We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Role of Pharmacological Agents in Nerve Regeneration after Peripheral Nerve Repair

Agon Mekaj and Ymer Mekaj

Additional information is available at the end of the chapter

Abstract

Peripheral nerve injuries are frequent and represent a significant pathology of the peripheral nervous system because, despite operative techniques and successful microsurgical repair, in most cases, the nerve repair is followed by scar formation. Numerous investigations have been carried out with the aim of finding pharmacological substances that can prevent scar formation and speed up the regeneration of repaired nerves. This chapter is dedicated to the efforts of many researchers to find different pharmacological agents with local effects on the improvement of nerve regeneration. Numerous experiments have been carried out in mice and rabbits using hyaluronic acid, tacrolimus, cyclosporin A, melatonin, vitamin B12, methylprednisolone, riluzole and potassium and calcium channel blockers. In the experimental animal studies, topical pharmacological agents were used at the site of peripheral nerve repair. The effect of these substances is most commonly studied in sciatic nerve injury in experimental animals. Their effects were evaluated using a variety of methods, such as morphological, biomechanical, electrophysiological and functional evaluation, and the above-mentioned substances, have been shown to have neuroprotective and neuroregenerative properties though different mechanisms.

Keywords: nerve injury, nerve regeneration, pharmacological agents, scar formation

1. Introduction

The peripheral nervous system (PNS) is very complex, being composed of the cranial nerves and the spinal nerves, which project from the spinal cord and pass through the intervertebral foramina of the vertebrae [1]. Peripheral nerves, composed of motor and sensory neurons, are considered as complex organs and are present in nearly all parts of the human body [2]. Motor neurons transmit processed information from the central nervous systems (CNS) to

skeletal muscles via efferent pathways, whereas collected information from periphery travels to the brain via afferent pathways, after they are translated into nerve signals [3]. Neurons are the main cells of the PNS, but there are two kinds of neuroglia in the PNS, namely Schwann cells and satellite cells. Neurons are made up of the body (soma) and their thin processes of the cell, which are called dendrites and axons. In the soma of the neuron, there are many types of organelles [4]. Based on the number of extensions that arise from the cell body, neurons can be multipolar (three or more extensions), bipolar (two extensions, one axon and one dendrite) or unipolar (only one extension), which are very short and divided in the form of a letter T [5].

Peripheral nerve injuries (PNIs) are very common, and automobile accidents are the most common cause of nerve trauma [6], with most cases (75%) occurring in the upper limbs [7]. However, the etiological factors of PNI are different in peace and conflict periods, and, historically, most knowledge of PNIs was developed during wars [8]. Nerve injuries can be caused by lacerations with sharp objects, penetrating trauma, stretching or crushing trauma, fractures and wounds [9]. Lacerations, especially those which were caused by a knife blade, are another common cause of PNIs, comprising 30% of serious injuries in some series [10]. Compression is another common cause of PNIs, including 'Saturday Night Palsy' caused by radial nerve compression, which causes entrapment neuropathies [11]. The most severe form of nerve injury is a transection, which is known as grade V neurotmesis, usually owing to a laceration from a knife, firearm or glass shard [12]. The neurotmesis is characterised with a full transection of the axons and connective tissue layers wherein complete discontinuity of the nerve is observed [13]. There are numerous classifications of nerve injuries, but of these classifications, the most widely accepted are those developed by Seddon and Sunderland [14, 15].

After a peripheral nerve sustains a traumatic injury, complex pathophysiologic changes, such as morphologic and metabolic changes, occur at the injury site [16]. Furthermore, these complex changes occur in the nerve cell body, in the proximal and distal segments to the injury site, as well as in the distal endings of both muscle end-plates and sensory receptors [17]. These changes are characterised by axonal degeneration, which follows a sequence of events within the zone of trauma extending both proximally and distally [18]. Disconnected axons and cell bodies (in proximal axon injuries) degenerate via chromatolysis [19]. The degenerative changes in the distal segment were first described by Waller in 1850 based on observations of frog glossopharyngeal and hypoglossal nerves after injury [20]. Wallerian degeneration starts almost immediately after axotomy and lasts for 3–6 weeks [17].

The regenerative process begins almost immediately after nerve injury. The first wave of axonal sprouting occurs within hours of axotomy [21]. Two days after this first wave of axonal sprouting, a second wave of this process of regeneration starts [22]. According to some authors, axons may branch once they reach the distal stump, and in these cases, one axon may give rise to several branches [23]. It is known that Schwann cells play an important role in nerve regeneration at the site of nerve injury because they elaborate processes that include physical conduits that lead axons to their targets [17]. The extension of Schwann cells' processes can limit the rate of axon regeneration more than axonal growth [24]. Regeneration of the damaged peripheral nerve depends on the microsurgical procedure performed. Currently, there are several operating techniques that can be used to repair injured nerves, such as direct epineural repair,

grouped fascicular repair, fascicular repair and nerve grafting [25]. However, there are some factors that influence the regeneration process after nerve repair, such as the nature, location and extent of damage, the extent and timing of repair, fascicular anatomy and patient factors (age, physical condition, metabolic disorders, avitaminosis and the presence of any disease).

In addition, recently some experimental studies have shown that nerve regeneration after its repair can be improved by some pharmaceutical agents, mainly used locally at the site of nerve repair. Drugs commonly used for this purpose include tacrolimus [26–29], hyaluronic acid and its derivatives [30–32], melatonin [33–35], methylprednisolone [36–39], vitamin B complex and vitamin B12, [40–42], calcium and potassium channel blockers [43, 44] and riluzole [45, 46]. These substances have neuroprotective and neuroregenerative properties, though different mechanisms contribute markedly to nerve regeneration.

Therefore, the main aim of this chapter is to present new insights into the mechanisms of action of many of the above-mentioned pharmacological agents on the prevention of perineural scar formation and on nerve regeneration after peripheral nerve surgery. However, it is understandable that complete regeneration and functional recovery will almost never be achieved, regardless of the operative technique used or the type of pharmacological agent applied.

2. Hyaluronic acid

Hyaluronic acid (HA) (CAS No. 9004-61-9) is a natural glycosaminoglycan formed by bonding N-acetyl-ᴅ-glucosamine with glucuronic acid [47]. It is a mucopolysaccharide, which occurs naturally in all living organisms, and is several thousands of sugars (carbohydrates) long. Disaccharide units are formed at the plasma membrane in vertebrates and some bacteria [48, 49]. HA is characterised by a very large number of disaccharide pairs (10,000 or more), so its molecular mass is approximately 4 million Da [50]. HA was discovered in bovine vitreous humour by Meyer and Palmer in 1934. These authors found that the HA contained two sugar molecules, one of which was uronic acid, and they proposed the name 'hyaluronic acid' [51], while the term 'hyaluronan' was introduced in 1986 by Endre Balazs to conform with the international nomenclature of polysaccharides [52]. HA is a major primary component in the extracellular matrix, but it has also been found intracellularly. HA has been isolated from many other sources, and its physicochemical structural properties and biological role have been studied in numerous laboratories [53]. The biosynthesis of HA has been studied for over six decades, but our understanding of the biochemical details of HA assembly is still incomplete. The enzyme responsible, HA synthase (HAS), is a membrane protein that requires only Mg^{2+} and two sugar-UDP substrates (GlcUA-UDP and GlcNAc-UDP) to polymerise HA chains [54]. In 1993, the hasA gene was identified and cloned, and the HAS protein from *Streptococcus pyogenes* was expressed [55, 56]. It was also demonstrated that only the HAS protein was required to synthesise HA [57]. It is known that mammalian genomes have three different HAS genes (HAS1, HAS2 and HAS3) that are expressed at specific times and in specific tissues during development, ageing and wound healing, as well as under normal and some pathologic conditions [58, 59].

HA has numerous biological functions, such as maintenance of the elastoviscosity of liquid connective tissues, for example, in joint synovial and eye vitreous fluid, control of tissue hydration and water transport, as well as supramolecular assembly of proteoglycans in the extracellular matrix. Furthermore, HA has various receptor-mediated roles in cell detachment, mitosis, migration, tumour development and metastasis, and inflammation [52, 60]. The predominant role of HA in organisms is unknown, but some clinical studies have demonstrated various physiological effects of exogenous HA. Exogenous HA enhances chondrocyte HA and proteoglycan synthesis, reduces the reproduction and activity of proinflammatory mediators, such as matrix metalloproteinases, and alters the behaviour of immune cells [61]. HA has also been successfully used in peripheral nerve surgery to reduce nerve adhesions during wound healing after nerve injury, which occur during ophthalmological, cardiovascular and dermatological procedures, including supplementing joint fluid in arthritis [32, 62, 63]. HA is known to reduce the extent of scar formation and nerve adhesions via the inhibition of lymphocyte migration, proliferation and chemotaxis of granulocyte phagocytosis and degranulation, and macrophage motility in order improve peripheral nerve regeneration [31, 64]. These functions are manifested during scavenging of reactive oxygen-derived free radicals, the inhibition of immune complex adherence to polymorphonuclear cells, the inhibition of leucocyte and macrophage migration and aggregation, and the regulation of fibroblast proliferation [65]. HA is an endogenous stimulator of interleukin-1 (IL-1) production, and IL-1 affects fibroblasts proliferation and collagenase production [30]. Therefore, according to Hiro et al., HA is an endogenous IL-1 inducer and may play important roles in the pathological and/or physiological changes of connective tissues [30]. It is known that HA is highly non-antigenic and non-immunogenic, because it has high structural homology across species and weak interactions with blood components [66]. HA's degradation products are thought to contribute in scar formation because the increased amounts of HA fragments from the action of hyaluronidase in HA induce increased scar formation. There are various commercial preparations of HA in different forms, such as films, microspheres, liposomes, fibres and hydrogels, which have been used for more than 20 years worldwide [63]. Although the above-mentioned commercial preparations of HA have mainly been used in animal studies, it also provides useful information regarding the effect of hyaluronate in the prevention of post-operative peridural scar adhesion after laminectomy in spine surgery; however, additional clinical trials regarding the use of HA-based gels should be performed to confirm its effects in human subjects [67, 68]. Use of the hyaluronic acid-carboxymethylcellulose membrane Seprafilm as a solid anti-adhesion barrier agent is one of the therapeutic approaches used to reduce postoperative scar formation and is effective in promoting peripheral nerve regeneration at the repair site [69]. In addition, HA-carboxymethylcellulose solutions improve nerve regeneration and reduce perineural scar formation and adhesion after sciatic nerve repair [70]. It has been confirmed that direct application of HA-carboxymethylcellulose in transected nerves may limit axonal outgrowth by contact with regenerating axons; therefore, HA-carboxymethylcellulose barriers may prove to be a tool to prevent neuroma formation through inhibiting axonal growth [71]. On the contrary, according to some other studies, the role of HA solution in axonal outgrowth is dose-dependent, because high dose of HA (100–1000 µg/ml) topically used is characterised by significantly increased axonal outgrowth compared with HA solution (10 µg/ml) applied in the control group, in which axonal outgrowth did not

occur [72]. Some authors describe the early effect on nerve regeneration of continuous local delivery of nerve growth factor (NGF) and the local incorporation of HA inside a newly manufactured nerve conduit material from fresh human amniotic membrane [73]. Additionally, other authors have demonstrated that the combination of vascular endothelial growth factor (VEGF) gene therapy and a HA film sheath-enriched microenvironment may synergistically promote peripheral nerve regeneration [74]. The microenvironment of neuron cells plays a crucial role in regulating neural development and regeneration [75].

