Hyperbaric oxygenation in peripheral nerve repair and regeneration

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Peripheral nerves are essential connections between the central nervous system and muscles, autonomic structures and sensory organs. Their injury is one of the major causes for severe and longstanding impairment in limb function. Acute peripheral nerve lesion has an important inflammatory component and is considered as ischemia-reperfusion (IR) injury. Surgical repair has been the standard of care in peripheral nerve lesion. It has reached optimal technical development but the end results still remain unpredictable and complete functional recovery is rare. Nevertheless, nerve repair is not primarily a mechanical problem and microsurgery is not the only key to success. Lately, there have been efforts to develop alternatives to nerve graft. Work has been carried out in basal lamina scaffolds, biologic and non-biologic structures in combination with neurotrophic factors and/or Schwann cells, tissues, immunosuppressive agents, growth factors, cell transplantation, principles of artificial sensory function, gene technology, gangliosides, implantation of microchips, hormones, electromagnetic fields and hyperbaric oxygenation (HBO). HBO appears to be a beneficial adjunctive treatment for surgical repair in the acute peripheral nerve lesion, when used at lower pressures and in a timely fashion (<6 hours). [Neurol Res 2007; **29**: 184–198]

Keywords: Peripheral nerve injury; nerve repair; nerve regeneration; hyperbaric oxygenation; growth factors; ischemia-reperfusion injury

INTRODUCTION

Reports of acute nerve injury can be traced to 3500 years ago, in a biblical story[1]. In the seventh century, Paulus Aegineta was the first to report the use of suture and agglutination to repair nerves[2]. The pioneer of peripheral nerve surgery was Gabriele Ferrara who described the technique of suturing stumps of a transected nerve[3].

Dysfunction of peripheral nerves results from damage to the neuron, to the Schwann cells or to the myelin sheath[4]. Many mechanisms of injury to peripheral nerves exist, including mechanical damage, crush injury, lacerations, penetrating trauma, stretch injury, high-velocity trauma, frostbites and iatrogenic injury[5]. Peripheral nerve injuries are relatively common[6]. They are one of the major causes for severe and longstanding impairment of limb function and remain as one of the most challenging and difficult reconstructive problems. I will classify the degree of injury, describe the surgical repair options and discuss adjunctive therapies, including hyperbaric oxygen.

CLASSIFICATION OF PERIPHERAL NERVE INJURY

The classification of nerve injury was described by Seddon[7] and later expanded by Sunderland and Bradley[2] and Mackinnon and Dellon[8]. The first-degree, neuropraxia, involves a temporary conduction block with demyelination of the nerve at the site of injury. It is a dysfunction and/or paralysis without loss of nerve sheath continuity and peripheral Wallerian degeneration. Electrodiagnostic study results are normal above and below the level of injury, and no denervation muscle changes are present. No Tinel's sign is present. Complete recovery occurs once the nerve has remyelinated at the damage area. Recovery may take up to 12 weeks[5,9].

A second-degree injury, axonotmesis, results from a more severe trauma or compression. The internal architecture is relatively preserved and can guide proximal axonal regeneration to reinnervate distal target organs[10]. This causes Wallerian degeneration distal to the level of injury and proximal axonal degeneration to at least the next node of Ranvier. In more severe traumatic injuries, it could extend beyond the next node. Electrodiagnostic studies demonstrate denervation changes in the affected muscles, and in cases of reinnervation, motor unit potentials (MUPs) are present[5].

In the third-degree injuries, axon continuity is disrupted by loss of endoneurial sheaths but the perineurium is preserved[9]. It is a more severe injury than the second-degree. Wallerian degeneration occurs and electrodiagnostic studies demonstrate denervation changes with fibrillations in the affected muscles. Because the endoneurial tubes are not intact, the regenerating axons may not reinnervate their original motor and sensory targets[5].

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In the fourth-degree injury, the nerve fasciculi are damaged but nerve sheath continuity is preserved[5,9]. It results in a large area of scar at the site of nerve injury and precludes any axon from advancing distal to the level of nerve injury. Electrodiagnostic studies reveal that denervation changes the affected muscles and no MUPs are present. No improvement of function is noted and the patient requires surgery to restore neural continuity, thus permitting axonal regeneration and motor and sensory reinnervation[5].

In the fifth-degree injuries, there is a complete transection of the nerve. They correspond to Seddon's classification of a neurotmesis lesion. It requires surgery to restore neural continuity. Electrodiagnostic studies are the same as in the fourth-degree injury[5,9]. Mackinnon introduced a sixth-degree injury to describe a complex peripheral nerve injury. It is a mixed nerve injury that combines the other degrees of injury[9].

PATHOPHYSIOLOGY

The physiologic response is different, depending on the type of injury. If the axon is spared, as in the first-degree injury, conduction is interrupted due to demyelination, but is reinstated whenever the aggravating stimulus is removed and the myelin layers are restored. If the axon, or more, is transected, causing a second- to fifth-degree injury, the response has two main phases, degeneration and regeneration, and takes substantially longer[11]. Pathologically, three phases were identifiable: phase 1 (0–3 hours): minimal pathologic changes and minimal edema, phase 2 (7–14 days): prominent fiber degeneration and endoneurial edema, and phase 3 (28–42 days): abundant small regenerating fiber clusters and minimal edema[12].

The first phase of axon injury, degeneration, was first described by Waller in 1850 (Ref. 13). Following injury, changes occur in the cell body, axon and the Schwann cell. Both distally and proximally, the axon begins to disintegrate and undergoes apoptosis. Local Schwann cells and macrophages clean up the apoptotic debris, creating long, clean endoneurial tubes. Once the debris has been removed, Schwann cells proliferate and organize themselves into columns that lie within the endoneurial tubes, creating the bands of Bunger[8,11,14].

