Melatonin and sciatic nerve injury repair: a current perspective

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Abstract: Peripheral nerve injury is an important clinical problem that can exert hazardous effects on the health of patients. For this reason, there are more studies conducted on the regeneration of the peripheral nerves via the usage of the nerves belonging to various animals with different types of lesions, ages, and by using different methods of assessment with regular follow-up. Contrary to data obtained through experimentation and clinical observation, no ideal way of treatment was found to increase the regeneration of the peripheral nerves. Finally, the effects of melatonin in the protection of peripheral nerves against trauma, especially the protection of sciatic nerve from pathological conditions, have come into attention in a wide group of scientists as there are beneficial effects of melatonin after surgery. While numerous studies indicate the melatonin's protective effects on the pathologies of nerves, there are also studies reporting its toxic effects on peripheral nerves. Melatonin is a widespread and crucial signaling molecule due to its features of free radical scavenging and anti-oxidation at both pharmacological and physiological conditions in vivo. In this context, although there are numerous studies elaborating the effects of melatonin in various tissues, its effects on peripheral nerves was documented in only a limited number of studies. The aim of this article was to perform a review of the knowledge in the literature on the subject of mostly beneficial or hazardous effects of melatonin on the repair of the damaged peripheral nerves.

Keywords: peripheral nerve injury, melatonin, regeneration, light and electron microscopy

What do we know about the peripheral nerve injury?

Peripheral nerve injury is an important clinical problem, which has only limited number of treatment options. It often occurs due to mechanical traumas and rarely as a result of surgical resection of tumors.¹ These injuries result in the damage or loss of musculoskeletal system, blood vessel, nerves, and basal membrane.^{2,3} Although some of the damage regenerates spontaneously, some severe traumatic damages result in the loss of motor, auditory, and autonomic functions.^{2,3}

Although there is no complete improvement in a majority of traumas, the amount of improvement may vary according to nerve type, grade of injury, and amount of fibrosis. Seddon⁶ and Sunderland⁵ demonstrated two basic classifications to scale the severity of the damage accepted today. Seddon⁶ classified the nerve injuries into three groups: neuropraxia, axonotmesis, and neurotmesis, and Sunderland⁵ further expanded the Seddon's classification⁶ to five degrees according to the damage grades of the tissues and the type of trauma. Sunderland's⁵ first- degree injury type

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is equivalent to Seddon's⁶ neuropraxia type injury, and only temporary transient loss of axonal integrity is not impaired. Sunderland's⁵ second-degree injury type is equivalent to Seddon's⁶ axonotmesis type injury. In this type of injury, there is no axonal integrity, but the endoneurium remains intact. Axonal regeneration is easy due to the presence of the endoneurium. Sunderland's⁵ third- degree injury type refers to injuries sustained in the axon, myelin sheath, and endoneurium but the perineum remains intact. Sunderland's⁵ fourth- degree injury type refers to injuries that occur in the axon, myelin sheath, endoneurium, and perineurium, and the nerve endurance is only provided by the epineurium. Spontaneous healing is not possible. Sunderland's⁵ fifth- degree injury type is equivalent to the neurotmesis type injury of Seddon's classification,⁶ which means that the nerve has been completely cut off and a complete functional loss has occurred. Spontaneous healing is not possible and surgical intervention is required.⁴⁻⁶

Current treatment modalities for peripheral nerve injuries are autologous nerve transplantation from sural, saphenous, or medial cutaneous nerves.⁷ It has been reported that the autologous nerve transplantation has fully recovered sensory restorations, whereas motor functions are recovered at a maximum of 40% level. Complications related to autograft include loss of function (motor and/or sensory), insufficient donor nerve tissue, and donor site morbidity.^{8,9} It is important to understand how nerve tissue repairs itself spontaneously, partly, to develop a repair strategy for greater damage.