HA and its derivatives may also promote regeneration of injured nerves through realignment of the fibrin matrix, and they can provide a suitable environment for axonal ingrowths [25]. In another animal experimental study, a single topical dose of HA enhanced the nerve regeneration process in hindlimb rat and rabbit models by preventing perineural scar formation after peripheral nerve repair [31, 76]. New data in the literature have shown that HA-based biomaterials have been applied in a wide range of medical and biological fields and play important roles in neural regeneration [75].

3. Tacrolimus

Tacrolimus, also known as FK506 or Fujimicin (C44H69O12), is a macrolide immunosuppressive drug that is approved for the prevention of allograft rejection [25], but it is well known that tacrolimus can also increase nerve regeneration and facilitate allografting of nerves via immunosuppression [18]. Chemically, tacrolimus is a 23-membered macrolide lactone. It is a powerful and selective anti-T-lymphocyte agent that was discovered in 1984 and later approved by the U.S. Food and Drug Administration (FDA). This agent, isolated from the fungus *Streptomyces tsukubaensis*, has a mechanism of action similar to that of cyclosporin A. The first preliminary report on FK506 was presented in 1986 at the 11th International Congress of the Transplantation Society [77], and the first experimental reports were published in 1987 [78–80]. These early reports demonstrated that FK506 is a potent immunosuppressant that acts *in vitro* via inhibiting interleukin 2 (IL-2) production, as well as by inhibiting the response of mixed lymphocyte cultures at concentrations 32–100 times lower than that of cyclosporin A [81]. It means that despite similar mechanisms of action, tacrolimus is 50–100 times more potent than cyclosporin A [82].

Tacrolimus is able to modulate the immune system, inhibit T-cell function by binding to FK binding proteins (FKBP) and mediate immunosuppression by inhibiting calcineurin and calcium and calmodulin-dependent phosphatase. The primary biological effect of calcineurin inhibition includes the decrease of the production of inflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin-2 and interferon-γ [28]. The drug's immunosuppressive effects are mediated largely through FKBP12, which is involved in intracellular calcium flux and cycle regulation [83]. Tacrolimus realises its effect by binding to its receptors (FKBP12 and FKBP52). The FKBP12 receptors are responsible for immunosuppressive effects, whereas the FKBP52 receptors are related to neuroregenerative effects. These effects of tacrolimus have been shown experimentally in multiple models of nerve injury during the past decade when

tacrolimus was used in sub-immunosuppressive doses, and these findings have stimulated interest in characterising its neurophysiologic effects on nerve regeneration [84]. Tacrolimus sustains nerve regeneration with both systemic and local administration [85]. *In vitro* and *in vivo* experimental models have proven that tacrolimus increases neurite elongation and accelerates the rate of peripheral nerve regeneration [29]. In addition, there is evidence from *in vivo* experimental studies that highlight that tacrolimus has a neuroprotective role in the central nervous system through its direct impact on its various cell populations [86]. Recently, some studies have demonstrated the effect of tacrolimus in spinal surgery where it was found that topical application of tacrolimus could inhibit fibroblast proliferation and prevent epidural scar adhesion after laminectomy in a rat model [87], and it is worth mentioning that tacrolimus has been successfully used topically in spinal cord trauma for neuroprotection and local regeneration [88].

Tacrolimus is an essential drug for the conventional immunosuppression regimen for solid organ transplantation. The use of tacrolimus in post-transplant immunosuppressive regimens can enhance nerve regeneration and the growth of axon sprouts into donor tissue [89]. It has been demonstrated that tacrolimus has a powerful effect on promoting axon regeneration through its immunosuppressive and neurotrophic action [90]. This (neurotrophic) action can be completely prevented *in vitro* by the addition of a monoclonal antibody against FKBP52 [84].

The topical effects of tacrolimus on peripheral nerve have not been well investigated to date and the exact mechanism by which tacrolimus affects nerve regeneration is unclear, but outcomes data, so far, have been promising [89]. Additionally, the results of the use of tacrolimus in peripheral nerve regeneration differ in the literature. The relative variability of the results of experimental studies of nerve injures can be explain by the variety of models and testing methods used [91]. Prior studies have shown that FK506-FKBP12 interaction may lead to a neuroregenerative effect through increased neuronal expression of a growth cone-associated protein GAP-43, but there is evidence that this occurs through inactivation of neuronal nitric synthetase [92, 93]. Moreover, axon regeneration from tacrolimus is realised predominantly through its binding to FKBP-12, which activates GAP-43 and the transforming growth factor (TGFb1) pathway [26]. Tacrolimus can promote peripheral nerve regeneration through reducing scar formation; however, little is known about how tacrolimus reduces scar formation [94, 95]. Que et al. suggest that tacrolimus-induced fibroblast apoptosis contributes to the suppression of fibroblast proliferation and then causes the reduction of scar formation in the damaged nerve; in fibroblasts, apoptosis of tacrolimus involves c-Jun N-terminal kinase (JNK) and extracellular-signal-regulated kinase [94].

Some studies have shown a positive effect of tacrolimus after it was used in different allograft and isographs. Earlier axon regeneration in allografts with FK506 compared to allografts without FK506 was demonstrated experimentally [96]. Systemic application of tacrolimus at doses of 0.6 mg/kg found the amount of myelin debris in autologous nerve grafts to be decreasing [97]. This can be explained by the reduction of macrophage infiltration after tacrolimus administration. The reduction of scar formation at the site of nerve repair by the above-mentioned mechanisms has been associated with better morphologic and nerve function recovery. Recently, we published two original articles that compare the effects of HA and FK-506 on

nerve regeneration. We found that our electrophysiological and biomechanical measurements as well as our results of functional evaluations indirectly indicate that the effects of HA and FK506 on nerve regeneration are similar [98, 99].

4. Cyclosporin A

Cyclosporin A is a neutral lipophilic cyclic undecapeptide that was isolated in 1971 from the fungus *Tolypocladium inflatum* and came into medical use in 1983 [100]. Its immunosuppressive function was first reported in 1972 by Sandoz Laboratories [101]. Discovery of cyclosporine A has revolutionised transplantation medicine, which requires the application of immunosuppressive therapy. Cyclosporin has been widely applied as an immunosuppressive substance in organ transplantation in association with other drugs, both in vital and non-vital organs, such as skin, nerve and muscles. It is known that cyclosporin A has similar immune-suppressing characteristics to tacrolimus, but tacrolimus has a more potent effect with equal volumes of drug [95]. There are data in the literature that cyclosporin A was used experimentally in order to investigate its anti-scarring effects on peripheral nerves both ultrastructurally and in gross post-surgical and histopathological analyses [102]. Cyclosporin's mechanism of action in nerve regeneration remains controversial [103]. However, probable mechanisms include inhibiting white blood cell proliferation and/or differentiation and inhibiting Ca^{2+}-dependent cell injury [104]. Besides the above-mentioned mechanism, cyclosporin A acts with other anti-inflammatory effects in preventing scar formation because it can block the transcription of cytokine genes in activated T cells, whereas on the another hand, it is well established that cyclosporin A inhibits the phosphatase activity of calcineurin through the formation of a complex with cyclophilin, which regulates nuclear translocation and subsequent activation of nuclear factor of activated T cells (NFAT) transcription factors [105]. Calcineurin plays an important role in the T-cell receptor-mediated signal transduction pathway and is identified as the common target for cyclosporin A and tacrolimus [106].

The role of cyclosporin A in peripheral nerve regeneration after peripheral nerve allografting has been investigated in experimental models immunosuppressed with cyclosporine for more than two decades [107]. It is worth mentioning that most of these studies were concentrated on allograft survival, rather than on the direct effect of cyclosporin A on peripheral nerve regeneration [103]. Some authors have investigated the efficacy of cyclosporine A in large- and small-diameter nerve grafts as well as in long and short allografts. They have found better nerve regeneration in large-diameter nerve grafts than in small-diameter nerve grafts, whereas with regard to the length of the grafted nerve, short nerve allografts give higher axon counts than long ones, the same as with autografts [108]. Recently published data suggest that even though cyclosporin A is effective at reducing graft rejection, axon regeneration is still superior in autografts versus immunosuppressed allografts [109]. Furthermore, cyclosporin A effectively prevented postoperative epineurial fibrosis on rat sciatic nerves after peripheral nerve surgery with no adverse effects after topical application [102]. The application of cyclosporin A in a silicon conduit neurorrhaphy resulted in improvement of functional recovery and quantitative morphometric indices of sciatic nerves in diabetic rats [110].

