STIMULATING FACTORS FOR THE REGENERATION OF PERIPHERAL NERVES

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Abstract

In a wounded nerve fiber, the distal segment separated from the nerve cell body, suffer an anterograde or Wallerian degeneration and within the proximal segment of the axon appear a retrograde or traumatic degeneration. The regeneration of peripheral nervous fibers is a complex process, with multiple interactions.

The factors that are stimulating the regeneration of the peripheral nervous fibers can be grouped in two categories: endogenous and exogenous. This article review some endogenous factors (the genetic factors and their expression, the growth factors, the endogenous antioxidant factors) and exogenous factors (the exogenous antioxidant factors, the hormonal supplements, the vitamins supplements, the neuroprotective drugs and the physical factors).

Keywords: peripheral nerve, degeneration, regeneration, stimulating factors.

FACTORI CARE STIMULEAZĂ REGENERAREA NERVILOR PERIFERICI

Rezumat

La nivelul unei fibre nervoase lezate, segmentul distal, separat de corpul celular, suferă o degenerare anterogradă sau wallerianã, iar segmentul proximal o degenerare retrogradă sau traumatică. Regenerarea fibrelor nervoase periferice este un proces complex, cu interacţiuni multiple.

Factorii care stimulează regenerarea fibrelor nervoase periferice pot fi grupaţi în două categorii: exogeni şi endogeni. Acest articol trece în revistă câţiva factori endogeni (factorii genetici şi expresia lor, factorii de creştere, factorii antioxidanţi endogeni) şi exogeni (factorii antioxidanţi exogeni, suplimentele hormonale, suplimentele vitaminice, medicamentele neuroprotectoare, factorii fizici) implicaţi în regenerarea nervoasă periferică.

Cuvinte cheie: nerv periferic, degenerare, regenerare, factorii stimulatori.

General considerations

The peripheral nerves contain groups of nerve fibers. In peripheral nerve fibers, axons are sheathed by *Schwann cells*, also called *neurolemmocytes* [1]. Axons and Schwann cells of nerves are enclosed within connective tissue layers.

Externally to the nerves there is a dense, irregular fibrous coat called *epineurium*, a typical dense connective tissue. Adipose tissue is often associated with the epineurium in larger nerves. The epineurium continues more deeply to fill the space between bundles of nerve fibers, called fascicles.

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Each *fascicle* is surrounded by the *perineurium*, a sleeve of specialized connective tissue formed by layers of flattened epithelial-like cells (perineurial cells). The cells of each layer of the perineurium are joined at heir edges by tight junctions, an arrangement that makes the perineurium a *blood-nerve barrier* to the passage of most macromolecules and has the important function of protecting the nerve fibers and helping maintain the internal microenvironment [1,2].

Within the perineurial sheath run the Schwann cell-covered axons and their enveloping connective tissue, the *endoneurium*. The endoneurium consists of a sparse layer of loose connective tissue that merges with an external lamina of type IV collagen, laminin, and other proteins produced by the Schwann cells [1]. Other than occasional fibroblasts, the only other connective tissue cells normally found within the endoneurium are mast cells and macrophages [2].

Tissue at he level of the endoneurium is poorly vascularized; metabolic exchange of substrates and wastes in this tissue depend on diffusion from an to the blood vessels through the perineurial sheath [2].

Mechanisms of degeneration

Injured fibers in peripheral nerves have a good capacity for regeneration and return of function. In a wounded nerve fiber, it is important to distinguish changes occurring proximal to the injury from those in the distal segment. The proximal segment maintains its continuity with the trophic center in the perikaryon (cell body) and can regenerate, while the distal segment separated from the nerve cell body, degenerates (*anterograde or Wallerian degeneration*). The proximal segment of the axon degenerates close to the wound for a short distance. This is a *retrograde or traumatic degeneration*, which usually extends for only one or a few internodal segments.