After injury, there is a great increase in the production of mRNA and proteins[15]. These products are transported down the axon, providing the material and energy for nerve elongation to the distal tip. The severed axons begin to sprout and contain an expanded region known as the growth cone. This is the site of axon elongation and sends out many small processes that seek specific markers, which influence the axon in its movements to preferentially select neural tissue and even exhibit a preference for endoneurial tubes with the same function[16].

The axon's response is regulated by neurotrophic factors that direct and attract the axon[17]. Neurotrophic factors induce maturation and elongation of the axon[14]. They are released by macrophages, Schwann cells and other supporting cells.

If the nerve is too far away, the axons are not strongly attracted to the distal end and eventually stop advancing, causing aberrant innervation at the end organ level[14,17].

Large gaps, greater than 15 mm, cannot be crossed reliably by axons. This is usually because proliferating Schwann cells or fibroblasts grow between the severe nerve ends and form a physical blockage[18]. If the axon sprouts stop proliferating and take residence in a different tissue, they will form a neuroma[19].

Neuroinflammation in primary demyelination and Wallerian degeneration is an ischemia-reperfusion (IR) injury and is mediated by cytokines and other immune mediators (*Figure 1*)[12,13,20–36]. IR injury contributes to both axonal degeneration and regeneration[13]. Reperfusion, by oxidative injury, worsened nerve function and aggravated fiber degeneration, but in the longer time frame, permitted fiber regeneration to occur[12].

During peripheral nerve injury, hypoxia creates several cellular shock stages that could be reversible or irreversible. The reversibility depends on the cells capability to maintain adenosine-5′-triphosphate (ATP) production[37,38]. Once ATP production is stopped, the cell can no longer maintain the homeostatic functions. This produces cytotoxic edema and release of calcium by the mitochondria[39].

ATP has many important functions on peripheral nerve repair and regeneration. It is important to maintain cellular functions and is also the energy source for axoplasmic transport. It is indispensable for the transport of nerve growth factors (NGF)[40]. There is a potential interplay between ATP and NGF in the signaling pathways triggered on their target cells[41]. ATP has neuritrogenic and trophic effects, which are comparable to those sustained by NGF and involve several overlapping pathways[42]. ATP exerts a protective effect on the neurons, which is valuable for nerve regeneration after nerve injury[42].

NERVE REPAIR

The surgical reconstruction of peripheral nerve damage is crucial. Although the surgical procedure may be optimal and an excellent rehabilitation program is conducted, the end results still remain unpredictable and complete functional recovery is still rare[43]. A nerve lesion is different from other tissue injury because it requires more than only local processes. Transection of axons has implications for the whole length of the neuron and the repair process involves outgrowth of neurites over very long distances. In addition, a nerve injury, in contrast to most other injuries of the body, has immediate functional consequences for the brain in terms of rapid functional reorganization in brain cortex[43].

From the biologic point of view, the physiologic result following nerve injury and repair is dependent on factors such as the extent of nerve cell survival after the injury, the rate and quality of axonal outgrowth, the orientation and specificity in growth of regeneration axons, the survival and state of end organs, and cortical