5. Melatonin

Melatonin, which is also known as *N*-acetyl-5-methoxytryptamine, is a hormone and was first identified in bovine pineal extracts [35]. Melatonin is the main hormone of the pineal gland and is an important signalling molecule that occurs in many organisms as well as in plants and fungi [33]. The pineal gland is in the middle of the brain and secretes melatonin, a hormone that regulates when you sleep at night and wake up in the morning, as well as other numerous aspects of circadian biology [111]. Melatonin has an effect on the morphologic features of the nerve tissue, suggesting its neuroprotective, free-radical scavenging and antioxidative and analgesic effects in degenerative diseases of peripheral nerves [25]. There are different opinions among authors regarding the protective effect of melatonin in stimulation of peripheral regeneration, because some authors have reported toxic effects of melatonin on peripheral nerves [33]. However, nowadays there is enough evidence from the literature showing that melatonin has a useful effect on axon length and sprouting after traumatic events to peripheral nerves [34]. The beneficial effects of melatonin administration on the recovery of injured nerves may be attributed to its antioxidant properties [112]. Melatonin has an effect on superoxide dismutase, which is an important antioxidative enzyme that is involved in redox regulation of regulative stress, and would exert melatonin's beneficial effects by preserving the superoxide dismutase reactivity following peripheral nerve injury [113]. The rhythm of melatonin defines the activity of glutathione peroxidase and consequently also glutathione reductase. It is thought that the involvement of melatonin in the control of redox processes depends on its high-affinity binding to cytosolic quinone reductase 2, previously believed to be a melatonin receptor [114]. Through a variety of experimental neuropathologies involving nitric oxide (NO), it was confirmed that melatonin exerts its neuroprotective role after peripheral axotomy via reduction of oxidative damage [115]. The neuronal isoform of nitric oxide synthetase (nNOS), an NADPH-dependent diaphorase, is considered to play a role in motoneuron death induced by nerve transection. In addition, it is known that exogenous melatonin can prevent neuropathy development via the inhibition of lipid peroxidation in renal tissue and the inhibition of TGF-β, which limits the effects against fibrosis [116]. Furthermore, data show that melatonin can significantly promote Schwann cell proliferation and can improve nerve regeneration after peripheral nerve injury via this mechanism both *in vitro* and *in vivo*. The functional recovery of damaged nerves was estimated by the amount of Schwann cells and the number of re-innervated muscle motor end-plate targets [117]. Furthermore, it is worth mentioning that Turgut et al. have experimentally demonstrated in rats that melatonin prevents neuroma formation after transacting the sciatic nerve by enhancing axonal regeneration [118]. Recently, some studies have shown the positive effect of melatonin on preventing scar formation, increasing nerve regeneration and improving functional recovery [119, 120]. However, the exact mechanisms by which melatonin limits fibrosis are currently unclear.

6. Methylprednisolone

Methylprednisolone is an anti-inflammatory pharmacological agent that has found widespread use in treatment of many pathological disorders in humans. It has also been experimentally

investigated intensely for preventing scar formation and nerve regeneration because it is considered to have a neuroprotective role. Generally, glucocorticoids are anti-inflammatory substances that are often used to alleviate tissue oedema and trauma-induced inflammatory response because they can down regulate the expression of pro-inflammatory factors, such as tumour necrosis factor-α and interleukin-1β [121]. These pro-inflammatory factors can increase the expression of induced nitric oxide synthase in the injured region, leading to nitric oxide production and cell apoptosis [122]. There are some mechanisms by which glucocorticoids can express their anti-inflammatory effects in central and peripheral nerve system. However, one possible mechanism by which methylprednisolone inhibits nerve inflammation is its inhibition of CD3-positive inflammatory cell infiltration of local tissue [123]. It was found that higher doses of methylprednisolone have a neuroprotective role in injured nerves through inhibition of oxygen-free radical-induced lipid peroxidation [124]. Therefore, through inhibition of lipid peroxidation, methylprednisolone can retard both anterograde and retrograde nerve degeneration after peripheral nerve injury. Moreover, the steroid expresses its anti-inflammatory effects via inhibition on responsive cells and consequently recruitment of macrophages [125]. Regarding these anti-inflammatory effects, inhibition of phospholipase A2 activity should be mentioned, along with prevention of granulocyte, mast cell and macrophage degranulation, inhibition of macrophage migration-inhibitory factor and stabilisation of the lysosomal membrane, which are beneficial for treating injured nerves [39]. In the same study, the effect of preoperative locally administered dexamethasone on the recovery of crushed nerves was examined, and the authors concluded that local dexamethasone is more effective than systemic dexamethasone [39]. Systemic application in rats of moderate doses of methylprednisolone, i.e. 15–30 mg/kg, can effectively increase peripheral nerve regeneration, and it was also found that local administration of the drug can have the same positive effects as those of systemic administration while reducing systemic side effects [126]. Moreover, it was found that dexamethasone loaded in silicone tubes can improve functional recovery and morphometric indices of the sciatic nerve, and it was confirmed that topical administration of dexamethasone on peripheral nerve offers the benefits of cost savings as well as avoiding the complications associated with systemic administration [38]. The effect of methylprednisolone in suppressing scar formation and improving axonal regeneration after transection and suture of rat peripheral nerves was described many years before in rats [36].

Recent experimental studies have demonstrated that topical application of methylprednisolone can be realised using various methods, for example, in various materials such as silicon tubes [38], amniotic membranes [125] and microsphere sustained-release membranes [126], in order to avoid the rapid destruction of methylprednisolone at the site of nerve repair.

7. Vitamin B12

Vitamin B12, also called cobalamin, is a water-soluble vitamin with multiple functions in organisms, although in comparison with other nutrients, the body needs them in relatively small amounts. It is naturally present in animal products, fish, meat, poultry, eggs, milk and milk products [127]. There are several forms of vitamin B12, which contains mineral cobalt, so for this reason, compounds with vitamin B12 activity are collectively called cobalamins and

the active forms are methylcobalamin and 5 deoxyadenosylcobalaminin [128]. Vitamin B12 as a coenzyme induces conversion of homocysteine to methionine in order to facilitate synthesis of nucleic acids and proteins. Therefore, it accomplishes the following essential nerve function, such as promotion of nerve regeneration owing to axoplasm flow within the neuraxon, in order to normalise the neuraxon's skeleton protein transportation as well as accelerate the formation of the myelin sheath [129]. Vitamin B12 in combination of B1 (thiamine) and B6 (pyridoxine) reduced degenerating processes in the nervous system, and therefore, this combination has been clinically administered [130]. Furthermore, this vitamin is involved in the metabolism of every cell in the human body, especially affecting DNA synthesis and fatty acid and amino acid metabolism [131]. It is known that B12 deficiency leads to deficiency in methionine, which is required for the synthesis of both phospholipids and myelin; therefore, it is an essential element in the maintenance of nerve functions because it induces synthesis of the myelin sheath and improves nerve conduction velocity. In addition, it was found that vitamin B12 increased the number of Schwann cells and myelinated nerve fibres, and the diameter of axons, through which effects it can promote the regeneration of myelinated nerve fibres and the proliferation of Schwann cells [132]. In addition, vitamin B12 has shown antioxidant properties because it is also a good scavenger of reactive oxygen species and is suggested to be a good neuroprotectant. Moreover, vitamin B complex or vitamin B12 can increase the expression of brain-derived neurotrophic factor (BDNF) in injured nerves at both mRNA and protein levels, therefore promoting the regeneration and functional recovery of injured nerves through increasing BDNF expression [41]. Some authors have shown that vitamin B12 provides a basis for more beneficial treatments of nervous disorders through both systemic and local delivery of high doses of methylcobalamin to target organs, which has been shown to have the potential to treat peripheral nerve injury [40]. Inasmuch as the amount of vitamin B complex and vitamin B12 vary in cases of crush nerve injuries, it is necessary to administrate these vitamins in the acute phase of nerve injury in order to enhance nerve regeneration [42].

8. Riluzole

Riluzole (2-amino-6-trifluoromethoxy-benzothiazol) is a benzothiazole anti-convulsant and the only U.S. Food and Drug Administration (FDA)-approved drug to treat amyotrophic lateral sclerosis (ALS) [133]. Riluzole is a sodium/glutamate antagonist that has been shown to have a neuroprotective effect, recently entered clinical testing for spinal cord injury [134] and currently is under Phase III clinical trial for the treatment of spinal cord injury (ClinicalTrials. gov: NCT01597518) [135]. Its neuroprotective effects are a result of the blockade of sodium channels and, consequently, prevention of Ca^{2+} overflow [136]. In experimental trials in animal models, it was successfully used to reduce symptoms in neurodegenerative disease and neural tissue injury, and these effects can be explained by its inhibition of presynaptic glutamate release through blocking voltage-gated sodium channels [133]. It is known that *in vitro* application of riluzole to adult dorsal root ganglion neurons gives a neuroprotective effect via promotion of neurite outgrowth in terms of number, length and branch [137]. For nerve

regeneration after nerve injury, neurite outgrowth of surviving neurons is very important in order to reinervate target tissue. In addition, riluzole inhibits neuro-excitotoxicity in animal models of neural injury, and soon after its administration, it can sufficiently reduce pain from nerve root compression and can prevent development of neuronal dysfunction in the nerve root and the spinal cord [138]. Recently, riluzole was clinically approved for the treatment of motor neuron disease, and experimental research is now underway for the assessment of its role on nerve regeneration processes after peripheral nerve injury [46].

9. 4-Aminopyridine

4-Aminopyridine (4-AP) is a potassium channel blocker with the chemical formula $C_5H_4N-NH_2$ that it used as a research tool in order to classify the subtypes of the potassium channel [139]. It has shown clinical efficacy in the treatment of neurological disorders such as multiple sclerosis [140]. The mechanisms of action of 4-AP can explain by its effect in allowing impulse conduction in demyelinated axons by blocking K^+ channels that allow leakage of K^+ from these axons and thereby enabling axons to restore the level of depolarisation required for propagation of action potentials [141]. Recently, there are data from literature that 4-aminopyridine is a potent small molecule with neuroregenerative properties that enhances both the speed and extent of functional recovery after acute peripheral nerve injury, because it promotes remyelination [142]. The same authors have found that 4-aminopyridine treatment enables differentiation between incomplete and complete lesions more rapidly compared with existing approaches [142].