Events after the injury

First studies on peripheral nerve damage are based on the experiments conducted by Augustus Waller in 1850 on the glossopharyngeal and hypoglossal nerves of frogs. Waller tried to observe the postdamage events on axons and tried to explain in his own words that a great number of small chambers were formed on the distal part of the peripheral nerve and Schwann cells lost their white substance (myelin sheaths).¹⁰ In this century, this process that we call "Wallerian degeneration" corresponds to inflammatory response that occurs in the peripheral nerve as a result of rupture or damage. A great number of genes and proteins (neurotrophic factors, cytokines, and cell adhesion molecules) take charge in all stages of Wallerian degeneration in a coordinated way.^{11,12} The inflammatory process after the peripheral nerve damage influences nerve repair in a positive way. Immunocytochemical analyses have confirmed that after the

sciatic nerve damage, T cells and macrophages migrate to the damage area in 2 days and they expand to the distal part in 4 days.¹¹⁻¹⁵ First, Wallerian degeneration starts with the destruction and degeneration of axoplasm and axolemma, and it is completed within 24 hours in small nerves and within 48 hours in greater nerves. After damage, intracellular and extracellular calcium concentration increase directly proportionally with the severity of damage.^{16,17} In *in vitro* study, increased calcium concentration has been shown to be a significant suppressive factor for the survival of Schwann cells.¹⁸ Axon destruction that starts with the flow of axonal protease and calcium continues with the help of various intrinsic factors.

As an early period response to axonal destruction, myelin protein synthesis is downregulated by Schwann cells.^{19,20} Immunohistochemical and *in situ* hybridization studies have shown that Schwann cells are the main source of early cytokine response.^{15,21,22} Later, in their study, Lin et al²³ have shown that inflammatory mediators such as TNF- α -IL-6, IL-1 β , MCP-1, Inos, and COX-2 are released not only from Schwann cells but also from glia cells, and after the nerve damage, nitric oxide (NO) inhibitors and anti-inflammatory cytokines save retinal ganglion cells from apoptosis by inhibiting microbial activation. With the discovery of Wallerian degeneration slow (WLDs) mutant mice in 1989, an important step has been taken to enlighten the nature of Wallerian degeneration.²⁴ While axon degeneration is delayed for longer than 2 weeks in WLDs mice, this process takes only 1.5 days in wild-type mice.²⁵ Studies on WLDs have shown that NMNAT and SARM-1 have significant roles in Wallerian degeneration.²⁶⁻²⁸

The pathological changes that occur in most peripheral neuropathies show a great deal of similarity. After removal of the above-mentioned myelin and axon residues in any active neuropathy, the loss of myelinated axons and increased endogenous collagen are observed.²⁹

Results of studies conducted in our laboratory show the remarkable decrease in myelin sheath thickness of axons and axon diameter (Figure 1).

In electron microscopic sections, newly formed myelinated and unmyelinated axons are noted in damaged nerves (Figure 2).

In the light of all this information, it is known that Wallerian degeneration is important for postdamage nerve repair and mediates protective response formation under damage. Axonal fragmentation, which initiates Wallerian

Figure 1 The light micrograph shows the Toluidine blue staining of the rat sciatic nerve in 500-nm resin-embedded sections.

Notes: (A) Normal peripheral nerve view with tightly packed nerves in normal axon diameter. (B) The view of the crushed nerve 30 days after injury. Abundant number of newly formed and small axons is observed in response to nerve damage. Wallerian degeneration and macrophage-mediated phagocytosis stages have been completed and nerve self-repair initiated. Arrow, myelinated axon; arrowhead, Schwann cell; asterisk, in some of the nerves, the myelin appears to consist of two separate rings. This is caused by the section passing through a Schmidt–Lanterman cleft.

Figure 2 The electron microscopic micrograph indicates the sciatic nerve distal to the nerve crush site from a crush-injured rat.