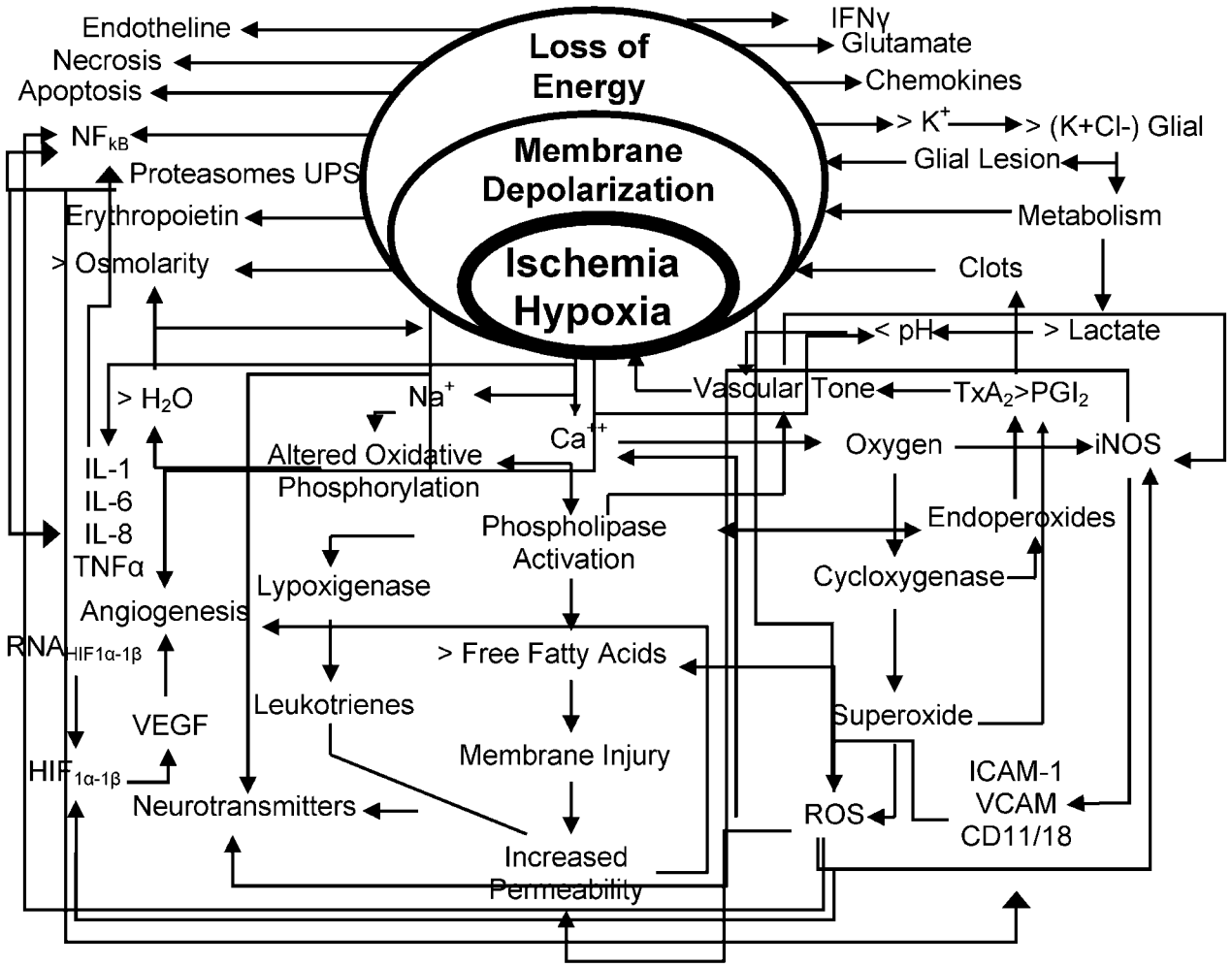

Figure 1: The core of the inflammatory cascades is the reduction of energy and subsequent mitochondrial dysfunction. They are very complicated and have several interactions between them. The only feasible way to stop the cascades is the timely restitution of energy before they all 'kick in'. This could be accomplished with the prompt application of hyperbaric oxygenation (HBO). It could explain the efficacy of HBO in the ischemia-reperfusion injury

reorganizational processes in somatosensory and motor brain cortex. From the clinical viewpoint, the outcome is often incomplete, as expressed in symptoms such as poor and abnormal sensory function, deficient motor function, cold intolerance, pain, impaired function, quality of life and problems at work, leisure and in social life[43].

The surgical approximation of severed nerve ends has reached the most desirable technical refinement. Nevertheless, nerve repair is not primarily a mechanical problem and microsurgery is not the only key to success. At most, the surgeon can manage to co-apt individual groups of fascicles, but the behavior of the separate axons inside individual fascicles cannot be addressed as they are regulated by biologic mechanisms at the molecular level[43].

Autologous nerve graft remains the standard of care; however, much effort is now focused on developing alternatives directed to the biologic mechanisms inside. Work has been carried out in basal lamina scaffolds, biologic and non-biologic structures in combination with neurotrophic factors and/or Schwann cells, tissues, immunosuppressive agents, reimplantation of avulsed nerve roots (brachial plexus surgery) and end-to-side anastomosis.

Other biologic factors may also be important tools to promote survival and regeneration processes of the severed ends, and improve the functional results. This has spearheaded new research into growth factors, cell transplantation, principles of artificial sensing, gene technology, gangliosides, implantation of microchips, hormones, electromagnetic fields and hyperbaric oxygenation (HBO) as potential adjuvant therapies. Nevertheless, these therapies have gained very limited clinical application.

SURGICAL ASPECTS OF NERVE REPAIR

The purpose of the surgical reconstruction is to align the proximal and distal nerve segments. In the last 30 years, there has been great advancement in the technical aspects of nerve reconstruction[44–56]. Direct muscular neurotization has been used for cases where the nerve has been avulsed from the muscle and the repair is

impossible[49]. The reimplantation of avulsed nerve roots with subsequent functional recovery has been tried for brachial plexus lesions in selected cases[50,57].

Autologous nerve grafts

Although this remains the gold standard in peripheral nerve repair and regeneration, autologous nerves are in limited supply, with the sural nerve graft being the primary source[58]. The purpose of such grafts is to provide a guide or conduit consisting of a basal lamina and Schwann cells that support axonal regeneration. The thickness of the graft is important so that it guarantees enough Schwann cells to synthesize neurotrophic factors, laminin and fibronectin in the basal lamina[59–61]. They have also been used in combination with autologous fibrin glue containing large number of platelets[62].

Another technique developed to improve axonal outgrowth is the pre-degeneration of the nerve graft that reduces the latency of repair[63]. Vascularized nerve grafts have also been described for extensive gaps, especially in crush injuries with massive skin defects and poor blood supply. Selective regeneration of motor and sensory axons has also been attempted[64].

Nerve allografts

Nerve allografts have been extensively investigated, but they require heavy immunosuppression to avoid rejection and failure[65–68]. Normally, this is accomplished with the use of cyclosporine and prednisone. Acellular allografts have been tried to reduce immunogenicity. There is still limited clinical experience of its use[69].

Basal lamina scaffolds from muscle

Any biologic tissue that contains basal lamina may serve as a bridge for nerve regeneration. Frozen and thawed muscle grafts have been used to bridge gaps in nerve continuity[70,71]. Regenerating axons grow readily into the empty basal lamina cylinders of such grafts containing laminin and fibronectin[43,72]. Migration of Schwann cells into the grafts is essential[73]. There is a critical length for the use of such grafts; however, with the introduction of a small nerve segment in the middle of the muscle graft, the conduits can be provided with an intermediate depot of Schwann cells to improve its regenerating potential[74]. There have been some clinical trials that have failed once they reach a critical length, probably due to insufficient supply of cells and neovascularization[75].

Other types of conduits

Venous grafts have been successful in bridging gaps in nerve continuity[76]. Various types of bioreabsorbable tubes have been used to bridge defects[77]. Silicone tubes can only be used together with various types of factors, cells and materials, to improve regeneration[78]. Animal models with multiple longitudinal synthetic filaments in the lumen have been used successfully to bridge extended nerve gaps[79]. Good results have also been reported in experimental models using biologic materials, such as collagen, as an extracellular matrix[80]. Other biologic grafts successfully used are biodegradable collagen grafts[81] with laminin[82] and fibronectin[83] which produce neurite-promoting factor[84] and axonal enlogation[85], teased tendons formed into a loose collagen roll[86], freeze-dried alginate gels[87], chitosan-PLA composite[88–90], 90 PLA/10 PLG nerve guides[91,92], glutaraldehyde cross-linking gelatin conduit[93] and expanded polytetrafluoroethylene tubes with autogenous vein[94].

