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Melatonin and its therapeutic actions on peripheral nerve regeneration

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Melatonin has many different roles in the human body, including its importance in circadian rhythms, sleep physiology, mental status, reproduction, tumour development, ageing, and many other physiologic processes. Although there are more than hundreds of studies on effects of melatonin in several tissues, its effects on peripheral nerve has been documented in a limited number of studies. This paper focused to review the available literature in terms of several actions and effects of melatonin (beneficial or toxic effects) on well-known peripheral nerve injury models. (Folia Morphol 2015; 74, 3: 283–289)

Key words: melatonin, peripheral nerve, traumatic injuries, neuroma formation

MELATONIN AND ITS PHYSIOLOGIC ACTIONS

Historical background

Melatonin (also known as N-acetyl-5-methoxytryptamine) (Fig. 1) is a hormone and is first identified in bovine pineal extracts. This hormone lightens the colour of frog melanocytes by its ability to aggregate melanin granules either after injection into the frog or after *in vitro* incubation with pieces of frog skin [5, 27]. Its melanocyte-lightening property is more than ten thousand times greater than that of noradrenalin [27].

Melatonin was first defined as the hormone that mediates the annual fluctuations in reproductive competence in seasonally breeding animals, as well as influencing numerous aspects of circadian biology [39].

Biosynthesis of melatonin

Melatonin is a highly lipophilic molecule, as well as having hydrophilic properties; rather than being stored in a secretory organ, upon biosynthesis it is released into bloodstream through capillaries [29]. Tryptophan plays a key role in biosynthesis of melatonin. Tryptophan is first converted by tryptophan hydroxylase to 5-hydroxytryptophan, which is decarboxylated to serotonin. Two enzymes (serotonin N-acetyltransferase and hydroxyindole O-methyltransferase) that are largely confined to the pineal gland catalyse the synthesis of melatonin from serotonin [5]. As the synthesis of melatonin increases, the hormone enters the bloodstream through passive diffusion. Melatonin synthesis and release are stimulated by darkness and inhibited by light (Fig. 1). In humans, melatonin secretion increases soon after the onset of darkness, peaks in the middle of the night (between 2 and 4 a.m.), and gradually falls during the second half of the night. Serum melatonin concentrations vary considerably according to age [5].

Physiologic effects of melatonin

Both *in vitro* [36, 37, 40] and *in vivo* studies [53] have shown that the neurohormone melatonin is a more potent free radical scavenger of the highly toxic hydroxyl
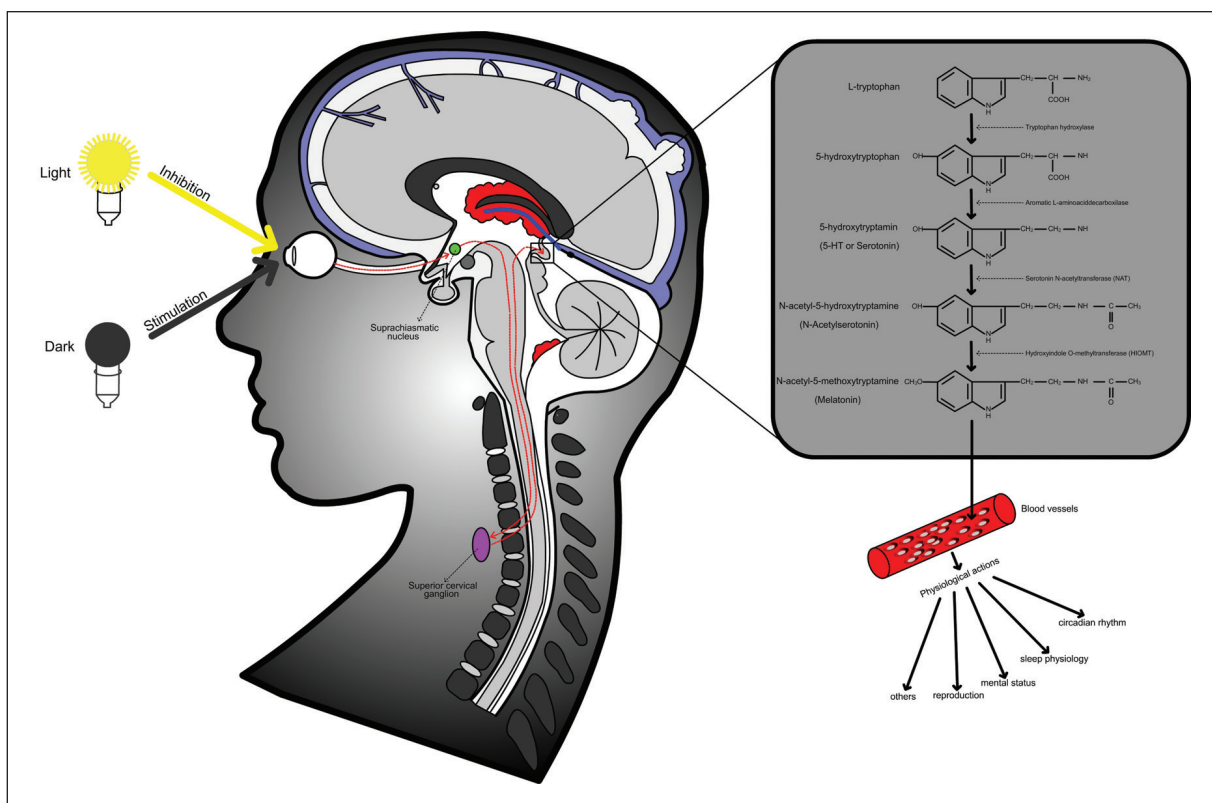
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Figure 1. Biosynthesis of melatonin.