Notes: (A) Normal peripheral nerve. (B) The view of the crushed nerve 30 days after injury. Sections were stained with lead citrate and uranyl acetate. Arrow, myelinated axon; arrowhead, unmyelinated axon; asterisk, newly formed myelinated axons. Bar indicates 1 mm length.

degeneration, starts with the mediation of first calcium flow and then calpain activation.³⁰ After axonal fragmentation, inflammatory cells of the complement system, chemokines, cytokines and signaling pathways are activated. These factors participate in fragmentation and axonal regeneration, and there are a great number of studies conducted on the roles of these factors.^{31–42}

Knowing the order of the events that take place after damage and the effect mechanisms of the factors in charge and thus understanding Wallerian degeneration are important for clarifying the most suitable treatment methods.

Structure, synthesis, and characteristics of melatonin

Melatonin, also known as *N*-acetyl-5-methoxytryptamine, is synthesized by the pineal gland product, which has basic functions such as the regulation of immune system, regulation of pituitary hormones, and photoperiod adaptation.^{43,44}

Melatonin is not synthesized in the fetal brain and maternal melatonin enters the fetal circulation through placenta.⁴⁵ Tryptophan acts as the initiator of melatonin biosynthesis, and it is taken from the circulation and turned into serotonin. Serotonin is later turned into *N*-acetylserotonin through AANAT enzyme, and *N*-acetylserotonin is further metabolized to melatonin through HIOMT enzyme.⁴⁶

Melatonin given intravenously is first hydroxylated in the liver by cytochrome P450 monooxygenases, and then it is conjugated with sulfate to form 6-sulfatoxymelatonin.^{47,48} Melatonin is also metabolized to kinuramine derivatives with oxidative pyrrole ring separation.⁴⁹ Primary separation product is either arylamine formamidase or *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK) deformalized to *N*1-acetyl-5-methoxykynuramine by hemoperoxidase.^{50,51} Evidences have suggested that pyrrole ring separation contributes to about one third of total melatonin catabolism; however, this rate can be much higher in some tissues. AFMK has been put forward to be the primary and major active metabolite of melatonin.⁵¹

Although melatonin is primarily known as an indole synthesized from the pineal gland, other organs have the feature to synthesize melatonin, which has local influences. In addition to its transducer effect on circadian rhythm, its other functions have been clarified in the past decades such as direct free radical scavenging and the regulation of the genes of antioxidant enzymes.^{52–56} Similar to the studies on the antioxidant properties of melatonin, its cell protective effect and potential disease-preventing characteristics have also been studied frequently.^{57–61} Currently, the “oxidant scavenger” effect of melatonin on radical and nonradical agents has been proven.^{58–62}

In addition, current studies indicate that melatonin production can occur in several sites such as astrocytes, the neuron in the brain.⁶³ In this context, melatonin synthesis is correlated with mitochondrial alterations. Mitochondria and chloroplasts can be considered the primary site of melatonin production in some cells such as neuron and glia.^{63,64}

The promoting effect of melatonin on peripheral nerve injury: general overview

Peripheral nerve trauma is common and repair generally occurs with scar tissue formation around the injury area.⁶⁵ Scar tissue formation leads to a block in neural conduction by creating a mechanical barrier for axonal regeneration. If scar formation can be prevented, the development of axonal extensions and so the regeneration process will

accelerate.^{66,67} Some agents have been used for previous in vivo experimental studies for this purpose.⁶⁷⁻⁶⁹ Although many experimental and clinical studies were performed to obtain the outcomes of peripheral nerve injury, the methods for improving peripheral nerve damage are limited in the literature.⁷⁰⁻⁷³