Retrograde signalling to the cell body of an injured nerve causes a change in gene expression that initiates reorganization of the perinuclear cytoplasm. The retrograde signalling is leading to the upregulation of a gene called c-jun, involved in early as well as later stages of nerve regeneration [2]. The onset of degeneration is accompanied by changes in the perikaryon: *chromatolysis* or dissolution of the RER and a consequent decrease in cytoplasmic basophilia; an increase in the volume of the perikaryon; and migration of the nucleus to a peripheral position in the perikaryon [1]. The chromatolysis is first observed within 1-2 days after injury and reaches a peak at about 2 weeks. The changes in the cell body are proportional to the amount of axoplasm destroyed by the injury; extensive loss of asoplasm can lead to death of the cell [2].

In the nerve segment distal to the injury the axon and the myelin, but not the connective tissue, degenerate completely and are removed by macrophages. The axonal cytoskeleton suffers a granular disintegration. Schwann cells downregulate expression of myelin-specific proteins and secrete several glial growth factors (GGFs). Schwann cells also initiate the removal of myelin debris; the resident macrophages are activated, migrate to the site of injury, proliferate and phagocytize the myelin debris. The disrupted blood-nerve barrier allows the influx of monocyte-derived macrophages from blood vessels. The process of myelin removal is usually completed within 2 weeks [2].

Mechanisms of regeneration

Growth starts as soon as debris is removed by macrophages. Macrophages produce cytokines which stimulate Schwann cells to secrete neurotrophins.

Division of dedifferentiated Schwann cells is the first step in the regeneration. Schwann cells proliferate within the connective tissue sleeve, giving rise to rows of cells (cellular bands of Bugner) that serve as guides for the sprouting axons formed during the reparative phase. Once the bands are in place, large numbers of sprouts begin to grow from the proximal stump. A growth cone develops in the distal portion of each sprout that consists of filopodia rich in actin filaments. The tips of the filopodia establish a direction for the advancement of the growth cone. They preferentially interact with proteins of the extracellular matrix, such as fibronectin and laminin found within the external lamina of the Schwann cell. Thus, if a sprout associates itself with a band of Bugner, it regenerates between the layers of external lamina of the Schwann cell. This sprout will grow along the band at a rate of about 3 mm/day [1,2]. Although many new sprouts do not make a contact with cellular bands and degenerate, their large number increases the probability of re-establishing sensory and motor connections. Axonal regeneration leads to Schwann cell redifferentiation.

The time is also important. A short-term denervation did not affect the collateral sprouts regeneration, but more prolonged denervation profoundly reduced collateral sprouts regenerated in the distal nerve stump [3].

Regeneration of transected peripheral nerves is a complex process involving the coordinated action of neuronal axons, glial cells, and fibroblasts [4]. We musn't forget that the mast cells, as well as Schwann cells, play a pivotal role in regeneration process after injury [5].

When there is an extensive gap between the distal and proximal segments of cut of the injured peripheral nerves, or when the distal segment disappears (amputation of a limb), the axonal sprouts do not establish contact with the appropriate Schwann cells, the sprouts grow in a disorganized manner, resulting in the mass of tangled axonal processes, forming a swelling, or *neuroma*, that can be the source of spontaneous pain.

Regeneration is functionally efficient only when the fibers and the columns of Schwann cells are directed to the correct place. In an injured mixed nerve, if regenerating sensory fibers grow into columns connected to motor end-plates that were occupied by motor fibers, the function of the muscle will not be re-established.

Factors stimulating the regeneration of the peripheral nerves

The factors that are stimulating the regeneration of the peripheral nervous fibers can be grouped in two categories: endogenous and exogenous.

The endogenous factors revised are: the genetic factors and their expression, the growth factors, the endogenous antioxidant factors. Between the exogenous factors we mention: the exogenous antioxidant factors, the hormonal supplements, the vitamins supplements, the neuroprotective drugs, and the physical factors.

Endogenous factors stimulating the regeneration of the peripheral nerves

• ***Genetic factors and their expression***

Numerous external inhibitory factors inhibit axonal

regeneration after injury. In response, neurons express various regeneration-associated genes to overcome this inhibition and increase the intrinsic growth capacity. The brain-expressed X-linked (Bex1) protein is over-expressed as a result of peripheral axonal damage. Bex1 antagonize the axon outgrowth inhibitory effect of myelin-associated glycoprotein [6].