10. Verapamil

Verapamil belongs to the class of medications called calcium channel blockers. Besides its effects in the cardiovascular system, recently some experimental studies have investigated the role of verapamil in the peripheral nervous system, and it has been shown to reduce scar formation through inhibiting fibroblast adhesion and proliferation *in vitro* [143]. However, it is not clear whether topical application of verapamil after surgical nerve repair *in vivo* could prevent scar formation and promote nerve regeneration [44]. Apparently, this role of verapamil consists of stimulation of the endogenous anti-inflammatory reaction and decreasing pro-inflammatory processes by a channel blocker, therefore causing pain modulation or nerve regeneration [43]. The effect of calcium channel blockers in the reduction of scar formation was first reported by Lee and Ping [144]. There are two mechanisms by which verapamil can prevent scar formation: by reducing the biological activity of cells through inhibiting signal transduction inside and outside fibroblasts, and by suppressing the synthesis and secretion of collagen and extracellular matrix through changing fibroblast morphology [44].

The overall effects and mechanisms of the above-mentioned pharmacological agents in the prevention of scar formation and improved nerve regeneration are presented in **Table 1**.

Pharmacological agents	Effects	Mechanisms of action	References
Hyaluronic acid	Reduce the extent of scar formation and nerve adhesions	Via proliferation and chemotaxis of granulocyte phagocytosis and degranulation, and macrophage motility	Ozgenel [31] and Park et al. [70]
		Stimulator of interleukin-1 (IL-1) production, which affects (decreases) fibroblast proliferation and collagenase production	Hiro et al. [30]
Tacrolimus (FK506)	Neuroprotective role via reduction of scar formation	Through encouraging fibroblast apoptosis, it contributes to the suppression of fibroblast proliferation. Fibroblast apoptosis by tacrolimus involves c-Jun N-terminal kinase (JNK) and extracellular-signal-regulated kinase	Que et al. [94]
	Neuroregenerative role	FK506-FKBP12 interaction may lead to a neuroregenerative effect through increased neuronal expression of growth cone-associated protein GAP-43 that probably occurs through inactivation of neuronal nitric synthetase	Dawson et al. [92] and Madsen et al. [93]
		By increased neurite elongation and accelerating the rate of nerve regeneration	Konofaos and Terzis [29]
Cyclosporin A	Anti-scarring effects and nerve regeneration on peripheral nerves	Probable mechanisms include inhibiting white blood cell proliferation and/or differentiation and inhibiting Ca^{2+}-dependent cell injury	Erkutlu et al. [104]
		It is well established that cyclosporin A inhibits the phosphatase activity of calcineurin through the formation of a complex with cyclophilin, which regulates nuclear translocation and subsequent activation of nuclear factor of activated T cells (NFAT) transcription factors	Matsuda and Koyasu [105]
Melatonin	Induces axon length and sprouting after traumatic events to peripheral nerves	Via its effect on superoxide dismutase, which is an important antioxidative enzyme that is involved in redox regulation of regulative stress	Chang et al. [113]
	Improves nerve regeneration	Via reduction of oxidative damage, melatonin exerts its neuroprotective role after peripheral axotomy in a variety of experimental neuropathologies that involve nitric oxide (NO)	Chang et al. [115]
	Limits fibrosis and neuroma formation	Through promoting Schwann cell proliferation	Chang et al. [117]
		Exact mechanisms through which melatonin imparts these effects are currently unclear	Turgut et al. [118]

Pharmacological agents	Effects	Mechanisms of action	References
Methylprednisolone	Neuroprotective role	By its inhibition of CD3-positive inflammatory cell infiltration in local tissue and consequently recruitment of macrophages	Feng and Yuan [123]
	Anti-inflammatory effects	Through inhibition of oxygen-free radical-induced lipid peroxidation	Hall [124]
Vitamin B12	Nerve regeneration	Owing to axoplasm flow within the neuraxon, in order to normalise the neuraxon's skeleton protein transportation as well as accelerating the formation of the myelin sheath	Wang et al. [129]
	Neuroprotectant	Through increasing the number of Schwann cells and myelinated nerve fibres, and the diameter of axons	Lopatina et al. [132]
		Through antioxidant properties, because it is also a good scavenger of reactive oxygen species	Sun et al. [41]
Riluzole	Neuroprotectant	Through blockade of sodium channels, and consequently prevention of Ca^{2+} overflow	Fehlings et al. [136]
	Inhibits neuro-excitotoxicity	Through reducing pain from nerve root compression, it can prevent development of neuronal dysfunction in the nerve root	Nicholson et al. [138]
4-Aminopyridine (4-AP)	Neuroregenerative properties	Through its effect in allowing impulse conduction in demyelinated axons by blocking K^+ channels that allow leakage of K^+ from these axons and thereby enabling axons to restore the level of depolarisation required for propagation of action potentials	Hayes [141]
Verapamil	Reduce scar formation and promote nerve regeneration	There are two mechanisms by which verapamil can prevent scar formation: by reducing the biological activity of cells through inhibiting signal transduction inside and outside fibroblasts, and by suppressing the synthesis and secretion of collagen and extracellular matrix through changing fibroblast morphology	Han et al. [44]

Table 1. The effects and mechanisms of pharmacological agents in nerve regeneration.

11. Discussion

There have been many efforts to diminish scar formation and perineural adhesion as well as to improve nerve regeneration after microsurgical nerve repair. Various surgical techniques and several different pharmacological agents have been used for this purpose. The evalua-

tion of the effects of these pharmacological agents in the prevention of scar formation and nerve regeneration in experimental animals is performed by electrophysiological measurements and through assessing functional recovery, whereas after sacrificing the animals, other methods have been used, such as macroscopic, histomorphometric, immunohistochemical and biomechanical techniques [70, 91, 98, 99]. Ozgenel found that nerves treated with HA have a significant reduction in perineural thickness compared to nerves treated with just saline ($P < 0.05$). Besides that, this author found better mean conduction velocities (MCVs) and faster functional recovery in HA-treated nerves (0.82 ± 0.08 m/s) compared with nerves treated with saline, in which the MCV was 0.76 ± 0.04 m/s ($P < 0.05$) [31]. Park et al. found that topical application of HA carboxymethylcellulose solutions in rats significantly reduced nerve adherence score and the number of cellular components compared with the saline group (control group) ($P < 0.05$) [70]. The authors concluded that HA carboxymethylcellulose solutions improved nerve regeneration and reduced perineural scar formation and adhesion after sciatic nerve repair [70]. Furthermore, the same results were demonstrated by Adanali et al. in rabbit sciatic nerves where they used HA carboxymethylcellulose membranes in the experimental group and saline in the control group; they observed that adhesion in the surrounding tissues was significantly less in the HA carboxymethylcellulose membranes group than in the saline group [69]. Ikeda et al. found that local application of HA in the sciatic nerve was the most effective at reducing extraneural and intraneural connective tissue, compared with the steroid and saline groups [145]. By electrophysiological measurements, Ikeda et al. also found that the latencies of the HA and steroid groups were much shorter than that of the neurolysis group (2.14 ± 0.20, 1.92 ± 0.11 and 1.91 ± 0.15 m/s, respectively), but longer than that of the control group (1.68 ± 0.07 m/s). Similar results were found by histological examination, because scar tissue in the neurolysis group was thicker and more voluminous than that in the HA group or the steroid group [145]. In addition, Zor et al. found significantly less scar formation ($P < 0.01$) and significantly higher peak amplitudes in rats ($P < 0.01$) that received a combination treatment of vascular endothelial growth factor gene therapy with HA [74].

Shahraki et al. demonstrated earlier axon regeneration in allografts with FK506 compared to allografts without FK506 ($P < 0.05$) [96]. Yan et al. found that short treatment courses of 10 and 20 days with FK506 (in the graft model) were sufficient to reduce functional recovery time by 15 and 21%, respectively, compared with negative controls assessed by walking track analysis [90]. In addition, via a functional study, Azizi et al. confirmed faster recovery of the regenerated axons in the inside-out vein graft/FK506 group than that for the inside-out vein graft without FK506 (control group) ($P < 0.05$). The same statistically significantly difference was found when comparing these groups regarding the mean gastrocnemius muscle weight ratio ($P < 0.05$) [91]. Que et al. showed that scar area had a significant positive correlation with the fibroblast number, as detected by linear correlation analysis [94]. Other authors reported that tacrolimus can increase the number of axons and their myelinated axons by 40% and reduce by half the time to neurological recovery [29]. Furthermore, Li et al. found that after application of FK506 loaded in a chitosan guide, the amplitude and velocity of compound muscle action potential (CMAP) reached 60 and 73% of the control values, respectively [146].

In order to compare the effects of HA and FK506 on peripheral nerve regeneration in rabbits after the drugs were topically applied at the site of sciatic nerve, we used electrophysiological, macroscopic and microscopic methods, while functional assessment was performed via

toe-spreading reflex. According to our results, HA and FK506 appear to have similar effects ($P > 0.05$) with respect to preventing scar formation and improving nerve regeneration compared with saline ($P < 0.05$) [80, 98]. However, it should be mentioned that we observed no significant differences in biomechanical properties in the HA and FK506 groups compared to the saline group ($P > 0.05$) [99].

Çetinalp et al. demonstrated that animals treated with cyclosporin A had statistically significant lower perineural adhesion and better separability than the saline group (control group) ($P < 0.0001$) [102]. However, these authors did not find a significant difference in the wound-healing characteristics or neurological functions between the treatment (cyclosporin A) group and the control group ($P > 0.05$) [102]. In an experimental rat sciatic nerve injection injury model established by penicillin G potassium injection, Erkutlu et al. randomly divided rats into three groups based on the length of time after nerve injury induced by cyclosporin A administration (30 minutes, 8 or 24 hours), recorded electrophysiological measurements (compound muscle action potentials, pre-injury, early post-injury [within 1 hour]and 4 weeks after injury) and then compared the results of the experimental groups with the control group. Finally, they found significant improvement of the compound muscle action potential amplitude value only when cyclosporin A was administered within 30 minutes of the injection injury ($P < 0.05$) [104].

Turgut et al. examined the gross morphology of neuroma formation in the proximal nerve segment via macroscopic and microscopic findings, and the surgical pinealectomy group without application of melatonin caused a proliferation of connective tissue and large neuroma formation at the proximal ends of transacted nerves compared with the surgical pinealectomy group and the group given melatonin ($P < 0.005$) [118]. In addition, Kaya et al. demonstrated a beneficial effect on axonal regeneration and functional recovery in the experimental group in which melatonin was applied after stripping of the epineurial vessels compared with other groups without melatonin application [119].