Microsurgery procedure is of great importance in peripheral nerve regeneration. In addition, regeneration procedure also depends on many factors such as location of injury and duration of the regeneration.⁷⁴ Especially, current data obtained from experimental studies have shown that pharmacological agents has a role in the repair mechanism.⁷⁵⁻⁷⁷ In this context, it has been suggested that melatonin, hyaluronic acid, methylprednisolone, and tacrolimus (FK506) are among the most used topical agents to accelerate the repair process by suppression of fibroblast proliferation in the damaged area. Hence, scar formation reduces in the nerve injury.⁷⁵ Melatonin has many physiological roles in vivo such as pharmacological modulation of circadian rhythm, regulation of blood pressure, and free radicals scavenging as mentioned above. Especially, melatonin has an active role in healing nerve damage because of its broad-spectrum antioxidant properties and its ability to be a powerful inhibitor of apoptosis. Hence, it is an alternative agent to prevent the scar formation around the damage area.^{79,80} In the peripheral nervous system, melatonin is effective on axon sprouting after trauma.⁸¹ From another point of view, the regeneration process is associated with the balance between Schwann cell proliferation and scar tissue formation.^{82,83}

Several studies showed that melatonin supplement decrease the scar formation in the nerve injury through inhibiting the collagen production.^{73,78,81,84} Similarly, Shokouhi et al⁸⁵ reported that low-dose melatonin supplement could decrease the myelin damage and axonal alterations in the peripheral nerve. In addition, Stavisky et al⁸⁶ pointed out that melatonin also plays a major role in the healing of a highly injured sciatic nerve by plasmalemma fusion. While studies on the effect of melatonin on the regeneration often deal with oxidative stress mechanisms, the effects of melatonin can be elaborated with molecular researches.

Antioxidant effects of melatonin on regeneration: key points for oxidative stress mechanisms

The role of antioxidants in the posttraumatic recovery of the damage in the peripheral and central nervous systems cannot be denied. After a neuronal trauma, it is believed that the primary cause of peripheral nerve injury is lipid

peroxidation, which progresses along the nerve fibers and is induced by free radicals. Therefore, the primary target of antioxidants is thought to be the scavenging of free radicals in the repair of degeneration.^{87,88} Some researchers reported that melatonin, which is a pineal hormone, has an neuroprotective and free radical scavenging effect on the peripheral nervous system.^{76,77,86,89,90} Because of the lipid solubility of melatonin, it easily penetrates the cell membrane and organelles. In addition, an antioxidant can penetrate the mitochondria of the cell. In this respect, it can also protect the mitochondria from oxidative damage.⁹¹ Melatonin supplementation is an effective antioxidant in the development of the peripheral nervous system. Especially, it has been proven to have an important role in the inhibition of axonal sprouting and collagen accumulation.^{92,93} Qiu et al⁶⁵ suggested that oxidative stress and free radical-induced lipid peroxidation negatively affect the repair of peripheral nerve damage. After peripheral nerve damage, melatonin provides oxidative action by stimulating superoxide dismutase enzyme, an important antioxidative enzyme. Superoxide dismutase enzyme acts as a redox regulator.⁹⁴ Melatonin stimulates antioxidant enzymes by inhibiting the posttraumatic polymorphonuclear infiltration.^{95,96} In addition, melatonin can reduce oxidative damage by preventing the levels of catalase, peroxidase, and ascorbate peroxidase such as superoxide dismutase (Figure 3).⁹⁷⁻⁹⁹

Nerve tissue does not contain highly oxidative defense mechanisms, and thus, lipid peroxidation negatively affects the integrity of the neuronal structure by disrupting membrane-binding receptors and enzymes. Melatonin can easily pass the physiological barriers in the lipid and aqueous environments.⁷⁹ Melatonin can enter into nucleus, and therefore, it protects DNA, intracellular proteins, and

Figure 3 The schema represents the receptor-dependent and receptor-independent effects of melatonin.

Notes: This figure shows the effect of melatonin and its metabolites on scavenging the reactive oxygen products through nonreceptor-independent actions.