and peroxyl radicals than some other known compounds [39]. Melatonin seems to be more effective than other known antioxidants (e.g., mannitol, glutathione, and vitamin E) in protecting against oxidative damage [5]. Therefore, melatonin may provide protection against oxidative damage in some diseases that cause degenerative or proliferative changes. However, these antioxidant effects require concentrations of melatonin that are much higher than peak nighttime serum concentrations [5]. Damage of the peripheral nerve also causes the production of free oxygen radicals [2]. When damage occurs to a peripheral nerve, both ischaemic and inflammatory processes begin. As a consequence of this process, free oxygen radicals and many toxic agents accumulate around the site of injury [2].

The neurohormone melatonin is a powerful antioxidant *in vivo* [39]. It prevents the toxicity of singlet oxygen and stimulates the antioxidative enzyme, glutathione peroxidase [39]. Glutathione peroxidase and, as a consequence, glutathione reductase activities follow the rhythm of melatonin. Involvement of melatonin in the control of redox processes has also been addressed by its high-affinity binding to cytosolic quinone reductase 2, previously believed to be a melatonin receptor [18].

Considering varied and potent antioxidant capability of melatonin, it has been reported that melatonin is an essential element of the antioxidant defence system of organisms [39]. Direct actions of melatonin in oxidative status may have important clinical implications considering the harmful role of free radicals in many disease processes [39].

Melatonin has many different roles in the human body, including its importance in circadian rhythms, sleep physiology, mental status, reproduction, tumour development, ageing, and many other physiologic processes [2]. Besides its well-known regulatory role on circadian rhythm, melatonin has other biological functions and a distinct metabolism in various cell types and peripheral tissues [28].

MELATONIN IN TRAUMATIC INJURIES OF PERIPHERAL NERVE

Effect of melatonin treatment to reduce scar formation

Advances in microsurgical instrumentation and great progress in nerve repair have provided the technical impetus for nerve reconstruction. However, the clinical

results for nerve repair are usually disappointing, and functional recovery is often suboptimal after peripheral nerve injury. Scar formation is a challenge for peripheral nerve surgeon. Traditionally, surgeons attempt to control collagen scar formation by close approximation of corresponding fascicles of the proximal to distal stumps [14, 19]. Although excellent coaptation has been achieved in matching proximal and distal nerve stumps and proper guiding axon regeneration toward the original target tissues, full recovery of nerve function does not guarantee in every patients [11].