Recently, Sadraie et al. found that at 8 weeks after surgery, sciatic functional index, withdrawal reflex latency test, electrophysiological values and histological results in the amniotic membrane with the betamethasone group were improved compared to those in the control and sham groups ($P < 0.05$) [125]. Furthermore, Feng and Yuan found better and faster functional recovery in the dexamethasone-administered group compared to other groups without dexamethasone ($P < 0.05$); therefore, they concluded that dexamethasone can promote functional recovery after sciatic nerve crush injury [123]. In addition, Sun et al. via morphological (by electron microscopy) and functional analysis observed that treatment with dexamethasone or vitamin B12 alone, or treatment with both agents, led to a much larger number of Schwann cells and myelinated nerve fibres compared with that in the saline group ($P < 0.05$) [41]. Okada et al. showed in a rat sciatic nerve injury model that continuous administration of high doses of methylcobalamin can improve nerve regeneration and functional recovery [40].

According to Shortland et al., a single dose of 0.1 μM riluzole was sufficient to promote neuronal survival in neonatal dorsal root ganglion cultures, whereas repeated riluzole administration was necessary in adult cultures. For both types of injuries, riluzole enhanced neurite outgrowth (number, length and branch pattern) significantly more on the injured side in comparison with the contralateral side [137].

Tseng et al. found that once-daily administration of 10 µg of 4-aminopyridine enhanced the speed of recovery from crush injury when it was used as early as 3 days post-injury in mice treated daily (beginning 24 hours post-injury), and a significant improvement (>25%) in gait function was confirmed over the control groups (vehicle-treated animals). Furthermore, at 5 and 8 days post-injury, 4-AP-treated mice showed statistically significant twofold greater levels of improvement than the control groups [142].

Han et al. carried out a study in which the right sciatic nerve of adult rats was transected and sutured, and then a gelfoam soaked with verapamil solution for 4 weeks was topically applied by them. The results showed that verapamil can inhibit the secretion of extracellular matrix from fibroblasts *in vivo* through suppression of type I and III collagen secretion. Verapamil also increased the total number of axons as well as the number of myelinated axons more than in the control group, in which gelfoam soaked with physiological saline was topically applied ($P < 0.05$) [44].

12. Conclusions

Generally, it should be mentioned that the most of the experimental research discussed in this chapter was conducted in rats and rabbits, in which the above-mentioned pharmacological agents have been applied (mainly locally in sciatic nerve). The success of the regenerative process of nerve repair in experimental research can be evaluated using a variety of methods, such as morphological, immunohistochemical, electrophysiological, biomechanical and functional evaluation. Some of the pharmacological agents described in this chapter are still only used for experimental purposes, whereas some of them are in clinical use for the treatment of various diseases, and now their neuroregenerative effects will also be explored in experimental animals. However, the success of nerve regeneration depends on the type and degree of nerve injury, age, repair time, operative techniques and the type of materials used. By combining appropriate dosages of these pharmacological agents with improved microsurgical techniques for nerve repair, better experimental results may be achieved in the future, encouraging clinical application of these agents. However, it is understandable that complete regeneration and functional recovery will almost never be achieved, regardless of the operative technique used or the type of pharmacological agent applied.

Author details

Agon Mekaj[1] and Ymer Mekaj[2]*

*Address all correspondence to: ymer.mekaj@uni-pr.edu

1 Clinic of Neurosurgery, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo

2 Institute of Pathophysiology, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo

References

[1]   McCance KL, Heuther SE. Pathophysiology: The Biologic Basis for Disease in Adults and Children. 5th ed. Philadelphia, USA: Elsevier Mosby; 2006. p 411.

[2]   Geuna S, Tos P, Titolo P, Ciclamini D, Beningo T, Battiston B. Update on nerve repair by biological tubulization. Journal of Brachial Plexus and Peripheral Nerve Injury. 2014;**9**:3. DOI: 10.1186/1749-7221-9-3

[3]   Madura T: Pathophysiology of Peripheral Nerve Injury, Basic Principles of Peripheral Nerve Disorders, Dr. Seyed Mansoor Rayegani (Ed.), ISBN: 978-953-51-0407-0, InTech; 2012. Chapter 1, pp 1-16.

[4]   Kerfeld CA, Sawaya MR, Tanaka S, Nguyen CV, Phillips M, Beeby M, Yeates TO. Protein structures forming the shell of primitive bacterial organelles. Science. 2005;**309**:936-938. DOI: 10.1126/science.1113397

[5]   Martini AI, Frederic ET. Anatomy and Physiology. 1st ed. Philipine: Pearson Education South Asia Pte. Ltd; 2007

[6]   Siemionow M, Brzezicki G. Chapter 8: Current techniques and concepts in peripheral nerve repair. International Review of Neurobiology. 2009;**87**:141-172. DOI: 10.1016/S0074-7742(09)87008-6

[7]   Kouyoumdjian JA. Peripheral nerve injuries: A retrospective survey of 456 cases. Muscle Nerve. 2006;**34**:785-788. DOI: 10.1002/mus.20624

[8]   Eser F, Aktekin LA, Bodur H, Atan C. Etiological factors of traumatic peripheral nerve injuries. Neurology India. 2009;**57**:434-437. DOI: 10.4103/0028-3886.55614

[9]   Robinson LR. Traumatic injury to peripheral nerves. Muscle Nerve. 2000;**23**:863-873

[10] Jacques L, Kline DG. Response of the peripheral nerve to physical injury. In: Crockard A, Hayward R, Hoff JT, editors. Neurosurgery: The Scientific Basis of Clinical Practice. 3rd ed, Vol. 1. London: Blackwell; 2000. pp. 516-525

[11] Burnett MG, Zager EL. Pathophysiology of peripheral nerve injury: A brief review. Neurosurgical Focus. 2004;**16**:E1

[12] Zochodne DW, Levy D. Nitirc oxide in damage, disease and repair of the peripheral nervous system. Cellular and Molecular Biology (Nosi-le-grand). 2005;**51**:255-267

[13] Menorca RM, Fussell TS, Elfar JC. Nerve physiology: Mechanisms of injury and recovery. Hand Clinics. 2013;**29**:317-330. DOI: 10.1016/j.hcl.2013.04.002

[14] Seddon HJ. Three types of nerve injury. Brain. 1943;**66**:237-288

[15] Sunderland S. Nerves and Nerve Injuries. 2nd ed. London: Churchill Livingston; 1978. pp 827-828.

[16] Flores AJ, Lavernia CJ, Owens PW. Anatomy and physiology of peripheral nerve injury and repair. American Journal of Orthopedics (Belle Mead NJ). 2000;**29**:167-173

[17] Geuna S, Raimondo S, Ronchi G, Di Spicio F, Tos P, Czaja Fornaro M. Chapter 3: Histology of the peripheral nerve and changes occurring during nerve regeneration. International Review of Neurobiology. 2009;**87**:27-46. DOI: 10.1016/S0074-7742(09)87003-7

[18] Grinsell D, Keating CP. Peripheral nerve reconstruction after injury: A review of clinical and experimental therapies. BioMed Research International. 2014;**2014**:698256. DOI: 10.1155/2014/698256

[19] Pfister BJ, Gordon T, Loverde JR, Kochar AS, Mackinnon SE, Cullen DK. Biomedical engineering strategies for peripheral nerve repair: Surgical applications, state of the art, and future challenges. Critical Reviews in Biomedical Engineering. 2011;**39**:81-124

[20] Waller A. Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. Philosophical Transactions of the Royal Society of London. 1850;**140**:423-429

[21] Fawcett JW, Keynes RJ. Peripheral nerve regeneration. Annual Review of Neuroscience. 1990;**13**:43-60. DOI: 10.1146/annurev.ne.13.030190.000355

[22] Mira JC. Effects of repeated denervation on muscle reinnervation. Clinics in Plastic Surgery. 1984;**11**:31-38

[23] Jenq CB, Jenq LL, Coggeshall RE. Numerical patterns of axon regeneration that follow sciatic nerve crush in the neonatal rat. Experimental Neurology. 1987;**95**:492-499

[24] Son YJ, Thompson WJ. Schwann cell processes guide regeneration of peripheral axons. Neuron. 1995;**14**:125-132

[25] Mekaj AY, Morina AA, Bytyqi CI, Mekaj YH, Duci SB. Application of topical pharmacological agents at the site of peripheral nerve injury and methods used for evaluating the success of the regenerative process. Journal of Orthopaedic Surgery and Research. 2014;**9**:94. DOI: 10.1186/s13018-014-0094-3

[26] Gold BG. FK506 and the role of immunophilins in nerve regeneration. Molecular Neurobiology. 1997;**15**:285-306. DOI: 10.1007/BF02740664

[27] Choi BH, Zhu SJ, Kim MY, Huh JY, Lee SH, Jung JH. Transplantation of cultured bone marrow stroma cells to improve peripheral nerve regeneration. International Journal of Oral and Maxillofacial Surgery. 2005:**34**:537-542. DOI: 10.1016/j.ijom.2004.10.017

[28] Tung TH. Tacrolimus (FK506): Safety and applications in reconstructive surgery. Hand (New York). 2010;**5**:1-8. DOI: 10.1007/s11552-009-9193-8

[29] Konofaos P, Terzis JK. FK506 and nerve regeneration: Past, present and future. Journal of Reconstructive Microsurgery. 2013;**29**:141-148. DOI: 10.1055/s-0032-1333314

[30] Hiro D, Ito A, Matsuta K, Mori Y. Hialuronic acid is an endogenous induces of inter-leukin-1 production by human monocytes and rabbit macrophages. Biochemical and Biophysical Research Communications. 1986;**140**:715-722

[31] Ozgenel GY. Effects of hyaluronic acid on the peripheral nerve scarring and regeneration in rats. Microsurgery. 2003;**23**:575-581. DOI: 10.1002/micr.10209

[32] Smit X, van Neck JW, Afoke A, Hovius SE. Reduction of neural adhesions by biodegradable autocrosslinked hyaluronic acid gel after injury of peripheral nerves: An experimental study. Journal of Neurosurgery. 2004;**101**:648-652. DOI: 10.3171/jns. 2004.101.4.0648

