234. Lykissas MG, Sakellariou E, Vekris MD et al. Axonal regeneration stimulated by erythropoietin: an experimental study in rats. J Neurosci Methods. 2007; 164:107-115.

235. Berkingali N, Warnecke A, Gomes P et al. Neurite outgrowth on cultured spiral ganglion neurons induced by erythropoietin. Hearing Res. 2008; 243:121-126.

236. Yin ZS, Zhang H, Gao W. Erythropoietin promotes functional recovery and enhances nerve regeneration after peripheral nerve injury in rats. AJNR . 31:509-515.

237. Aydin A, Ozden BC, Karamursel S, Solakoglu S, Aktas S, Erer M. Effect of hyperbaric oxygen therapy on nerve regeneration in early diabetes. Microsurg. 2004; 24:255-261.

238. Bakker DJ. Hyperbaric oxygen therapy and the diabetic foot. Diabetes/Metabol Res and Reviews. 2000; 16 Suppl 1:S55-58.

239. Tuma Junior P, Dias MD, Arrunategui G et al. Effect of hyperbaric oxygen on the regeneration of experimental crush injuries of nerves. Rev Hosp Clin Fac Med Sao Paulo. 1999; 54:81-84.

240. Haapaniemi T, Nylander G, Kanje M, Dahlin L. Hyperbaric oxygen treatment enhances regeneration of the rat sciatic nerve. Exp Neurol. 1998; 149:433-438.

241. Santos PM, Zamboni WA, Williams SL, Covey JF, Kienstra MA. Hyperbaric oxygen treatment after rat peroneal nerve transection and entubulation. Otolaryngol Head Neck Surg. 1996; 114:424-434. ❑