The injured sciatic nerve can be distinguished by the preferential upregulation of genes involved in axonal processes and plasticity (Chl1, Epha5, Gadd45b, Jun, Nav2, Nptx1, Nrcam, Ntm, Sema4f), inflammation and immunity (Arg1, Lgals3, Megf10, Panx1), growth factors/cytokines and their receptors (Clcf1, Fgf5, Gdnf, Gfrα1, Il7r, Lif, Ngfr/p75(NTR), Shh), and cell adhesion and extracellular matrix (Adam8, Gpc1, Mmp9, Tnc) [7]. Molecular inflammatory mediators such as cytokines (IL-1, IL-6, IL-10, and TNF-alpha, among others), transcription factors (NF-kappaB, c-Jun), the complement system and arachidonic acid metabolites have been shown to modulate Wallerian degeneration [8]. An immediate therapy with TNF-alpha antagonist supports axonal regeneration after peripheral nerve injury [9].

Schwann cells, derived from the embryonic neural crests, differentiate by expressing transcription factor Sox-10 [2]. During myelin formation, compaction of the sheath corresponds with the expression of transmembrane myelin-specific proteins, such as protein 0 (P0), a peripheral myelin protein of 22 kilodaltons (PMP-22), and myelin basic protein (MBP). Mutations in human genes encoding P0 produce unstable myelin and may contribute to the development of demyelinating diseases. P0 and PMP-22 are expressed only in the myelin of the PNS [2]. After axonal injury, Schwann cells downregulate expression of myelin-specific proteins. The PMP22 gene is located on chromosome 17p11.2, and defects in PMP22 gene have been implicated in several common inherited peripheral neuropathies [10].

Myelin sheath thickness is regulated by a growth factor called neuregulin (NRG1) that acts on Schwann cells, a transmembrane protein expressed on the axolemma (cell membrane) of the axon [2]. NRG1 and epidermal growth factor receptor (ErbB) signaling pathways control Schwann cells during axonal regeneration in an injured peripheral nervous system. A persistent supply of recombinant NRG1 to the injury site could improve axonal growth and recovery of sensory and motor functions in rats during nerve regeneration [11].

Major histocompatibility complex (MHC) class I expression by neurones and glia constitutes an important pathway that regulates synaptic plasticity. The upregulation of MHC class I after treatment with interferon beta (IFN beta) accelerates the response to injury. In the peripheral nerve, IFN beta-treated animals showed increased S100, GAP-43 and p75NTR labelling coupled with a significantly greater number of regenerated axons [12]. Also, the expressions of growth-associated protein-43 (GAP-43) mRNA and protein may be upregulated after brachial plexus injury, and GAP-43 protein is possibly associated with the axon regeneration and function reconstruction [13].

Using rodent models of nerve repair, Parrinello et al. (2010) find that ephrin signaling between fibroblasts and Schwann cell progenitors, involving the stemness factor Sox2, is required for nerve regeneration [4]. Ephrin-B/EphB2 signaling between fibroblasts and Schwann cells results in cell sorting, followed by directional collective cell migration of Schwann cells out of the nerve stumps to guide regrowing axons across the wound [14].

Schwann cells synthesize neurotrophic factors and cytokines that are crucial for the repair of the injured nerve. The receptor for advanced glycation end products (RAGE) and its ligand S100B, which are secreted by Schwann cells, are required for the repair of the injured peripheral nerve in vivo [15].

Schwann cells drastically change their phenotype following peripheral nerve injury. The posttranscriptional regulation of protein expression in Schwann cells may be involved in their phenotypic changes required for efficient nerve degeneration/regeneration. ZNRF1 is an E3 ubiquitin ligase, whose expression is upregulated in the Schwann cells following nerve injury. During nerve degeneration/regeneration, glutamine synthetase (GS) expression is controlled mostly by ZNRF1-dependent proteasomal degradation. Schwann cells increase oxidative stress upon initiation of nerve degeneration, which promotes carbonylation and subsequent degradation of GS. GS expression regulates Schwann cell differentiation; i.e., increased GS expression promotes myelination via its enzymatic activity. Among the substrates and products of GS, increased glutamate concentration inhibited myelination and yet promoted Schwann cell proliferation by activating metabotropic glutamate receptor signaling [16].