[33] Odaci E, Kaplas S. Melatonin and nerve regeneration. Chapter 16. International Review of Neurobiology. 2009;**87**:317-335. DOI: 10.1016/S0074-7742(09)87016-5

[34] Turgut M, Kaplan S. Effects of melatonin on peripheral nerve regeneration. Recent Patents on Endocrine Metabolic & Immune Drug Discovery. 2011;**5**:100-108

[35] Behram Kandemir Y, Sarikcioglu L. Melatonin and its therapeutic actions on peripheral nerve regeneration. Folia Morphologica (Warsz). 2015;**74**:283-289. DOI: 10.5603/FM.2015.0043

[36] Nachemson AK, Lundborg G, Myrhage R, Rank F. Nerve regeneration and pharmacological suppression of the scar reaction at the suture site. An experimental study on the effect of estrogen progesterone, methylprednisolone-acetate and cis-hydroxyproline in rat sciatic nerve. Scandinavian Journal of Plastic and Reconstructive Surgery. 1985;**19**:255-260

[37] Boa F, Chen Y, Dekaban GA, Weaver LC. Early anti-inflammatory treatment reduces lipid peroxidation and protein nitration after spinal cord injury in rats. Journal of neurochemistry. 2004;**88**:1335-1344

[38] Mahammadi R, Azad-Tirgan M, Amini K. Dexamethasone topically accelerates peripheral nerve repair and target organ reinnervation: A transected sciatic nerve model in rat. Injury. 2013;**44**:565-569. DOI: 10.1016/j.injury.2012.10.013

[39] Suslu H, Altun M, Erdivanli B, Turan SH. Comparison of the effect of local and systemic dexamethasone on the rat traumatic sciatic nerve model. Turkish Neurosurgery. 2013;**23**:623-629. DOI: 10.5137/1019-5149.JTN.7761-13.0

[40] Okada K, Tanaka H, Temporin K, Okamoto M, Kuroda Y, Moritomo H, Murase T, Yoshikawa H. Methylcobalamin increases Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model. Experimental Neurology. 2010;**222**:191-203. DOI: 10.1016/j.expneurol.2009.12.017

[41] Sun H, Yang T, Li Q, Zhu Z, Wang L, Bai G, Li D, Li Q, Wang W. Dexamethasone and vitamin B12 synergistically promote peripheral nerve regeneration in rats by upregulating the expression of brain-derived neurotrophic factor. Archives of Medical Science. 2012;**8**:924-930. DOI: 10.5114/aoms.2012.31623

[42] Altun I, Kurutas EB. Vitamin B complex and vitamin B12 levels after peripheral nerve injury. Neural Regeneration Research. 2016;**11**:842-845. DOI: 10.4103/1673-5374.177150

[43] Darwish Iel S, Dessouky IS. Potential neuroprotective role of verapamil in experimentally-induced chronic sciatic nerve constriction in mice. British Journal of Medicine and Medical Research. 2015;**8**:781-789. DOI: 10.9734/ BJMMR/2015/17908

[44] Han AC, Deng JX, Huang QS, Zheng HY, Zhou P, Liu ZW, Chen ZB. Verapamil inhibits scar formation after peripheral nerve repair *in vivo*. Neural Regeneration Research. 2016;**11**:508-511. DOI: 10.4103/1673-5374.179075

[45] Leinster VH, Robson LG, Shortland PJ. Differential effects of riluzole on subpopulations of adult rat dorsal root ganglion neurons *in vitro*. Neuroscience. 2010;**166**:942-951.DOI: 10.1016/j.neuroscience.2009.12.058

[46] Ghayour MB, Abdolmaleki A, Behnam-Rassouli M. The effect of Riluzole on functional recovery of locomotion in the rat sciatic nerve crush model. European Journal of Trauma and Emergency Surgery. DOI: 10.1007/s00068-016-0691-4

[47] Balazs EA, Laurent TC, Howe AF, Varga L. Irradiation of mucopolysaccharides with ultraviolet light and electrons. Radiation Research. 1959;**ll**:149-164

[48] Laurent TC. Biochemistry of hyaluronan. Acta oto-laryngologica Supplementum 1987; **442**:7-24

[49] Laurent TC, Laurent UBG, Fraser JR. Functions of hyaluronan. Annals of the Rheumatic Diseases. 1995;**54**:429-432

[50] Becker LC, Bergfeld WF, Belsito DV, Klaassen CD, Marks Jr JG, Shank RC, Slaga TJ, Snyder PW. Cosmetic Ingredient Review Expert Panel, Andersen FA. Final report of the safety assessment of hyaluronic acid, potassium hyaluronate, and sodium hyaluronate. International Journal of Toxicology. 2009;**28**:5-67. DOI: 10.1177/1091581809337738

[51] Meyer L, Palmer J. The polysaccharide of the vitreous humor. Biological Chemistry. 1934; **107**:629–634

[52] Balazs EA, Laurent TC, Jeanloz RW. Nomenclature of hyaluronic acid. Biochemical Journal. 1986;**235**:903

[53] Kreil G. Hyaluronidases-A group of neglected enzymes. Protein Science. 1995;**4**:1666-1669. DOI: 10.1002/pro.5560040902

[54] Weigel PH. Hyaluronan synthase: The mechanism of initiation at the reducing end and a pendulum model for polysaccharide translocation to the cell exterior. International Journal of Cell Biology. 2015;**2015**:367579. DOI: 10.1155/2015/367579

[55] DeAngelis PL, Papaconstantinou J, Weigel PH. Isolation of a Streptococcus pyogenes gene locus that directs hyaluronan biosynthesis in acapsular mutants and in heterologous bacteria. Journal of Biological Chemistry. 1993;**268**:14568-14571

[56] Dougherty BA, van de Rijn I. Molecular characterization of hasA from an operon required for hyaluronic acid synthesis in group A streptococci. Journal of Biological Chemistry. 1994;**269**:169-175

[57] DeAngelis PL, Weigel PH. Immunochemical confirmation of the primary structure of streptococcal hyaluronan synthase and synthesis of highmolecular weight product by the recombinant enzyme. Biochemistry. 1994;**33**:9033-9039

[58] Tien JY, Spicer AP. Three vertebrate hyaluronan synthases are expressed during mouse development in distinct spatial and temporal patterns. Developmental Dynamics. 2005;**233**:130-141

[59] Törrönen K, Nikunen K, Kärnä R, Tammi M, Tammi R, Rilla K. Tissue distribution and subcellular localization of hyaluronan synthase isoenzymes. Histochemistry and Cell Biology. 2014;**141**:17-31. DOI: 10.1007/s00418-013-1143-4

[60] Toole BP, Wight TN, Tammi MI. Hyaluronan-cell interactions in cancer and vascular disease. Journal of Biological Chemistry. 2002;**277**:4593–4596

[61] Mekaj Y, Mekaj A. Prevention of failed back surgery syndrome with applications of different pharmacological agents: A review article. Therapeutic Targets for Neurological Diseases. 2015;**2**:e507

[62] Medina JM, Thomas A, Denegar CR. Knee osteoarthritis: Should your patient opt for hyaluronic acid injection? Journal of Family Practice. 2006;**55**(8) 669-675

[63] Necas J, Bartosikova L, Brauner P, Kolar J. Hyaloronic acid (hyaloronan): A review. Veterinarni Medicina. 2008;**52**:397-411

[64] Burd DA, Greco RM, Regauer S, Longaker MT, Siebert JW, Garg HG. Hyaloronan and wound healing: A new perspective. British Journal of Plastic Surgery. 1991;**44**:579-584

[65] Balazs EA, Denlinger JL: The role of hyaluronic acid in arthritis and its therapeutic use. In: Peyron JG, editor. Osteoarthritis: Current Clinical and Fundamental Problems. Geigy: Basel; 1984. pp. 165-174

[66] Amarnath LP, Srinivas A, Ramamurthi A. *In vitro* hemocompatibility testing of UV-modified hyaluronan hydrogels. Biomaterials. 2006;**27**:1416-1424

[67] Chen JM, Lee SH, Tsai TT, Niu CC, Chen LH, Chen WJ. Anti-adhesive effect of hyaluronate in a rabbit laminectomy model. Biomedical Journal 2014;**37**:218-224

[68] Isik S, Taskapilioğlu MÖ, Atalay FO, Dogan S. Effects of cross-linked hight-moleculare-weight hyaluronic acid on epidural fibrosis: Experimental study. Journal of Spine & Neurosurgery. 2015;**22**:94-100. DOI: 10.3171/2014.10.SPINE131147

[69] Adanali G, Verdi M, Tuncel A, Erdogan B, Kargi E. Effect of hyaluronic acid-carboxymethylcellulose membrane on extraneural adhesion formation and peripheral nerve regeneration. Journal of Reconstructive Microsurgery. 2003;**19**:29-36. DOI: 10.1055/s-2003-37188

[70] Park JS, Lee JH, Han CS, Chung DW, Kim GY. Effect of hyaluronic acid-carboxymethyl-cellulose solution on perineural scar formation after sciatic nerve repair in rats. Clinics in Orthopedic Surgery 2011;**3**: 315-324. DOI: 10.4055/cios.2011.3.4.315

[71] Agenor A, Dvoracek L, Leu A, Hunter DA, Newton P, Yan Y, Johnson PJ, Mackinnon SE, Moore AM, Wood MD: Hyaluronic acid/carboxymethyl cellulose directly applied to transected nerve decreases axonal outgrowth. J Biomed Mater Res B Appl Biomater. 2017;105(3):568-574. DOI: 10.1002/jbm.b.33576

[72] Torigoe K, Tanaka HF, Ohkochi H, Miyasaka M, Yamanokuchi H, Yoshidad K, Yoshida T. Hyaluronan tetrasaccharide promotes regeneration of peripheral nerve: *In vivo* analysis by film model method. Brain Research. 2011;**1385**:87-92. DOI: 10.1016/j.brainres.2011.02.020

[73] Mohammad JA, Warnke PH, Pan YC, Shenaq S: Increased axonal regeneration through a biodegradable amnionic tube nerve conduit: Effect of local delivery and incorporation of nerve growth factor hyaluronic acid media. Annals of Plastic Surgery. 2000;**44**: 59-64

[74] Zor F, Deveci M, Kilic A, Ozdag MF, Kurt B, Sengezer M, Sönmez TT. Effect of VEGF gene therapy and hyaluronic acid film sheath on peripheral nerve regeneration. Microsurgery. 2014;**34**:209–216. DOI: 10.1002/micr.22196.