A neuroma is a collection of disorganised nerve fibres sprouted from proximal stump of the injured nerve emanating from an interruption of axonal continuity. Excessive collagen formation can create a mechanical barrier to axonal regeneration, and the resulting scar formation can hinder axonal regrowth [21, 24, 31]. A variety of techniques have been proposed to decrease the neuroma formation characterised by accumulation of connective tissue at the lesion site [15, 19, 31].

It has been reported that recovery of peripheral nerve depends on a balance between Schwann cell regeneration and scar tissue formation [10, 21]. Previous studies have shown that exogenous melatonin administration can reduce scar formation in nerve stump and can prevent collagen production in the granulation tissue in the peripheral nerve of the pinealectomised animals [12, 33, 56].

Weichselbaum et al. [60] demonstrated that in pinealectomised rats, wound healing is slower than in controls, and this effect can be reversed by treatment with melatonin. Cunnane et al. [8] and Drobnik and Dabrowski. [12] reported that tissue collagen content in pinealectomised rats was found to be elevated due to low melatonin level. A variety of growth factors may play role in collagen synthesis. The immunohistochemical profile of transforming growth factor (TGF-β1) and basic fibroblast growth factor (bFGF) in the lesion site of the sciatic nerve, after pinealectomy and exogenous melatonin administration, was investigated by Turgut et al. [55]. They reported that TGF-β1 and bFGF immunoreactivity was most intense in the epineurium in animals that had undergone pinealectomy, while negative or weakly positive immunoreactivity was observed in animals in control and melatonin-treated groups [55].

Proliferative effects of melatonin on Schwann cells

A recent study demonstrated that melatonin significantly promoted Schwann cell proliferation in both *in vitro* and *in vivo* conditions following peripheral nerve injury and improved nerve regeneration [7]. The increased expression of Schwann cells induced by melatonin was clearly expressed by cell counting and the proliferative assay in spontaneous immortalised rat Schwann cell line derived from long-term culture of rat primary Schwann cells (RSC 96 cells) as well as by quantitative bromodeoxyuridine (BrdU) immunostaining and S-100 immunoexpressions in the lesioned nerves [7].

The proliferative effect of melatonin on Schwann cells has further been demonstrated to be mediated by MT1 receptor-dependent phosphorylation of extracellular signal-regulated kinases (ERK1/2) pathway [7, 28]. Increased ERK1/2 activation would transmit signals into perikarya of the nerve for transcriptional regulation of genes related to Schwann cell differentiation and proliferation [54, 62]. It has been suggested that ERK activation is important in the establishment of a regeneration-promoting extracellular environment in proximal and distal stumps of transected nerves [1, 50]. Seo et al. [49] also reported that increased ERK1/2 activation would contribute to Schwann cell proliferation and play an important role in sciatic nerve regeneration.

Effect of melatonin on re-innervation of the motor end plates

It is believed that the major reason for the poor functional recovery after a peripheral nerve lesion is collateral branching and regrowth of axons to incorrect muscles. It has been suggested that correct re-innervation of the neuromuscular junctions, rather than collateral axonal branching at the lesion site, may be the critical limiting factor for recovery of function after peripheral nerve injury [17]. Chang et al. [7] recently demonstrated that re-innervated motor end plates (as labelled by PGP 9.5 and a-bungarotoxin) following peripheral nerve injury was much higher in the animals receiving melatonin treatment when compared to that of saline-treated ones.

Effect of melatonin treatment on apoptosis and neuronal cell death

Melatonin is able to reduce not only apoptotic cell death in the central nervous system [38] but also the death of rat motoneurons induced by sciatic nerve transection in neonatal rats [44]. Rogerio et al. [44] reported a significant decrease in motor neuron death in the neonatal spinal cord after administration of

melatonin at doses of 1, 5, 10, and 50 mg/kg before and at 7 time intervals after sciatic nerve cut injury. In their study, neuronal survival rate was higher in those animals treated with lower doses of melatonin rather than higher doses.