In an experiment studying the healing of injured peripheral nerves in vivo, rabbit facial nerves were injured. At 4 weeks post-injury, cross-section images of facial nerves showed that axons treated with recombinant human bone morphogenetic protein-2 (rhBMP-2) were denser and thicker, and levels of tau protein were increased. It is concluded from these data that rhBMP-2 may affect injured facial nerve regeneration by inducing more neurons to return to embryonic patterns of tau gene expression [17].

- ***Growth factors***

The growth factors *nerve growth factor* (NGF), *glial-derived neurotrophic factor* (GDNF), and *epidermal growth factor* (EGF) activate the MAP-kinase Erk1/2 via receptor tyrosine kinase signalling. NGF and GDNF are established factors in regeneration and sensitization of nociceptive neurons. EGF did not induce mechanical hyperalgesia, but blocked PGE2-induced sensitization [18]. The delivery of GDNF from the fibrin-based delivery system promotes motor nerve regeneration at a level similar to an isograft in the femoral motor nerve

model [19]. The inactivation of GDNF signaling leads to defects of innervation [20].

Recent bioengineering strategies for peripheral nerve regeneration have been focusing on the development of alternative treatments for nerve repair. The incorporation of *nerve growth factor* (NGF) into aligned fibrous nerve guidance conduits (NGCs), combined physical guidance cues and biomolecular signals to closely mimic the native extracellular matrix (ECM). This method could greatly promote peripheral nerve regeneration [21]. Collagen nerve conduits loaded with the synergistically acting glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) improve early axonal regeneration in a 10 mm rat sciatic nerve gap model [22]. Transfected HEK-293 cells can be regulated to inducibly produce bioactive NGF in vivo over prolonged periods. This tissue-engineered nerve construct including the NGF delivery system is able to improve peripheral nerve regeneration and functional recovery and appears to be superior to nerve isografts [23].

The *brain-derived neurotrophic factor* (BDNF) is the sole neurotrophin upregulated in sensory neurons after peripheral nerve injury. BDNF induce the cell body response in injured sensory neurons and their intrinsic ability to extend neurites, but BDNF does not appear to be necessary for maintaining the response once it is induced [24].

Basic *fibroblast growth factor* (bFGF) and *insulin-like growth factor* (IGF)-1 have multiple effects on cells, including proliferation, differentiation, and survival. Both bFGF and bIGF decreased growth cone collapse after tetracaine-induced injury in vitro [25]. Local administration of insulin-like growth factor-I (IGF-I) has been shown to increase the rate of axon regeneration in a model of crush-injured and freeze-injured rat sciatic nerves [26]. The treatment strategy of peripheral nerve grafting with acidic fibroblast growth factor (aFGF), combined with physical therapy is effective to treat spinal cord injury [27].

The *placental growth factor* (PlGF) has properties like monocyte activation/attraction, ability to increase expression of pro-inflammatory molecules, as well as neuroprotective effects. An experiment which studied PlGF expression under physiological and degenerative conditions and explored its role in Wallerian degeneration (WD), using a model of sciatic nerve transection in wild-type and Pgf(-/-) mice, showed dynamic changes of PlGF expression, from periaxonal in normal nerve to Schwann cells (SCs) 24h postinjury, in parallel with a p65/NF-κB recruitment on Pgf promoter. After injury, SC proliferation is reduced by 30% in absence of PlGF. Macrophage invasion is significantly delayed in Pgf(-/-) mice compared with wild-type mice, which results in worse functional recovery. MCP-1 and proMMP-9 exhibit a 3-fold reduction of their relative expressions in Pgf(-/-) injured nerves, as demonstrated by cytokine array. In conclusion, this work originally describes PlGF as a novel member of the cytokine network of WD [28].

The *granulocyte-colony stimulating factor* (G-CSF), protects α-motoneurons, improves functional outcome, and increases life expectancy. Intraspinal delivery improves efficacy of G-CSF treatment in an amyotrophic lateral sclerosis mouse model while minimizing the systemic load of G-CSF [29].