Abbreviations: MT1, melatonin receptor 1; SOD, superoxide dismutase.

membranes lipids from oxidative stress.⁹¹ Melatonin reduces the oxidative stress by affecting NO and NO synthase after peripheral axotomy. It is known that melatonin exerts antioxidant effects by mimicking the effects of calcium channel blockers.⁹² It is believed that melatonin is an electron donor for the detoxification of free radicals. Thus, it protects the sciatic nerve from ischemia via reducing lipid peroxidation.^{100,101} In addition, according to the study by Atik et al,⁹² melatonin has a direct effect on the independent receptors of toxic radicals. Therefore, it has high potential affinity for peroxy and hydroxyl radicals via receptor for inactivation of free radicals. It has antioxidant properties with its affinity to receptors (Figure 4).¹⁰²

Melatonin has antioxidant, circadian rhythm regulator, and immunoregulator properties as well as anti-inflammatory properties.^{103–105} The anti-inflammatory effect of melatonin reduces the formation of free radicals accompanying inflammatory response.¹⁰⁵ Melatonin reduces inflammatory mediators and activates the antioxidant enzymes by signal transduction pathways. Especially, the nuclear factor 2 (Nrf2) expression has an important role in the activation of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, and it is believed that melatonin affects the antioxidant enzymes with Nrf2 signaling pathway (Figure 5).^{51,103,106} When the antioxidant effects of melatonin are examined in general, it can be seen that there is a fairly wide spectrum of antioxidants

Figure 4 Melatonin is an endogenously synthesized and secreted hormone by the pineal gland and possesses intense antioxidant activity.

including reduction of synthesis of adhesion molecules and pro-inflammatory cytokines.¹⁰⁷ Because melatonin has no pro-oxidative activity, the melatonin molecule is not easily oxidized, does not undergo autooxidation, and does not enter the hydroxyl radical-generating reactions in the redox cycle. More importantly, unlike other antioxidants, melatonin does not show toxic effects at very high doses (300 mg/day) and even for as long as 5 years.¹⁰⁸

Melatonin and Schwann cells: extracellular signal-regulated kinase (ERK) pathway

After injury, a number of endogenous factors have been identified that are effective in maintaining the vitality of axons and axonal growth.^{109,110} Schwann cells play a key role in peripheral nerve regeneration by regulating axonal proliferation.¹¹¹ In addition, Schwann cells secrete various neurotrophic factors such as bFGF and nerve growth factor, which play a major role in regeneration of peripheral nervous system and development.^{112,113} In addition, bFGF and TGF- β are important for Schwann cell activity. Turgut et al¹¹⁴ studied the expression of these growth factors, which have important role in the control of neuroma formation and collagen accumulation in rats that underwent pinealectomy. Also this study showed that melatonin supplementation suppressed proximal neuroma and contributed to repair mechanism.

Metallothionein 1 melatonin receptor, which depends on the phosphorylation of ERK1/2, plays a role in the proliferative effect of melatonin on Schwann cells (Figure 6).^{115–117} Harrisingh et al¹¹⁸ suggested that Schwann cells stimulated by “damage signals” could be considered as a regenerative cell type in response to peripheral nerve injury. In this context, Ras/Raf/mitogen-activated protein kinase/ERK signaling regulates the differentiation of Schwann cells.¹¹⁸ In addition, ERK/MAPK signaling activity stimulates Schwann cell differentiation. Syed et al¹¹⁹ studied the quantitative model of ERK/MAPK activity and found that lower levels induce myelination, whereas higher levels induce Schwann cell differentiation and proliferation. In addition, ERK/MAPK activity is needed at basal level for the differentiation of the progenitors.¹¹⁹ Similarly, Seo et al¹²⁰ suggested that an increase in the ERK1/2 activation promotes the Schwann cell proliferation, and thus, it is crucial in the repair of sciatic nerve. This point of view shows that exogenous melatonin administration can promote the Schwann cell proliferation and also increase the reinnervations.¹²¹

Figure 5 Schematic representation shows the role of NF-κB and Nrf2 pathways on the effect of melatonin on the injury.