[75] Yang MH, Chen KC, Chiang PW, Chung TW, Chen WJ, Chu PY, Chen SC, Lu YS, Yuan CH, Wang MC, Lin CY, Huang YF, Jong SB, Lin PC,Tyan YC. Proteomic profiling of neuroblastoma cells adhesion on hyaluronic Acid-Based surface for neural tissue engineering.BioMed Research International. 2016;**2016**:1917394. DOI: 10.1155/2016/1917394

[76] Mekaj AY, Manxhuka-Kerliu S, Morina AA, Duci SB, Shahini L, Mekaj YH. Effects of hyaluronic acid and tacrolimus on the prevention of perineural scar formation and on nerve regeneration after sciatic nerve repair in a rabbit model. European Journal of Trauma and Emergency Surgery. DOI: 10.1007/s00068-016-0683-4.

[77] Wallemacq PE, Reding R. FK506 (tacrolimus), a novel immunosuppressant in organ transplantation: Clinical, biomedical, andanalytical aspects. Clinical Chemistry. 1993;**39**: 2219-2228

[78] Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, Goto T, Okuhara M, Kohsaka M, Aoki H, Ochiai T. FK-506, a novel iinmunosuppreasant isolated from a streptomyce8. II. Immunosuppressive effect of FK-506 *in vitro*. Journal of Antibiotics (Tokyo). 1987;**40**:1256-1265. DOI.org/10.7164/antibiotics.40.1256

[79] Sawada S, Suzuki G, Kawase Y, Takaku F. Novel immunosuppressive agent, FK506. *In vitro* effects on the doned T cell activation. Journal of Immunology. 1987;**139**:1797-1803

[80] Goto T, Kino T, Hatanaka H, Nishiyama M, Okuhara M, Kohsaka M, Aoki H, Imanaka H. Discovery of FK 506, a novel immunosuppreasant isolated from Streptomycea tsukubaensis. Transplantation Proceedings. 1987;**19**:4-8

[81] Ochiai T, Nakima K, Nagata M, Suzuki T, Asano T, Uematsu T, Goto T, Hori S, Kenmochi T, Nakagoori T. Effect of a new immunosuppressive agent, FK 506, on heterotopic cardiac allo. transplantation in the rat. Transplantation Proceedings. 1987;**19**:1284-1286

[82] Pleyer U, Lutz S, Jusko WJ, Nguyen KD, Narawane M, Ruckert D, Mondino BJ, Lee VH, Nguyen K. Ocular absorption of topically applied FK506 from liposomal and oil formulations in the rabbit eye. Investigative Ophthalmology & Visual Science. 1993;**34**:2737-2742

[83] Tung TH. Clinical strategies to enhance nerve regeneration. Neural Regeneration Research. 2015;**10**:22-24. DOI: 10.4103/1673-5374.150641

[84] Yeh C, Browers D, Hadlock TA. Effect of FK506 on functional recovery after facial nerve injury in the rat. Archives of Facial Plastic Surgery. 2007;**9**:333-339. DOI: 10.1001/archfaci.9.5.333

[85] Toll EC, Seifalian AM, Birchall MA. The role of immunophilin ligands in nerve regeneration. Regenerative Medicine. 2011;**6**:635-652. DOI: 10.2217/rme.11.43

[86] Saganová K, Gálik J, Blaško J, Korimová A, Račeková E, Vanický I. Immunosuppressant FK506: Focusing on neuroprotective effects following brain and spinal cord injury. Life Sciences. 2012;**91**:77-82. DOI: 10.1016/j.lfs.2012.06.022

[87] Yan L, Li X, Wang J, Sun Y, Wang D, Gu J, He J, Hu H, Chen G, Wang Q, Feng X. Immunomodulatory effectiveness of tacrolimus in preventing epidural scar adhesion after laminectomy in rat model. European Journal of Pharmacology. 2013;**699**:194-199. DOI: 10.1016/j.ejphar.2012.11.037

[88] Bavetta S, Hamlyn PJ, Burnstock G, Lieberman AR, Anderson PN. The effects of FK506 on dorsal column axons following spinal cord injury in adult rats: Neuroprotection and local regeneration. Experimental Neurology.1999;**158**:382–393. DOI: 10.1006/exnr.1999.7119.

[89] Arun A, Abt NB, Tuffaha S, Brandacher G, Leto Barone AA. Nerve regeneration in vascularized composite allotransplantation: Current strategies and future directions. Plastic and Aesthetic Research. 2015;**2**:226-235. DOI:10.4103/2347-9264.158853.

[90] Yan Y, Sun HH, Hunter DA, Mackinnon SE, Johnson PJ. Effect of short-term FK506 administration on accelerating nerve regeneration. Neurorehabilitation and Neural Repair. 2012;**26**: 570-580. DOI: 10.1177/1545968311431965

[91] Azizi S, Mohammadi R, Amini K, Fallah R. Effects of topically administered FK506 on sciatic nerve regeneration and reinnervation after vein graft repair of short nerve gaps. Neurosurgical Focus. 2012, **32**:E5-10. DOI: 10.3171/2012.1.FOCUS11320.

[92] Dawson TM, Steiner JP, Dawson VL, Dinerman JL, Uhl GR, Synder SH. Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthetase and protects against glutamate neurotoxicity. Proceedings of the National Academy of Sciences of the United States. 1993;**90**: 9808-9812

[93] Madsen JR, MacDonald P, Irwin N, Goldberg DE, Yao GL, Meiri KF, Rimm IJ, Stieg PE. Tacrolimus (FK506) increase neuronal expression of GAP-43 and improves functional recovery after spinal cord injury in rats. Experimental Neurology. 1998;**154**: 673-683. DOI: 10.1006/exnr.1998.6974

[94] Que J, Cao Q, Sui T, Du S, Kong D, Cao X. Effect of FK506 in reducing scar formation by inducing fibroblast apoptosis after sciatic nerve injury in rats. Cell Death & Disease. 2013;**4**:e526-e510. DOI: 10.1038/cddis.2013.56

[95] Behram Kandemir Y, Sarikcioglu L. Immunophilin ligands in peripheral nerve regeneration. Folia Morphol (Warsz). 2015;**74**:407-413. DOI: 10.5603/FM.2015.0100

[96] Shahraki M, Mohammadi R, Najafpour A. Influence of tacrolimus (FK506) on nerve regeneration using allografts: A rat sciatic nerve model. Journal of Oral and Maxillofacial Surgery. 2015;**73**:1438 e1–1438 e 9. DOI: 10.1016/j.joms.2015.03.032

[97] Fansa H, Keilhoff G, Altmann S, Plogmeier K, Wolf G, Schneider W. The effect of the immunosuppressant FK 506 on peripheral nerve regeneration following nerve grafting. Journal of Hand Surgery British. 1999;**24**:38-42

[98] Mekaj AY, Morina AA, Manxhuka-Kerliu S, Neziri B, Duci SB, Kukaj V, Miftari I. Electrophysiological and functional evaluation of peroneal nerve regeneration in rabbit following topical hyaluronic acid or tacrolimus application after nerve repair. Nigerian Postgraduate Medical Journal. 2015;**22**:179–184. DOI: 10.4103/1117-1936.170738

[99] Mekaj AY, Morina AA, Lajqi S, Manxhuka-Kerliu S, Kelmendi FM, Duci SB. Biomechanical properties of the sciatic nerve following repair: Effects of topical application of hyaluronic acid or tacrolimus. International Journal of Clinical and Experimental Medicine. 2015;**8**:20218–20226

[100] Watts R, Clunie G, Hall F, Marshall T. Rheumatology.1 edition. New York, USA. Oxford University Press: 2009. p. 558. ISBN 9780199229994.

[101] Borel JF, Feurer C, Gubler HU, Stahelin H. Biological effects of cyclosporin A: A new antilymphocytic agent. Agents Actions. 1976;**6**:468–475

[102] Çetinalp NE, Albayrak SB, Ismailoğlu Ö, Temiz NÇ, Solmaz İ, Tanriöver G, Demir N: Topical Application of Cyclosporine Reduces Epineurial Fibrosis: Gross Postsurgical, Histopathological and Ultrastructural Analysis in a Rat Sciatic Nerve Model. Turk Neurosurg. 2016 [Epub ahead of print]. DOI: 10.5137/1019-5149.JTN.17871-16.1.

[103] Meirer R1, Babuccu O, Unsal M, Nair DR, Gurunluoglu R, Skugor B, Meirer B, Siemionow M. Effect of chronic cyclosporine administration on peripheral nerve regeneration: A dose–response study. Annals of Plastic Surgery. 2002;**49**:96-103

[104] Erkutlu I, Alptekin M, Geyik S, Geyik AM, Gezgin I, Gök A. Early cyclosporin A treatment retards axonal degeneration in an experimental peripheral nerve injection injury model. Neural Regeneration Research. 2015;**10**:266-270. DOI: 10.4103/1673-5374.152381

[105] Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. Immunopharmacology. 2000;**47**:119-125

[106] Sun L, Youn HD, Loh C, Stolow M, He W, Liu JO. Cabin 1, a negative regulator for calcineurin signaling in T lymphocytes. Immunity. 1998;**8**:703-711

[107] Bain JR, Mackinnon SE, Hudson AR, Falk RE, Falk JA, Hunter DA, Makino A. Preliminary report of peripheral nerve allografting in primates immunosuppressed with cyclosporine. Transplantation Proceedings. 1989;**21**:3176-3177

[108] Muramatsu K, Doi K, Kawai S. Nerve regenerating effect of short-course administration of cyclosporine after fresh peripheral nerve allotransplantation in the rat: Comparison of nerve regeneration using different forms of donor nerve allografts. Microsurgery. 1995;**16**:496-504

[109] Hellenbrand DJ, Kaeppler KE, Ehlers ME, Thompson CD, Zurko JC, Buchholz MM, Springer AR, Thompson DL, Ibrahim RK, Hanna A. Immunohistochemical assessment of rat nerve isografts and immunosuppressed allografts. Neurology Research. 2016;**38**:1094-1101