Terminolateral anastomosis

End-to-side anastomosis has been proposed in situations in which the proximal segment of a severed nerve trunk is not available[95]. It is used to induce collateral sprouting from intact axons in the healthy nerve. The collateral sprouts from the donor nerve will reinnervate the distal segment of the injured nerve trunk[96]. Animal experimental models have shown good ingrowth in sensory and motor fibers[97–101].

NEUROTROPHIC FACTORS

There has been substantial development in the field of neurotrophic factors. The cellular and molecular basis for the survival and outgrowth of neurons shows an enormous complexity[102–104]. The key factor for the regeneration following axotomy is the survival of nerve cell bodies which is facilitated by multiple neurotrophic factors. These factors are divided into three major groups: the neurotrophins, neuropoietic cytokines and fibroblast growth factors[105–108]. There are additional groups of neurotrophic factors such as the insulin-like growth factor, epidermal growth factor[109,110], leukemia-inhibiting factor, glial-derived neurotrophic factor[111], transforming growth factor-beta 1 (TGF-β1)[112] and pleiotrophin[113].

The actions of growth factors are exerted by their binding to particular classes of tyrosine kinase (Trk) receptors and a low-affinity NGF present on the surface of the responsible cells. Intracellular signaling and subsequent gene activation follow the activation of the receptor site (ATP is needed for this process).

The neurotrophin family includes NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5) and neurotrophin-6 (NT-6)[43]. NGF mRNA is constituently expressed in healthy nerves and up-regulated following nerve injury in the distal segments[114]. Trophic factors are transmitted by the retrograde transport along the axon and used to sustain survival and essential activities of the nerve cell body[115]. Macrophages are important not only in myelin degradation and nerve remodeling, but also in the production of neurotrophic factors after nerve injury, probably through the release of interleukin-1β (IL-1β)[116]. Schwann cells in the injured nerve trunk also produces growth factors such as NGF[117], insulin-like growth factor[118], ciliary neurotrophic factor[119] and BDNF[120]. Glial cell line-derived neurotrophic factor

(GDNF) stimulates Schwann cells to migrate and enhances myelination[121].

NGF has a key role in sensory neurons survival and neurite outgrowth but has almost no influence on motor neurons[122]. NGF can only influence neurons with high-affinity NGF receptor (TrkA). Motor neurons do not contain TrkA receptor genes and they respond only to TrkB and TrkC.

BDNF supports survival of motor neurons in culture and acts as a trophic factor[123]. In anterior spinal horns, it prevents cell death following axotomy[124]. Its effects are mediated by TrkB and TrkC receptors.

NT-3 binds to TrkC receptors and promotes survival in sensory and motor neurons and differentiation responses in sensory and parasympathetic neurons[41,121]. NT-4/5 binds to TrkB receptors in motor neurons, supports survival and increases the ability of motor neurons to innervate skeletal muscle fibers in co-cultures in rat spinal cord and human muscle[124,125]. NT-6 acts preferably on sympathetic and sensory neurons[126].

OTHER FACTORS

Several other factors can facilitate the regeneration of nerve cell bodies and are being developed as putative adjunctive therapies to autologous nerve grafts.

Betamethasone

Betamethasone has been systemically administered perioperatively to enhance nerve recovery after induced nerve crush injury. Short-term perioperative administration of betamethasone has a beneficial effect on the recovery of the injured rat sciatic nerve[127].

Pyrroloquinoline quinone

Pyrroloquinoline quinone (PQQ) has been tested in animal models to promote nerve regeneration of transected sciatic nerve. It has a remarkable effect on nerve regeneration, sciatic nerve function, sciatic nerve function index, electrophysiologic index and morphologic appearance[128].

Hypothalamic proline-rich peptide

Proline-rich peptide-1 (PRP-1) is produced by neurosecretory cells of hypothalamic nuclei (paraventricular nucleus and supraoptic nucleus), 3 and 4 weeks following rat sciatic nerve transection. Histochemical and electrophysiologic findings provide evidence for reinnervation of the injured side by complete coalescence of transected fibers together with restoration of the motor activity[129].

Low-dose FK506 and anti-CD40 ligand

Low-dose immunomodulatory agents (FK506) in combination with anti-CD40 ligand used in mice with tibial nerve grafting, exhibited robust nerve regeneration without disrupting immune unresponsiveness[130].

Thrombin and peptide thrombin receptor agonist PAR1

Experiments demonstrate a dose-dependent facilitating effect of thrombin and thrombin receptor agonist PAR1 (TRAP6) on regeneration of mouse peripheral nerve after crush injury. The maximal neurotrophic effect was observed at low concentrations[131].

Triiodothyronine

Local administration of triiodothyronine (T3) at the level of transected rat sciatic nerve increases the number and diameter of regenerated axons. Local T3 treatment significantly enhances the expression of superior cervical ganglion 10, a regulator of microtubule dynamics in growth cones that could provide a mechanism by which T3 enhances peripheral nerve regeneration[132].

Neuroactive steroids

Progesterone, dihydroprogesterone, tetrahydroprogesterone, dihydrotestorenone and 3 alpha-diol, stimulate the expression of two important proteins of the myelin of peripheral nerves, the glycoprotein Po and the peripheral myelin protein 22. Neuroactive steroids not only control the expression of these proteins, but also influence the morphology of myelin sheaths and axons[133].

Peripheral benzodiazepine receptor

Peripheral benzodiazepine receptor (PBR) expression increases in small dorsal root ganglion sensory neurons after peripheral nerve injury. It has a role in the early regenerative response of small caliber sensory axons[134].

Activating transcription factor 3

Peripheral nerve compression induces nuclear translocation of activating transcription factor 3 (ATF3), a transcription factor associated with survival and regeneration of sensory neurons. The response is related to duration of compression and partly correlated to function[135].

Cell adhesion molecules

Adhesion molecules, such as N-CAM, L1, the myelin-associated glycoprotein and transient axonal glycoprotein-1, correlate with axonal growth, advancement and regeneration[136,137].