A *ciliary neurotrophic factor* (CNTF)-coated polylactide-co-glycolide/chitosan nerve conduit could be an alternative artificial nerve conduit for nerve regeneration [30].

• ***Endogenous antioxidant factors***

The oxidative stress in nerve injury

Developing a successful treatment strategy for neuropathic pain has remained a challenge among researcher and clinicians. Various animal models have been employed to understand the pathogenic mechanism of neuropathic pain in experimental animals.

Nitric oxide (NO), the smallest signalling molecule known, is produced by three isoforms of NO synthase (NOS). Inducible NOS (NOS II) can be expressed in many cell types in response to lipopolysaccharide, cytokines, or other agents. Inducible NOS generates large amounts of NO that have cytostatic effects on parasitic target cells. Neuronal NOS (nNOS, NOS I) is constitutively expressed in central and peripheral neurons and some other cell types. Its functions include synaptic plasticity in the central nervous system (CNS), central regulation of blood pressure, smooth muscle relaxation, and vasodilatation via peripheral nitrergic nerves [31].

Oxidative stress and large amounts of nitric oxide (NO) are implicated in the pathophysiology of neuronal injury and neurodegenerative disease [32]. Nitric oxide (NO) signalling results in both neurotoxic and neuroprotective effects in CNS and PNS neurons, respectively, after nerve lesioning. NO-cGMP ignalling pathway through nNOS activation is involved in neuroregeneration after nerve lesioning [33].

The peripheral nerve suture and/or treatment of NOS inhibitors can maintain the homeostasis of oxidative stress-related biomarkers, especially nNOS in neuronal cell bodies. These actions may thus facilitate the axonal regeneration [34].

The endogenous antioxidant factors

Anti-oxidative stress responses are crucial for the survival of nerve-injured motor neurons. The two major redox systems, GSH/GSHr and TRX1/TRXr1, are simultaneously activated in injured neurons, and likely provide protection of injured neurons against oxidative stress [35].

After sciatic nerve transection (SNT), there is an increase in GPx activity, in GSH content, and in the GSH/GSSG ratio 3 days after surgery. nNOS expression is upregulated 7 days post SNT. These observations support

the role of the gluthatione system in pain physiology and highlight the involvement of NO as an important molecule related to nociception [36].

In an experimental sciatic nerve injury group rats, compared to control group, were observed: significant ($P<0.05$) increases in oxidative stress markers (such as malondialdehyde (MDA) and advanced oxidation protein products (AOPP)), significant increases in antioxidants (such as glutathione peroxidase (GPx), superoxide dismutase (SOD)) and decreases in the GSH and catalase activities [37].

Exogenous factors stimulating the regeneration of the peripheral nerves

• ***Exogenous antioxidant factors***

The (-)-epigallocatechin gallate (EGCG), one of the green tea polyphenols, has potent antioxidant effects against free radical-mediated lipid peroxidation in ischemia-induced neuronal damage. EGCG can reduce NADPH-d/nNOS reactivity and thus may enhance motor neuron survival time following peripheral nerve injury [32].

The flavonoid baicalein (5,6,7-trihydroxyflavone) has been reported to counteract sorbitol accumulation, activation of 12/15-lipoxygenase, oxidative-nitrosative stress, inflammation, and impaired signaling in models of chronic disease. Baicalein targets several mechanisms implicated in diabetic peripheral neuropathy [38].

Pralidoxime has ameliorative potential in attenuating the painful neuropathic state associated with tibial and sural nerve transection, which can be attributed to decrease in oxidative stress and calcium levels. Administration of pralidoxime (10, 20 mg/kg intraperitoneally (i.p.)) for 14 days attenuated cold allodynia, mechanical, hot and cold hyperalgesia [39].

Another two antioxidants, acetyl-l-carnitine and resveratrol, have neuroprotective abilities. Oxidative stress and secondary excitotoxicity, due to cellular energy deficit, are major factors playing roles in 3-nitropropionic acid (3-NPA) induced mitochondrial dysfunction. Acute or chronic exposure to 3-NPA also leads to neuronal degeneration. Treatment with resveratrol prevented the functional effects of 3-NPA exposure, whereas treatment with acetyl-l-carnitine, prevented paresis [40].