[110] Mohammadi R, Shariati H, Sharghi N, Naghipour M. Peripheral nerve regeneration using silicone rubber chamber combined by local administration of cyclosporin a in streptozotocin induced diabetic rats. International Journal of Oral and Craniofacial Science. 2015;**1**:019-026

[111] Wolrath L. Functional anatomy of the human pineal gland. In: Reiter RJ, editor. The Pineal Gland. New York: Raven Press; 1984. pp. 285-322

[112] Kaya Y, Sarıkcıoğlu L, Aslan M, Kencebay C, Demir N, Derin N, Angelov DN, Yıldırım FB. Comparison of the beneficial effect of melatonin on recovery after cut and crush sciatic nerve injury: A combined study using functional, electrophysiological, biochemical, and electron microscopic analyses. Child's Nervous System. 2013;**29**:389-401. DOI: 10.1007/s00381-012-1936-0

[113] Chang HM, Huang YL, Lan CT, Wu UI, Hu ME, Youn SC. Melatonin preserves superoxide dismutase activity in hypoglossal motoneurons of adult rats following peripheral nerve injury. Journal of Pineal Research. 2008:**44**:172-180. DOI: 10.1111/j.1600-079X.2007.00505.x

[114] Hardeland R, Coto-Montes A, Poeggeler B. Circadian rhythms, oxidative stress, and antioxidative defense mechanisms. Chronobiology International. 2003;**20**:921-962

[115] Chang HM, Ling EA, Lue JH, Wen CY, Shieh JY. Melatonin attenuates neuronal NADPH-d/NOS expression in the hypoglossal nucleus of adult rats following peripheral nerve injury. Brain Research. 2000;**873**:243-251

[116] Abdel-Wanis ME, Kawahara N. The role of neurofibromin and melatonin in pathogenesis of pseudoarthrosis after spinal fusion for neurofibromatous scoliosis. Medical Hypotheses. 2002;**58**:395-398. DOI: 10.1054/mehy.2001.1467

[117] Chang HM, Liu CH, Hsu WM, Chen LY, Wang HP, Wu TH, Chen KY, Ho WH, Liao WC. Proliferative effects of melatonin on Schwann cells: Implication for nerve regeneration following peripheral nerve injury. Journal of Pineal Research. 2014;**56**:322–332. DOI: 10.1111/jpi.12125

[118] Turgut M, Uyanikgil Y, Baka M, Tunç AT, Yavasoglu A, Yourtseven ME, Kaplan S. Pinealectomy exaggerates and melatonin treatment suppresses neuroma formation of transected sciatic nerve in rats. Journal of Pineal Research. 2005;**38**:284-291. DOI: 10.1111/j.1600-079X.2004.00205.x

[119] Kaya Y, Savas K, Sarikcioglu L, Yaras N, Angelov DN. Melatonin leads to axonal regeneration, reduction in oxidative stress, and improved functional recovery following sciatic nerve injury. Current Neurovascular Research. 2015;**12**:53-62

[120] Yanilmaz M, Akduman D, Sagun ÖF, Haksever M, Yazicilar O, Orhan I, Akpolat N, Gök U. The effects of aminoguanidine, methylprednisolone, and melatonin on nerve recovery in peripheral facial nerve neurorrhaphy. Journal of Craniofacial Surgery. 2015;**26**:667-672. DOI: 10.1097/SCS.0000000000001503

[121] Li H, Zhang L, Xu M. Dexamethasone prevents vascular damage in early-stage non-freezing cold injury of the sciatic nerve. Neural Regeneration Research. 2016;**11**:163-167. DOI: 10.4103/1673-5374.175064

[122] Regina G, Angiletta D, Impedovo G, Robertis GD, Fiorella M, Carratu MR. Dexamethasone minimizes the risk of cranial nerve injury during CEA. Journal of Vascular Surgery. 2009;**49**:99-102. DOI: 10.1016/j.jvs.2008.08.049

[123] Feng X, Yuan W. Dexamethasone enhanced functional recovery after sciatic nerve crush injury in rats. Biomed Research International. 2015:627923. DOI: 10.1155/2015/627923

[124] Hall ED. The neuroprotective pharmacology of methylprednisolone. Journal of Neurosurgery. 1992;**76**:13-22. DOI: 10.3171/jns.1992.76.1.0013

[125] Sadraie SH, Parivar K, Arabi F, Moattari M, Kaka G, Mansouri K. Study of transected sciatic nerve repair by amniotic membrane with betamethasone in adult albino wistar rats. Archives of Iranian Medicine. 2016;**19**:612-617. DOI: 0161909/AIM.003

[126] Li Q, Li T, Cao XC, Luo DQ, Lian KJ. Methylprednisolone microsphere sustained-release membrane inhibits scar formation at the site of peripheral nerve lesion. Neural Regeneration Research. 2016;**11**:835-841. DOI: 10.4103/1673-5374.182713.

[127] McDowell LR. Vitamins in Animal and Human Nutrition, Iowa State University Pres. USA; 2008.

[128] Goyal V. Vitamin B12—A brief overview. IAIM. 2015;**2**:211-216

[129] Wang SA, Yang J, Zhang GB, Feng YH, Wang F, Zhou PY. Effect of mecobalamin treatment on the recovery of patients with posterior communicating artery aneurysm inducing oculomotor nerve palsy after operation. European Review for Medical and Pharmacological Sciences. 2015;**19**:2603-2607

[130] Jolivalt CG, Mizisin LM, Nelson A, Cunha JM, Ramos KM, Bonke D, Calcutt NA. B vitamins alleviate indices of neuropathic pain in diabetic rats. European Journal of Pharmacology. 2009;**612**:41-47. DOI: 10.1016/j.ejphar.2009.04.028

[131] Yamada K. Chapter 9. Cobalt: Its role in health and disease. In: Sigel A, Sigel H, Sigel Roland KO, editors. Interrelations Between Essential Metal Ions and Human Diseases.Metal Ions in Life Sciences Netherlands: Springer; 2013 pp. 295-320. DOI: 10.1007/978-94-007-7500-8_9

[132] Lopatina T, Kalinina N, Karagyaur M, Stambolsky D, Rubina K, Revischin A, Pavlova G, Parfyonova Y, Tkachuk V. Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth de novo. PLoS One. 2011;**6**:e17899. DOI: 10.1371/journal.pone.0017899

[133] Doble A. The pharmacology and mechanism of action of riluzole. Neurology. 1996;**47**(6 Suppl 4):S233-S241

[134] Wu Y, Satkunendrarajah K, Teng Y, Chow DS, Buttigieg J, Fehlings MG. Delayed post-injury administration of riluzole is neuroprotective in a preclinical rodent model of cervical spinal cord injury. Journal of Neurotrauma. 2013;**30**:441-452. DOI: 10.1089/neu.2012.2622.

[135] Satkunendrarajah K, Nassiri F, Karadimas SK, Lip A, Yao G, Fehlings MG. Riluzole promotes motor and respiratory recovery associated with enhanced neuronal survival and function following high cervical spinal hemisection. Experimental Neurology. 2016;**276**:59-71. DOI: 10.1016/j.expneurol.2015.09.011

[136] Fehlings MG, Wilson JR, Karadimas SK, Arnold PM, Kopjar B. Clinical evaluation of a neuroprotective drug in patients with cervical spondylotic myelopathy undergoing surgical treatment: Design and rationale for the CSM-protect trial. Spine (Philadelphia Pa 1976). 2013;**38**:S68-S75. DOI: 10.1097/BRS.0b013e3182a7e9b0.

[137] Shortland PJ, Leinster VH, White W, Robson LG. Riluzole promotes cell survival and neurite outgrowth in rat sensory neurones *in vitro*. European Journal of Neuroscience. 2006;**24**:3343-3353. DOI: 10.1111/j.1460-9568.2006.05218.x

[138] Nicholson KJ, Zhang S, Gilliland TM, Winkelstein BA. Riluzole effects on behavioral sensitivity and the development of axonal damage and spinal modifications that occur after painful nerve root compression. Journal of Neurosurgery-Spine. 2014;**20**:751-762. DOI: 10.3171/2014.2.SPINE13672

[139] Solari A, Uitdehaag B, Giuliani G, Pucci E, Taus C. Aminopyridines for symptomatic treatment in multiple sclerosis. Cochrane Database of Systematic Reviews. 2001;(4):CD001330. DOI: 10.1002/14651858.CD001330.

[140] Smith C, Kongsamut S, Wang H, Ji J, Kang J, Rampe D. *In Vitro* electrophysiological activity of nerispirdine, a novel 4-aminopyridine derivative. Clinical and Experimental Pharmacology and Physiology. 2009;**36**:1104-1109. DOI: 10.1111/j.1440-1681.2009.05200.x

[141] Hayes KC. The use of 4-aminopyridine (fampridine) in demyelinating disorders. CNS Drug Reviews. 2004;**10**:295-316

[142] Tseng KC, Li H, Clark A, Sundem L, Zuscik M, Noble M, Elfar J. 4-Aminopyridine promotes functional recovery and remyelination in acute peripheral nerve injury. EMBO Molecular Medicine. 2016;**8**:1409-1420. DOI: 10.15252/emmm.201506035

[143] Lundborg G. A 25-year perspective of peripheral nerve surgery. evolving neuroscientific concepts and clinical significance. Journal of Hand Surgery (American Volume). 2000;**25**:391-414. DOI: 10.1053/jhsu.2000.4165

[144] Lee RC, Ping JA. Calcium antagonists retard extracellular matrix production in connective tissue equivalent. Journal of Surgical Research. 1990;**49**:463-466

[145] Ikeda K, Yamauchi D, Osamura N, Hagiwara N, Tomita K. Hyaluronic acid prevents peripheral nerve adhesion. British Journal of Plastic Surgery. 2003;**56**:341-347

[146] Li X, Wang W, Wei G, Wang G, Zhang W, Ma X. Immunophilin FK506 loaded in chitosan guide promotes peripheralnerve regeneration. Biotechnology Letters. 2010;**32**: 1333-1337. DOI: 10.1007/s10529-010-0287-8