Therapeutic dose of melatonin

In human studies, delivered doses of melatonin varied in a very large range, from 0.1 mg to 2000 mg [2]. After intravenous administration melatonin is rapidly distributed and eliminated [20], while bioavailability of orally administered melatonin varies widely [5]. Melatonin dosages over 0.5 mg are accepted as pharmacologically therapeutic [5, 45].

It has been reported that plasma melatonin levels reached a steady-state after 60 min and 120 min after melatonin administration [30]. In another study, the maximal mean serum melatonin value reached 20 min after a single subcutaneous injection of melatonin in hamsters [57]. Moreover, peak serum melatonin levels were observed after 60–150 min and remained stable for approximately 90 min after administration of crystalline melatonin to 5 young male volunteers [59].

Because there is no consensus on the therapeutic dose of melatonin, different doses have been frequently used in animal studies. Shokouhi et al. [51] investigated the neuroprotective effects of melatonin on neural fibre damage and lipid peroxidation after a blunt sciatic nerve trauma, comparing the effects of low dose (10 mg/kg) and high dose (50 mg/kg) of melatonin. According to their results, low-dose melatonin (10 mg/kg) reduced trauma-induced myelin breakdown and axonal changes in the sciatic nerve. However, high-dose melatonin (50 mg/kg) almost entirely neutralised any ultrastructural changes. Therefore, they suggested that melatonin has a potent neuroprotective effect at a dose of 50 mg/kg and it can preserve peripheral neural fibres from lipid peroxidative damage after blunt sciatic nerve trauma [51]. Peripheral nerve functions in the ovariectomised rats receiving melatonin (5 or 20 mg/kg) daily for either 2 or 6 weeks have also been examined [13].

Rogerio et al. [44] compared 1, 5, 10, and 50 mg/kg melatonin doses on motor neuron death in the neonatal spinal cord after sciatic nerve cut injury. Very low doses of melatonin have also been studied. Cunnane et al. [8] administered melatonin (30 μg/100 g body weight) after surgical pinealectomy procedure. They found low collagen content in the lesion site, demonstrating the opposite effect of melatonin application on elevated collagen level caused by pineal gland removal [12]. Atik et al. [2] reported that beneficial effects of melatonin cannot be achieved at physiologic doses. Therefore they proposed higher doses and a longer duration of administration should be planned in experimental paradigms [2]. In line of Atik et al. [2], a potent neuroprotective effect to preserve peripheral neural fibres from lipid peroxidative damage after blunt trauma achieved by Shokouhi et al. [51] especially at a dose of 50 mg/kg. In our previous studies we also administered 50 mg/kg melatonin after sciatic nerve injury [22, 23].

Effect of disruption of melatonin treatment

Melatonin concentrations in the body are typically lower during the day and reach to maximal levels at night in the dark [59]. In a recent study, we studied the effect of exogenous melatonin treatment on cut or crush injuries of the sciatic nerve and demonstrated that melatonin treatment had significant beneficial effect on sciatic nerve injury [22]. This treatment was performed in the light period and its effects were studied on cut and crush injury models of the sciatic nerve. In another recent study we re-addressed the beneficial effect of melatonin treatment and tried to improve this beneficial effect on cut injury by disruption of the circadian rhythm induced by light-at-night [23]. Although we used the same dose of melatonin as used in previous study [22] and found beneficial effect on sciatic nerve injury evaluated by functional, ultrastructural, biochemical tests; circadian rhythm disruption induced by light-at-night surprisingly did not demonstrate the same effect as expected [23]. We think that further studies need to be conducted to elucidate mechanism of this unexpected effect of melatonin in disrupted circadian rhythm.

Combination of melatonin with other substances

Kaplan et al. [21] studied postoperative intraperitoneal melatonin administration after intraoperative autologous platelet gel application in the collagen nerve conduit. They found that combination of melatonin with autologous platelet gel enrichment and the use of melatonin alone did not improve nerve regeneration. In the light of this negative effect, they suggested that effects of melatonin on nerve regeneration may not be because of the inhibition of collagen formation [21].