77 kDa muscle-derived protein

Histologic and immunohistochemical evaluations suggested that 77 kDa muscle-derived protein (MDP77) treatment accelerates Schwann cell migration, followed by enhanced maturation of regenerating axons, resulting in functional recovery of both the nerves and the atrophied denervated muscle in rats[138].

Galectin-1

Galectin-1 (gal-1) was the first identified member of the galectin family of beta-galactosidase-binding

proteins released by Schwann cells. It has been implicated in the regenerative response of axons following peripheral nerve injury. Gal-1 has been shown to promote axonal regeneration through the activation of macrophages to secrete an axonal regeneration-promoting factor[139–141].

Transplanted cells

Transplantation of Schwann cells, bone marrow stromal cells, mesenchymal cells and pluripotent embryonic stem cells, has demonstrated contribution to myelin repair[142–149].

Regeneration-associated gene

After peripheral nerve axotomy, a sequence of events including glial activation and axonal regrowth leads to functional recovery of the afflicted pool of motoneurons. As a consequence of nerve injury, there is an increase in the expression of 60 genes with the sustained up-regulation of one specific gene encoding the hematological and neurological expressed sequence-1. It is associated with nervous system development and nerve regeneration[150].

Electroacupuncture

In a model of crushed sciatic nerve in rabbits, electroacupuncture promoted nerve regeneration[151].

Low-frequency pulsed electromagnetic field

Low-frequency pulsed electromagnetic field (PEMF) was ineffective on rat sciatic nerve regeneration, in a model of crushed sciatic nerve in rats[152].

Low intensity ultrasound

Low intensity ultrasound (LIUS) in combination with poly(DL-lactic acid-co-glycolic acid) conduits was found to have significantly greater number and area of regenerated axons at the mid-conduit of implanted grafts. LIUS stimulation on silicone groups was found to induce a mass of fibrous tissues that covered the nerve conduits and retarded axon regeneration[153].

HBO

HBO is an approved adjunctive treatment for several conditions[154]. It has proven to be an effective treatment in the IR injury[155–158]. HBO reduces the IR injury through several mechanisms. First, through hyperoxygenation, its primary mechanism of action, it maintains the viability of the marginal tissue (penumbra)[159]. This hyperoxygenation also creates other secondary mechanisms that are responsible for wound healing and neovascularization[159]. When used in a timely fashion, it can modify the pathophysiology of the IR injury[155].

Increase in oxygen tensions allows the tissues to maintain ATP and other high energy compounds levels. It re-establishes aerobic metabolism and inhibits the elevation of lactate levels. Others have shown that HBO restores not only ATP levels, but also creatine phosphokinase, guanosine triphosphate and uridine triphosphate[160–162]. HBO promotes the production of gluthathione, the principal non-enzymatic body defense against reactive oxygen species (ROS)[160].

HBO reduces the liberation of calcium and thus the increase in phospholipase A2 and cyclooxygenase-2. The protection exerted through the blockage of the arachidonic acid cascade, with the subsequent reduction of leukotrienes, thromboxanes and prostaglandins, protects against the no flow state of the IR injury[163,164]. By blocking nuclear transcription factor kappa B, HBO reduces the inflammatory response created by its up-regulation. It reduces substantially the production of the proinflammatory cytokines, especially IL-1, IL-6, IL-8, tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ) and platelet activating factor (PAF)[165–179].

HBO can inhibit the conversion of xanthine oxidase, reducing the oxidative stress in the reperfusion stage of IR injury[177]. This effect prevents the production of ROS and tissue damage. HBO also prevents endothelial damage and the expression of intercellular adhesion molecule-1 (ICAM-1), soluble intercellular adhesion molecule-1 (slCAM) and integrin beta2 (Refs. 156–158 and 180–182). These effects occur at both the local and systemic levels[183–185].

HBO has protective effects over mitochondrial dysfunction. It restores the electron flux through the I–IV complex, and reduces the formation of ROS and damage of mitochondrial DNA. By reducing the oxidative stress and concomitant oxidative damage, it prevents apoptosis and damage created by the glutamate cascade and down-regulates the Nogo-A, NG-R and RhoA system, preventing further damage to the nervous system[186].

Besides the favorable effects that hyperbaric oxygen exerts through oxygenation and protection against IR injury, it could have a very important protective effect through the antioxidant response that hyperbaric oxygen itself produces. Thus, the oxidative stress caused by HBO could indeed inhibit an oxidative damage[187,188]. This could be considered as the 'hyperbaric oxygen paradox' in the IR injury.

HBO also promotes the production of enzymatic antioxidants such as Mn, Cu/Zn superoxide dismutase, gluthathione peroxidase and catalase[189–193]. There is also an elevation of the most important non-enzymatic antioxidant system, the glutathione/cysteine system[160]. This protective effect appears after the first hour of exposure and can still be found 24–72 hours after the last HBO treatment. It is also well known that a preconditioning with hyperbaric oxygen can prevent damage caused by IR injury[190].

Among the key protective antioxidant effects, we can find increase production of anti-inflammatory cytokines (IL-10), reduced production of inducible neuronal nitric oxide synthase and neuronal nitric oxide synthase, reduction in ROS production and up-regulation of key antioxidant and anti-apoptotic factors such as BCL-2, heme oxygenase-1 and heat-shock protein 70 and 72 (Refs. 194–205).

The antioxidant response to HBO may be as important as the oxygenation effects of breathing 100% oxygen at pressure, especially in the acute conditions that exhibit IR injury. This dual process could have an important protective effect in acute conditions. It appears that the energy crisis caused by the reduction of the cellular ATP could also be part of the pathophysiology of chronic degenerative diseases[206]. The difference would be then in the magnitude and speed of the decline of ATP. In the acute and rapid fall of ATP necrosis and apoptosis results, but in the mild chronic reduction of ATP, cellular dysfunction and a more subtle cellular damage occur[207].