Notes: Melatonin induces the antioxidant protection. ROS increases the NF-κB transcription and cytokines. In addition, relationship between inflammation and lipid peroxidation is explained in the schema and the role of them on apoptosis.

Abbreviations: IL, interleukin; NF-κB, nuclear factor-κB; Nrf2, nuclear factor 2; ROS, reactive oxygen species; TNF, tumor necrosis factor; ICAM, intercellular adhesion molecule; SOD, superoxide dismutase; GST, glutathione S-transferase.

Controversies on dose-dependent effects of melatonin on regeneration

After trauma, an increase in lipid peroxidation occurs. Significant increases in lipid peroxidation have been shown at 1st, 24th, and 48th hours in studies performed.¹²² It was determined that the rate of peroxidation returned to its preinjury level, and melatonin had maximal chronic neuroprotective effect after 48 hours.¹²³ In addition, it was suggested that melatonin is neuroprotective at doses of 1–50 mg/kg.^{85,114,122} Especially, Shokouhi et al⁸⁵ reported that melatonin at 50 mg/kg dose has a potentially positive effect on preserving the neural fibers. Similarly, Kaya et al^{124,125} studied the effect of melatonin at 50 mg/kg dose on nerve injury.

In clinical studies, melatonin doses range from 0.1 to 2,000 mg.⁹² More than 0.5 mg of melatonin dosage shows pharmacologically therapeutic effect.^{126,127} Rogerio et al¹²⁸ investigated the effect of melatonin on motor neuron death in the spinal cord after sciatic nerve injury in their study and compared the doses of 1, 5, 10, and 50 mg/kg of melatonin for this purpose. They reported that 1–50 mg/kg melatonin is effective in decreasing the neuronal death. In addition, the neuroprotective effect is fully active even at the lowest dose,

although toxic effects may possibly occur at doses of 50–100 mg/kg.¹²⁸ In addition, Cunnane et al¹²⁹ previously studied low doses (30 µg/100 g) of melatonin after the pinealectomy process. They reported that melatonin reduced the collagen in the injury area.¹²⁹ However, Atik et al⁹² suggested that the physiological dose of melatonin could not be sufficient for its beneficial effects. Melatonin shows the receptor-mediated activity at physiological doses. However, pharmacological concentrations of melatonin are required for the receptor-independent activity.¹³⁰ Turgut et al¹³¹ reported the positive effect of melatonin on reducing the neuroma formation in the sciatic nerve injury by enhancing axonal regeneration.

Shokouhi et al⁸⁵ compared the effects of low (10 mg/kg) and high dose (50 mg/kg) of melatonin on lipid peroxidation in the experimental sciatic nerve injury. In this study, they aimed to assess the dose-dependent neuroprotective and antioxidant activity of melatonin on injury. They found the beneficial effects of high-dose melatonin on axonal damage compared to that of low-dose melatonin.⁸⁵ Contrarily, Gul et al¹³² reported no dose-dependent effect of melatonin in the decreasing of lipid peroxidation in the sciatic nerve-injured rat after spinal cord clamping. Rogerio et al¹²⁸ investigated the effect of melatonin at doses of 1, 5, 10, and 50 mg/kg on

Figure 6 Schematic representation indicates the role of ErbB2 receptor on inducing the Schwann cell proliferation through Ras/Raf/MEK/ERK pathway.
Abbreviations: ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase.

sciatic nerve injury and observed the significant decrease in motor neuron death in the melatonin-treated groups. According to this study, administration of the low dose of melatonin is more effective for the neuronal survival rather than a high concentration of melatonin.¹²⁸ At this point, it can be said that the high dose of melatonin causes the toxic effect. Another study conducted by Chang et al¹⁰⁰ suggested that melatonin decreased the expression of NO synthase in the hypoglossal nerve injury and a dose-dependent neuroprotective effect of it is due to antioxidant properties.