The effect of melatonin treatment in cut and crush injuries

The effect of the melatonin treatment has frequently been studied in neurotmesis (cut injury) model. However, in a previous study we studied its effect on axonotmesis (crush injury) and neurotmesis (cut injury) models of sciatic nerve [22]. We found that rats treated with melatonin demonstrated better structural preservation of the myelin sheaths compared to the vehicle-treated animals. The biochemical analysis also confirmed the beneficial effects of melatonin displaying lower lipid peroxidation and higher superoxide dismutase, catalase, and glutathione peroxidase activities in sciatic nerve samples in comparison to vehicle-treated animals.

MELATONIN IN OTHER TYPES OF PERIPHERAL NERVE INJURIES

Penetrating injury, crush, traction, ischaemia, and less common mechanisms such as thermal, electric shock, radiation, percussion, and vibration may be one of the aetiological factors of peripheral nerve injury [41, 42]. Ischaemia–reperfusion (I/R) is a problem that occurs in a variety of clinical conditions and leads to endoneurial oedema, segmental demyelisation, axonal degeneration, conduction failure, and multifocal or diffuse loss of nerve fibres [3, 32, 33]. Reperfusion after ischaemic insult of the tissue may elevate the production of various reactive oxygen species which damage a variety of molecules leading to cellular dysfunctions [16].

Several studies have investigated the effect of melatonin on I/R-injury. It has been well documented that melatonin provides protection against I/R injury of the liver [48], heart [25, 46], intestine [9], and brain [34, 52, 58]. However, effect of melatonin on I/R injury in the peripheral nerves has only been studied by Sayan et al. [47]. They demonstrated that melatonin treatment prevented increase in the malondialdehyde levels after I/R injury of the sciatic nerve. Melatonin also restrained an I/R induced decrease in the superoxide dismutase enzyme activity. Moreover, they found that pretreatment with melatonin resulted in fewer morphologic alterations in terms of the ultrastructural features of the sciatic nerve.

TOXICITY AFTER MELATONIN ADMINISTRATION

Although there are numerous studies that mention beneficial effects of melatonin treatment on peripheral nerve pathologies, there are some experimental and clinical studies that report a toxic effect of melatonin on peripheral nerves [33]. Lehman and Johnson [26] mentioned potential drug interaction of melatonin with Zoloft (an antidepressant drug also known as Sertraline). Combined use of melatonin, Zoloft, and a high-protein diet resulted in a melatonin//dopamine imbalance in the retina, manifesting as a toxic optic neuropathy [26].

Melatonin is known to interact with neuronal microtubules [6] and competes with microtubule--disruptive drugs like Vinblastine and Colchicine [4, 61]. Piezzi and Cavicchia [35] studied morphological changes in microtubules of toad sciatic nerve under melatonin administration and they found that the majority of microtubules of sciatic nerve of both control and melatonin-treated groups showed disintegration after 2 h of cooling. Thereafter, while microtubules were reformed in the control group nerves, the disorganisation of microtubules persisted in the sciatic nerves treated with melatonin when room temperature was restored (in toad sciatic nerve, microtubules are thermodependent). Their integrity, which is disrupted by low temperature, is restored when the nerves are placed at room temperature [43].

CONCLUSION AND FUTURE PERSPECTIVE

Beneficial effects of melatonin treatment on neuroma formation at the lesion site and on recovery of function in peripheral nerve seem a particularly attractive treatment option in clinic. Although there are few studies reporting toxic effect of melatonin administration, there are great numbers of studies on beneficial effects of melatonin on peripheral nerves. We think that further studies should be carried on peripheral nerves to clearly refine beneficial or toxic effects of melatonin administration in low, therapeutic and higher doses.

Although different agents have been studied on their effect on peripheral nerve regeneration, novel drugs for peripheral nerve injury will also be required. We think that detailed studies will clarify therapeutic effects of melatonin and the use of melatonin as a neuroprotective agent could be possible in the clinical practice

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