HBO can also exert its beneficial effect in peripheral nerve repair and regeneration by enhancing or preventing the production of growth factors. Yu *et al.* found that HBO reduced the gene expression of GDNF after 1 day of injury in the HBO group, as confirmed by immunohistochemical staining[208]. Some of the growth factors, such as basic fibroblast growth factor (bFGF), are ineffective in stimulating healing under ischemic conditions even at high doses. But when treated with HBO, growth factors recover their function and become highly effective again ($p<0.05$)[209]. HBO increases the production of bFGF, vascular endothelial growth factor and TGF-β1. They have the ability to respond to hyperoxia directly, which causes changes in cell signaling pathways involved in cellular proliferation and growth factor production[210]. HBO has a synergistic effect with several growth factors[211]. Another factor that is influenced by HBO is NT-3. It reduces the ischemia-induced downregulation of NT-3 mRNA level 4 hours post-ischemia and significantly increased cell survival 7 days after reperfusion. As mentioned previously, NT-3 is an important neurotrophic factor involved in peripheral nerve repair and regeneration[212].

HBO used for peripheral nerve injury started more than 30 years ago[213]. Several studies have documented the effectiveness of HBO in models of acute and delayed crush injury and regeneration. Zhao[214] reported 114 patients treated microsurgically. Fifty-four of them were given HBO with good results in 89% of the cases ($p<0.05$), compared with the control group ($n=60$). He suggested the importance of prompt combined treatment.

Zamboni *et al.*[215] used a rat sciatic nerve model ($n=36$). The nerve was mobilized, stripped of extrinsic blood supply, transected and repaired in an epineural fashion with microsurgical technique. The animals were then randomized into two groups: with and without HBO. The protocol used was 2.5 ATA/90 min/BID/7 days. Nerve recovery was assessed weekly for 10 weeks [walking track analysis from which the nerve function index (SFI) was calculated for each animal]. SFI reached statistical significance at weeks 7–10. The results suggested functional recovery with the protocol used.

Bradshaw *et al.*[216] tried a sciatic nerve crush model in rabbits ($n=30$). Six different oxygen environments were used and HBO was started 4 days after injury. The regenerative morphology of the nerves was evaluated with transmission electron microscopy and light microscopy. At week 7, the HBO groups resembled normal uncrushed nerves, with nerve fibers uniformly distributed throughout the section. Myelination was also similar to normal nerves. Collagen and blood vessels were more evident in the HBO treatments at lower pressures than at higher pressures. The nerves of the surface oxygen and ambient or hyperbaric air groups were edematous and contained disarrayed nerve fibers (*Table 1*). HBO can accelerate a peripheral nerve recovery from a crush injury.

Santos *et al.* conducted two studies. In the first one[217], they used HBO in rats with transected peroneal nerves and entubulated with a Silastic channel. The changes evaluated were acute edema, functional recovery and histology. The protocol used was 2.5 ATA/90 min/BID/7 days and then four times a day for other 7 days. Thirteen weeks after the initial injury, elicited muscle force measurements demonstrated no significant improvement from hyperbaric oxygen treatment of injured nerves. There were no significant differences between groups in histologic evaluation of nerve area, myelinated axon number, myelinated axon area, myelin thickness and blood vessel number. In the second study[218], Santos *et al.* also developed a reliable hypoxic nerve injury model. They used 48 rats in a controlled and blinded trail of the injury model followed by treatment with hyperbaric oxygen, and the model was evaluated with a functional model. In the HBO group, a 12% improvement in function 5 days after treatment was demonstrated ($p<0.03$), but no long-term or histologic benefit was seen.

Haapaniemi *et al.* did several models for sciatic rat nerve regeneration[219], axonal outgrowth in grafts in sciatic rat nerves[220], nerve regeneration in acellular nerve and muscle grafts in rats[221], and early regeneration in nerve injury[222]. Nerve regeneration was evaluated using a pinch-reflex test 3, 4 and 5 days following surgery and with neurofilament staining at day 4. The regeneration distance was significantly longer in the HBO group (3.3 ATA/45 min/0, 4 and 8 hours postoperatively/TID). They concluded that HBO stimulated axonal outgrowth following a nerve crush lesion.

In the axonal outgrowth grafts model ($n=40$), the sciatic nerve was transected and a 10 mm long segment from the opposite side was immediately sutured as a nerve graft. The HBO group ($n=17$) was treated with 3.2 ATA/45 min, repeated 4 and 8 hours postoperatively and the TID for 7 days. The outgrowth was

Table 1: Excerpted from Bradshaw (216)

Group	O_2 (%)	Pressure (kPa) (ATA)	Edema	Myelination
Control	21	101 (1)	0	3
I	21	101 (1)	2	1
II	100	101 (1)	3	1
III	21	202 (2)	1	1
IV	100	202 (2)	1	2
V	100	242 (2.4)	1	2
VI	100	303 (3)	0	2