It is important to determine the minimum dose of melatonin in peripheral nerve regeneration. Ulugol et al¹³³ demonstrated that high dose of melatonin prevented thermal hyperalgesia caused by nerve damage. In mice, intracerebroventricular and intraperitoneal administration of melatonin prevented the hyperalgesia by L-arginine–NO and opioidergic pathways in the neuropathic pain. However, it had no effect on the mechanical allodynia.¹³³ Opioidergic and gamma-aminobutyric acid ergic systems have an important role in the effects of melatonin.^{134,135} Similarly, Mantovani et al¹³⁵

showed the antidepressant effect of melatonin in the mouse through L-arginine–NO pathway and *N*-methyl D-aspartate receptors. Also it can be concluded that useful doses of melatonin contribute to the repair of peripheral nerve injury by attenuating the oxidative stress mechanisms.

Comparison of beneficial effects of melatonin and some agents on nerve repair: summary of current studies

In recent years, several experimental studies focusing on the effect of melatonin on peripheral nerve injury have been performed. In addition, many studies have compared the regenerative effect of melatonin with other antioxidants and have introduced new combinations of treatments. The formation of collagen scar tissue in peripheral nerve injury is a clinically important problem. Scar formation blocks the axons from sprouting into the appropriate distal fascicles, thus delaying the regeneration process. Control of the collagen accumulation in the neuroma formation was experimented using various chemical and physiological methods, but a functional success could not be achieved.¹³¹ In another study by Turgut et al,⁸¹ the effect of exogenous melatonin administration on scar formation was examined in the damaged nerves. After pinealectomy, they observed fibroblast proliferation by using stereological techniques. In addition, after melatonin administration, regenerating axons were observed at the proximal nerve ends. They demonstrated the inhibitory effect of melatonin on collagen accumulation. Furthermore, they suggested that surgical pinealectomy causes an increase in the collagen accumulation. However, melatonin administration suppresses this accumulation after pinealectomy.⁸¹ The role of TGF- β and bFGF collagen production and thus scar formation is known. In this regard, another study by Turgut et al¹¹⁴ on pinealectomized rats revealed that expression of TGF- β 1 and bFGF was suppressed by melatonin using immunohistochemical methods. In a study of pinealectomized chickens, Turgut et al¹³⁶ also examined the neuroprotective effects of melatonin. Neonatal pinealectomized chickens suggested negative effects on sciatic nerves. They showed that melatonin has a regulatory effect on collagen content.¹³⁶

Stavisky et al⁸⁶ investigated the effects of cyclosporine A, glial-derived neurotrophic factor, and methylprednisolone, as well as the effects of melatonin on polyethylene glycol-induced repair in the sciatic damage. They measured conduction of compound action potentials in the lesion area

after polyethylene glycol fusion. In *in vivo* and *in vitro* preparations, a significant increase in the improvement for melatonin-administrated group was determined compared to control Krebs saline. They suggested that in combination with polyethylene glycol fusion, melatonin provides rapid repair of crush-type injuries of the spinal cord.⁸⁶ Similarly, Daglioglu et al¹³⁷ designed a study on beneficial effects of melatonin at appropriate doses on peripheral nerve injury. They suggested that melatonin administration could be successful in the treatment of peripheral nerve injury.¹³⁷ Moreover, Zencirci et al¹³⁸ examined the functional effects of melatonin on sciatic nerve crush injury. The researchers applied melatonin at doses of 5 and 20 mg/kg for 21 days. In this study, melatonin showed a positive effect on the sciatic function index. In addition, while melatonin increases the conduction velocities, it decreases latency according to the study by Zencirci et al,¹³⁸ and the regenerative effect of the melatonin has been shown to be dose independent. Possibly the application of melatonin at 5 and 20 mg/kg intraperitoneally may show similar antioxidant effect.¹³⁸ However, Atik et al⁹² suggested that beneficial effects of melatonin in nerve regeneration could be seen at physiological doses. Kaplan et al⁸³ studied the effects of intraperitoneal melatonin administration after intraoperative platelet gel on sciatic nerve regeneration. Platelet gel application had a positive effect on nerve regeneration, but platelet gel application did not show the same effect when combined with melatonin. Administration of melatonin alone or in combination with platelet gel did not show any positive effect on nerve regeneration. This failure of regeneration reveals that melatonin does not play a role in inhibiting collagen formation.⁸³ In other respects, 30 μ g/100 g melatonin was used in this study.⁸¹ This dose may be inadequate to show antioxidant activity of melatonin. However, Kaya et al⁷¹ suggested that administration of 50 mg/kg melatonin showed improvement effects on nerve injury reducing oxidative stress.