Rimonabant treatment reduced oxidative stress in peripheral nerve, as revealed by the ability of the compound to counteract the reduced glutathione (GSH) depletion. This rimonabant-induced improvement might favour the nerve regeneration; accordingly, the histological analysis of sciatic nerves showed a marked degeneration of myelinated fibers in diabetic mice, that was substantially reduced after rimonabant administration [41].

Rosiglitazone, a peroxisome proliferator-activated receptor gamma (PPAR gamma) agonist, in a model of tibial and sural nerve transection-induced neuropathic pain in rats, has an ameliorative potential in attenuating the painful state, which may be attributed to anti-inflammatory actions: attenuated oxidative stress, myeloperoxidase activity and calcium levels [42].

Dietary restriction

Dietary restriction is an efficient means of defying age-related oxidative damage and maintaining a younger state in peripheral nerves. A drastic decline in the expression of glial and neuronal proteins in myelinated peripheral nerves occurs with age, which is significantly ameliorated by lifelong calorie restriction. With age appears a prominent accumulation of polyubiquitinated substrates, which are associated with the conglomeration of distended lysosomes and lipofuscin adducts. The occurrence of these structures is notably less frequent within nerves of age-matched rodents kept on a lifelong reduced calorie diet. Markers for lipid peroxidation, inflammation, and immune cell infiltration are all elevated in nerves of ad libitum-fed rats, whereas food restriction is able to attenuate such deleterious processes with age [43].

• ***Hormonal supplements***

Melatonin exhibits an array of biological activities, including antioxidant and anti-inflammatory actions. The nitric oxide pathway is involved in the protective effect of melatonin against chronic constriction injury (CCI)-induced behavioral and biochemical alterations in rats. Chronic administration of melatonin (2.5 or 5 mg/kg, ip) significantly attenuated hyperalgesia, cold allodynia and oxidative damage in sciatic nerves as compared to CCI group [44]. Melatonin modulates neuroinflammation by decreasing NF-κB activation cascade and oxidative stress by increasing Nrf2 expression [45]. Axonal shrinkage and myelin changes were not prominent histopathologically in melatonin-treated group compared to the neural injury group. The neuroprotective effects of melatonin determine a significantly lower lipid peroxidation and myeloperoxidase activity. Thus melatonin can improve neural healing [46]. By evaluating the functional nerve recovery using the sciatic functional index (SFI), the melatonin treatments were showed to increase the SFI values in the injured sciatic nerves [47].

A promoting effect of *thyroid hormones* has been established on the maturation of central and peripheral nervous systems. After a nerve-sparing-like injury in a rat model, administration of triiodothyronine (T3) improved neuroregeneration and recovery [48]. The local administration of T3 significantly increased the number and the myelination of regenerated axons [49].

Glucocorticoids improve the symptoms of peripheral nerve disorders; the effects of glucocorticoids are mainly anti-inflammatory. Schwann cells express glucocorticoid receptors (GR), and glucocorticoids enhance the rate of myelin formation in vitro [50].

Using the *mineralocorticoid* receptor (MR) antagonist spironolactone, an experimental research demonstrated that MR plays an active role in the function

of the intact sciatic nerve: MR is required for walking ability and participates in the control of the accumulation of the mRNA for several endogenous genes [51]. This can lead furthermore to a possible active role of MR in nerve regeneration.

Erythropoietin (EPO) has been shown to have beneficial effects in a variety of nerve injury models. In a rat model of peripheral nerve surgery, in EPO-treated rats there was a significant increase in the axon diameter, myelin thickness, and total number of nerve fibers, as well as the degree of maturity of regenerated myelinated nerve fibers; also the motor function significantly improved [52].

• ***Vitamins supplements***

The ectonucleotidase activities in synaptosomes and platelets and some parameters of oxidative stress were altered after a demyelinating event on the nervous system. The treatment with vitamin E modulated adenine nucleotide hydrolysis and altered oxidative stress parameters [53].