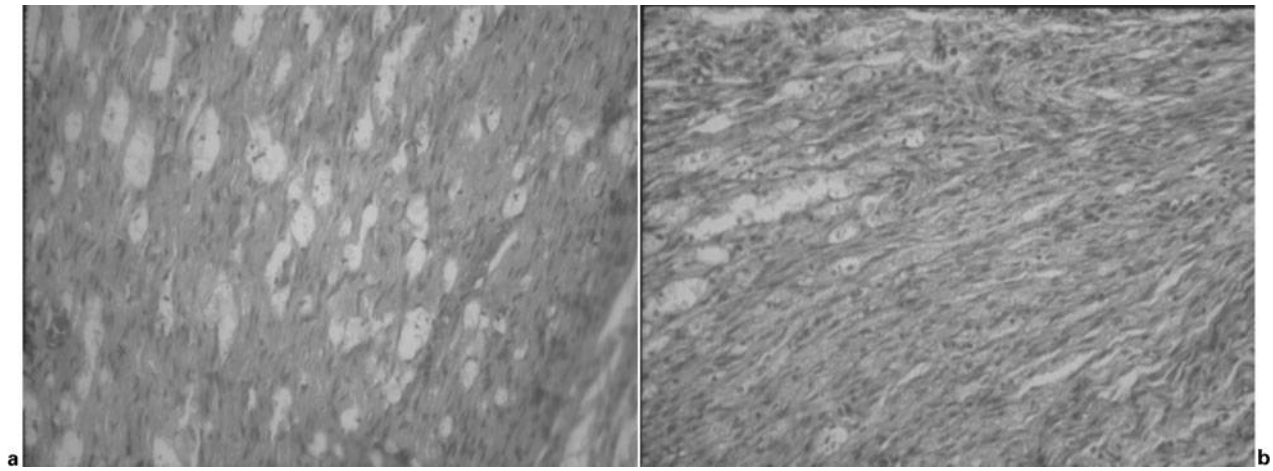

Figure 2: (A) Histology of Non-treated group. There is characteristic of Wallerian degeneration of the nerve. There is also, reduction of the Schwann cells, edema, demyelination and loss of cytostructure. There is moderate to severe infiltration of macrophages and neutrophils with formation of granuloma. (B) Histology of HBO2 Group. There is conservation of the Schwann cell architecture, discrete demyelination and little edema. There is no inclusion of neutrophils or macrophages and no granuloma is observed in the nerve fibers. Although the fibers appear to be thinner, probably due to remodelling

evaluated by immunohistochemical staining of neurofilaments in the nerve grafts. It was significantly longer in animals treated with HBO.

In the acellular nerve and muscle grafts model, both grafts were made acellular by freeze-thawing and then used to bridge a 10 mm gap in the sciatic nerve on the left and right sides, respectively. The HBO protocol used was 2.5 ATA/90 min/BID/7 days. Ten days after surgery, the Schwann cell migration and invasion of macrophages were examined. It was concluded that HBO had no effect on regeneration process in acellular nerve grafts, in contrast with fresh cellular nerve grafts.

In the last report, they compared two models, a crush injury model to a nerve transection and repair model. The protocol used was 2.5 ATA/90 min/BID/7 days. The animals were evaluated with walking track analysis up to twice weekly. The experiments were terminated after 90 days when the tetanic force was measured in the tibial anterior and gastrocnemius muscles. No statistically significant differences were found. They concluded that HBO was not effective in the restoration of gait or the muscular strength after 90 days in the nerve-injured rats.

Tuma *et al.*[223] used a crush sciatic rat nerve model that was assessed by functional evaluation using walking track analysis. The functional indexes did not differ from the untreated group. They concluded that HBO had no effect on functional recovery after nerve injuries.

Perez-Bolde *et al.*[224] used a rat sciatic nerve anastomosis model (n=18). The functional evaluation with electromyography was carried out before and after neurorraphy, and every 5 days up to 20 days when the animals were killed and a histologic analysis was performed. The HBO protocol was 2.0 ATA/90 min/BID/7 days and then QD for 7 more days. There was a statistical significance in the treatment group by day 10 ($p<0.05$) and by day 20 ($p<0.01$). In the histologic analysis, there was conservation of the Schwann cell architecture, discrete demyelination and little edema in the HBO group, in contrast with the control group that had marked reduction of Schwann cells, large edema, demyelination and loss of Schwann cell architecture. There was also moderate to severe infiltration of macrophages and neutrophils within the formation of granulomas (Figure 2). They concluded that early HBO could help reduce the peripheral nerve damage in crush injuries.

Eguiluz *et al.*[225] used a transection rat sciatic nerve model with repair by microsurgical technique (n=40). Nerve recovery was assessed by nerve conduction studies 7 and 14 weeks after surgery. Histopathologic analysis was carried out after 7 and 14 weeks. In the HBO groups, there was a statistical significance at week 7 ($p<0.03$) in conduction velocities and amplitude, and in the number of blood vessels. The foot/ankle angle showed better response at weeks 7 and 14. Nevertheless, the untreated group had a higher number of axons and vessels at week 7 ($p=0.03$), whereas at week 14, there was no significant difference. Although there were more axons and myelins, it appeared to be less functional than in the HBO-treated group (Figure 3). They suggested that HBO could improve functional recovery in this model.

CONCLUSIONS

Acute peripheral nerve injury is one of the major causes for severe and longstanding impairment of limb function. Up to now, the surgical repair has been the golden standard of care. Acute peripheral nerve lesion has a very important inflammatory component and is considered as an IR injury. Nevertheless, nerve repair is not primarily a mechanical problem and microsurgery is not the only key to success. There are many biologic aspects that contribute to nerve repair and regeneration and can improve the functional results. HBO has been proposed as one of the adjunctive treatments that could enhance these processes.