Yanilmaz et al⁷³ suggest that some agents may have a positive effect on recovery after nerve palsy. In this respect, they examined the effect of melatonin, aminoguanidine, and methylprednisolone on facial nerve damage. They observed a decrease in the myelin debris of melatonin-administrated group. This study showed an increase in regeneration of the facial nerve in aminoguanidine- and melatonin-administrated groups; however, methylprednisolone was insufficient to prevent myelin degeneration. In particular, most efficient agent was aminoguanidine on reducing the collagen accumulation and preventing degeneration.⁷³ In this context, the appropriate dose may not have been administered for the neuroprotective

effect of melatonin on the facial nerve. Onger et al⁷⁶ investigated the effects of 50 mg/kg melatonin on sciatic nerve damage compared to the effects of acetyl-L-carnitine (50 mg/kg) and leptin (1 mg/kg). On the other hand, Onger et al⁷⁷ studied the peripheral nerve damage in the obese rats using the same antioxidant agents. According to their data, melatonin has no regenerative effect on obese rats. Contrarily, leptin and acetyl-L-carnitine could stimulate the myelination and regeneration in the sciatic nerve injury. Melatonin has anti-obesity effect. In this context, they claimed that melatonin led to decrease in the white adipose tissue mass and increase in the activity of brown adipose, thus energy requirement could not meet for regeneration. In addition, adequate stem cells cannot be obtained from the white fat tissue. However, acetyl-L-carnitine accelerates the pass of long-chain fatty acids through the inner membrane of mitochondria and has a function in the requiring neuronal energy after nerve injury.⁷⁷

In a recent study, Salehi et al¹³⁹ aimed to increase the efficiency of regeneration by allogenic Schwann cell transplantation using a biologic transporter. In this study, a conduit made of polyurethane and gelatin nanofibers was prepared and filled in with platelet-rich plasma and melatonin. They observed that this conduit enhanced the regeneration of the damaged site. However, functional success has not been achieved in this method as far as autograft is applied.¹³⁹

As a result, studies in recent years have focused on introducing new methods, including the local application of antioxidants, rather than the systemic application of them to accelerate the regeneration process in nerve crush injury.

Conclusion

Peripheral nerve injuries are still among the vital clinical issues, and thus, the research for advanced knowledge about this condition and its treatment still continues.⁹² Regarding this, some studies demonstrate that pineal neurohormone melatonin has effects on the physiological and histological properties of the nerve tissue, hinting at its effects of antioxidant, analgesia, and free radical scavenging in the degenerative peripheral nerve disorders. It is acknowledged that melatonin has beneficial effects on the length of the axons, the sprouting after the damage to peripheral nerves.^{93,96,140} Nevertheless, some studies indicate the hazardous effects of melatonin on peripheral nerves.^{141,142} To shed light on the beneficial or detrimental effects of melatonin treatment in low and high doses, further clinical and experimental studies should be performed.

Disclosure

The authors report no conflicts of interest in this work.

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