Methylcobalamin is a *vitamin B12* analog and is necessary for the maintenance of the nervous system. It promotes neurite outgrowth and neuronal survival and these effects are mediated by the methylation cycle. In a rat sciatic nerve injury model, continuous administration of high doses of methylcobalamin improves nerve regeneration and functional recovery [54].

• ***Neuroprotective drugs***

A short-term course of high-dose *atorvastatin* pretreatment can protect against sciatic nerve crush injury through modifying intracellular or extracellular environments, making it favorable for regeneration. Atorvastatin improved injury-induced neurobehavioral/ electro-physiological changes and axonal loss. Damage-associated alterations, including structural disruption, oxidative stress, inflammation, and apoptosis, were attenuated by atorvastatin. After injury, regeneration-associated genes, including growth-associated protein-43, myelin basic protein, ciliary neurotrophic factor, and collagen, were upregulated by atorvastatin. The suppression of extracellular signal-regulated kinase, AKT, signal transducer and activators of transcription-1, and necrosis factor-kappaB and the elevated activation of c-Jun N-terminal kinase, Smad2/3, and activating protein-1 were associated with the neuroprotective action of atorvastatin [55].

Botulinum neurotoxin serotype A (BoNT/A) is able to counteract neuropathic pain induced by chronic constriction injury (CCI) to the sciatic nerve both in mice and in rats. The behavioral improvement was accompanied by structural modifications, as revealed by the expression of cell division cycle 2 (Cdc2) and growth associated protein 43 (GAP-43) regeneration associated proteins, and by the expression of S100β and glial fibrillary acidic protein (GFAP) Schwann cells proteins. These are demonstrating long-lasting anti-allodynic and anti-hyperalgesic effects of BoNT/A in animal models of neuropathic pain, together with an acceleration of regenerative processes in the injured nerve [56].

Ginsenoside Rg1 (GRg1) is one of the bioactive compounds extracted from ginseng. GRg1 is capable of promoting nerve regeneration after nerve injuries, suggesting the possibility of developing GRg1 a neuroprotective drug for peripheral nerve repair [57].

Immunophilin ligand *FK506* has been treated as adjunct therapy for nerve repair due to its potent neurotrophic and neuroprotective actions. A rat sciatic nerve defect model treated with FK506-releasing chitosan guide showed more mature appearance of myelinated fibers 8 weeks after surgery [58].

Neuronal survival and regeneration are neurotrophin dependent and require increased aerobic capacity. *Acetyl-l-carnitine* (ALCAR) facilitates this need and prevents neuronal loss. ALCAR not only increases the number of regenerating nerve fibres, but also morphologically improves the quality of regeneration and target organ reinnervation [59].

• ***Physical factors***

Pulsed magnetic fields (PMFs) have well-known beneficial effects on nerve regeneration. Following the injury, peripheral nerves were very sensitive to repetitive stimulation. Application of PMF ameliorate and may restore the abnormal electrophysiological activities in signaling or aberrant ion channel functions of nerves, such as hyperpolarizing afterpotentials and delayed depolarizations [60].

The timing of *electrical stimulation* (ES) after peripheral nerve transection may enhance axonal regeneration and functional recovery [61]. The application of ES for 30 minutes immediately following crush injury is effective to promote nerve regeneration in a rat sciatic nerve model [62]. Also, an electrical stimulation with an implantable electrical stimulator (biphasic current pulse), applied between the proximal and the distal nerve stumps (in the rat sciatic nerve with 7 mm gap) showed that the electrical stimulation, especially for two weeks of stimulation, could accelerate functional regeneration of the severed nerve [63]. The therapeutic actions of ES on myelination are mediated via enhanced brain-derived neurotrophic factor (BDNF) signals, which drive the promyelination effect on Schwann cells at the onset of myelination [64].

Operant conditioning of the spinal stretch reflex or its electrical analog, the H-reflex, produces spinal cord plasticity and can thereby affect motoneuron responses to primary afferent input. The H-reflex up-conditioning may improve functional recovery after nerve injury and repair [65].

Conclusions

The neuroprotective and stimulating effects of the exogenous factors might be used successfully in clinical practice. The stimulation of the peripheral nerve regeneration still remains an open challenge to modern medicine.

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