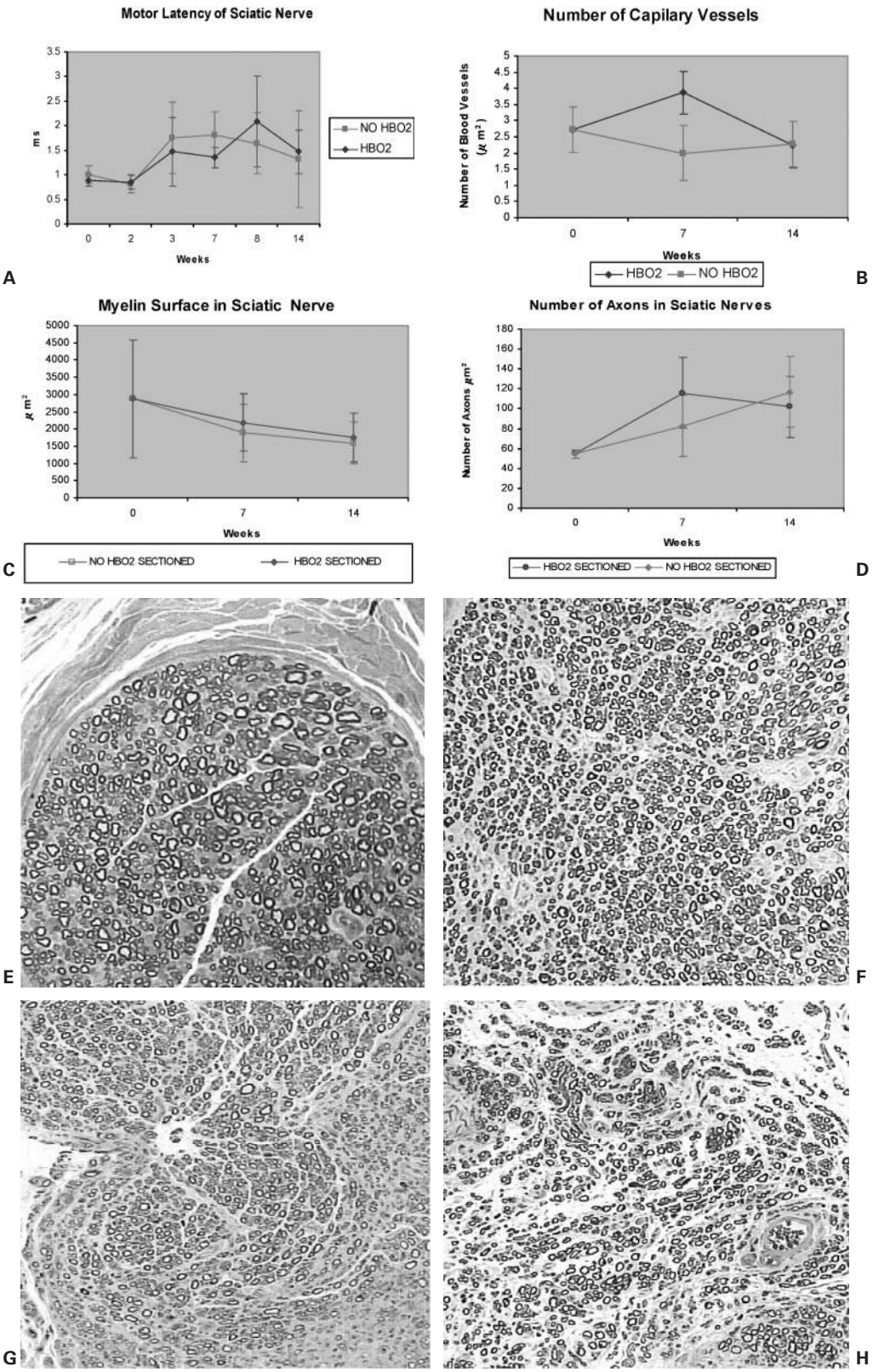

Figure 3: (A) At week 7, there was a significant increase in latency ($p<0.03$) in the non-treated group. The significance was lost at week 14. (B) There was a statistical significance in the HBO_2 group at week 7 in the number of blood vessels. (C) The amount of myelin was higher in the HBO_2 group throughout the 14 weeks. (D) Number of axons. There is a statistical significant increase (70.8%) in the number of axons at week 7 in the HBO_2. The significance was lost at week 14. (E) Representative histological features from sciatic rat nerve. Numerous middle size axons covered by myelin (black rings) and occasional small blood vessels from the sciatic rat nerve at 7 weeks in the control group. (F) Sciatic nerve at week 7 in the HBO_2 group, showing numerous axons and small blood vessels. (G) An apparent lower number of axons and blood vessels in the control group at 14 weeks. (H) Increased number of axons in the HBO2 group (magnification $\times 200$, toluidin blue staining)

HBO will promote survival of marginal tissue (penumbra), reduce the edema and improve the microcirculation, brake the vicious cycle of edema–hypoxia–edema, enhance healing, promote the up-regulation of growth factors and improve neovascularization. At the cellular level, it will maintain the tissue levels of ATP, restore mitochondrial dysfunction, inhibit, prevent or reduce the IR injury and have significant antioxidant and anti-apoptotic effects.

All of these mechanisms will enhance acute peripheral nerve repair and regeneration. Nevertheless, as with other treatments tried before, the research success cannot be directly extrapolated into clinical benefits. There have been non-favorable results when HBO has been employed for this injury.

It appears that the non-favorable results are encountered in those research protocols that use pressures higher than 2.0 ATA (>2.02 kPa). The possible explanation is the importance of ATP and other high energy compounds in the regeneration of peripheral nerve[226–231]. Almost 30 years ago, Holbach *et al.*[232] proved that ATP production was reduced when treatment pressures were above 1.5 ATA. This could explain why results are less favorable when pressures higher than 2.0 ATA are used. Actually, Bradshaw *et al.* described that the best results in the multiple groups used were found at lower pressures (<2.0 ATA). This could also correlate with the 'oxygen balance'. If too high pressures are used for an IR injury, the balance could tilt to the oxidative stress side and could generate too much ROS that could not be sufficiently compensated by the antioxidant capabilities, at the time needed. In this case, HBO could have even deleterious effects.

Timing is also very important. The treatment window for acute peripheral nerve lesions appears to be ~6 hours. If HBO treatment is started after this window, it could also create negative effects on the tissue. This could also explain the contradictory results encountered with the use of HBO.

We have found that if applied early, HBO enhances nerve repair, regeneration and functional recovery, as early as 10 days after the lesion. The effects are maintained after 14 weeks, which suggests that it is not a short-term effect. What was interesting was that the number of axons, myelin, blood vessels and functional tests were statistically significant at the 7 week mark, but lost significance at week 14, except for the functional test that remained unchanged.

It appears that there is a remodelling process at the site of neurorraphy in the HBO-treated group, which did not occur in the non-treated group. Apparently, there was a persistent effect of growth factors and/or other stimuli that did not end in functional recovery for the group that did not receive HBO.

HBO could affect the pathophysiology of acute peripheral nerve injury that seems to translate to a better correlation between research studies and clinical outcome. In conclusion, HBO holds much promise as an effective therapy; however, more prospective randomized controlled studies are needed to establish the utility of HBO in improving outcomes in peripheral nerve injury.

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