New Prophylactic and Therapeutic Strategies for Spinal Cord Injury

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Melatonin production by the pineal gland in the vertebrate brain has attracted much scientific attention. Pineal melatonin is regulated by photoperiodicity, whereas circadian secretion of melatonin produced in the gastrointestinal tract is regulated by food intake. Thus, the circadian rhythm of pineal melatonin depends upon whether a species is diurnal or nocturnal. Spinal cord injury (SCI) involves damage to the spinal cord caused by trauma or disease that results in compromise or loss of body function. Melatonin is the most efficient and commonly used pharmacological antioxidant treatment for SCI. Melatonin is an indolamine secreted by the pineal gland during the dark phase of the circadian cycle. Neurorehabilitation is a complex medical process that focuses on improving function and repairing damaged connections in the brain and nervous system following injury. Physical activity associated with an active lifestyle reduces the risk of obesity, cardiovascular disease, type 2 diabetes, osteoporosis, and depression and protects against neurological conditions, including Parkinson’s disease, Alzheimer’s disease, and ischemic stroke. Physical activity has been shown to increase the gene expression of several brain neurotrophins (brain-derived neurotrophic factor [BDNF], nerve growth factor, and galanin) and the production of mitochondrial uncoupling protein 2, which promotes neuronal survival, differentiation, and growth. In summary, melatonin is a neural protectant, and when combined with therapeutic exercise, the hormone prevents the progression of secondary neuronal degeneration in SCI. The present review briefly describes the pathophysiological mechanisms underlying SCI, focusing on therapeutic targets and combined melatonin and exercise therapy, which can attenuate secondary injury mechanisms with minimal side effects.

Key Words: Spinal cord injury, Melatonin, Exercise therapy, Secondary damage, Neuroprotectant

INTRODUCTION

Spinal cord injury (SCI) is a serious and devastating neurological disorder that may result in the loss of sensory and motor function and, depending on the extent of injury, may lead to paralysis and death [1,2]. The primary causes of SCI are disease (e.g. polio, spina bifida, Friedrich’s ataxia) or trauma, as may occur in motor vehicle accidents, falls, acts of violence, or sports; thus, the victims of SCI are often young adults. Furthermore, because SCI often results in permanent disability and poor quality of life, it represents an enormous financial burden on society that includes the cost of medical care and lost productivity.

Several therapeutic agents have been shown to be effective for SCI including antioxidants (e.g., 21-aminosteroids),
free radical scavengers (e.g., vitamin C, E), calcium channel blockers (e.g., nimodipine), sodium channel blockers, magnesium, NMDA/AMPA-kainate receptor antagonists, gangliosides, COX inhibitors, and neurotrophic factors [3-8]. Although these agents have been used in clinical trials, their neuroprotective capabilities are limited. The corticosteroid methylprednisolone has demonstrated significant neurological benefits in humans when given at high doses following acute SCI [9-12], and it is currently the only approved pharmacotherapy for SCI. However, treatment with methylprednisolone is controversial given its limited efficacy and potentially serious side effects, including immunosuppression and increased risk of infection (e.g., pneumonia, urinary tract infection, sepsis), hyperglycemia, adrenal insufficiency, and death [2,13,14]. Thus, a significant need exists for more effective and safer pharmacotherapies and a therapeutic strategy to improve functional recovery in patients with SCI. The present review briefly describes the pathophysiological mechanisms underlying SCI, focusing on therapeutic targets and combined melatonin and exercise therapy, which can attenuate secondary injury mechanisms with minimal side effects.

**POTENTIAL THERAPEUTIC TARGETS FOR SECONDARY DAMAGE IN SCI**

Two types of injury underlie SCI pathophysiology. Primary injury at the lesion site causes necrotic cell death within minutes to hours after the insult and is unlikely to be reversible despite therapeutic intervention [15]. Secondary injury develops over days or weeks in the rostral and caudal penumbra surrounding the original lesion. Secondary injury may be a prime target for therapeutic intervention because it involves a number of cellular and molecular events, such as oxidative stress, ischemia/reperfusion injury, phospholipase activity, intracellular Ca\(^{2+}\) influx, glutamate excitotoxicity, reactive oxygen species (ROS) production, inflammatory cell damage, apoptosis, and activation of multiple cell death proteases including calpains and caspasas [16-21]. Furthermore, these factors interact with one another. Given the wide range of mechanisms that can contribute to neuronal damage, a combination of multi-active drugs and a modified treatment regimen using two or more agents that target several pathways in SCI may be more effective for neurological recovery than any single treatment alone.

A recent review indicated that oxidative stress associated with secondary SCI caused DNA damage in the injured spinal cord [22]. Moreover, DNA single- and double-strand breaks have been detected in a variety of SCI injury models at acute and chronic time points [23-25]. Mounting evidence suggests that various neuroprotective agents contribute to direct or indirect prevention of DNA damage after SCI [26-29]. Huang et al. [24] demonstrated that administration of the omega-3 polyunsaturated fatty acid docosahexaenoic acid following compression SCI in rats reduced oxidative stress-associated changes, including lipid peroxidation, protein oxidation, and nucleic acid oxidation. Furthermore, injection of a monoclonal antibody against CD11d integrin, an integral protein for leukocyte adhesion, attenuated oxidative stress-related DNA oxidation and prevented infiltration of macrophages into the injured spinal cord after severe compression injury [30].

**MELATONIN AND EXERCISE AS A MULTIACTIVE NEUROPROTECTANT IN SCI**

Because multiple pathophysiological pathways are co-activated following SCI, a combination of pharmacological agents or one multiactive agent may be more effective than treatment using one pharmacological agent or a single therapeutic approach. Our previous studies demonstrated a synergistic effect of melatonin plus treadmill exercise in animal models of SCI [31]. Furthermore, we have demonstrated that melatonin plus exercise therapy attenuates the early progression of secondary injury following SCI [32].

Melatonin is a potent multiactive neuroprotective agent, and we are currently investigating the hormone’s efficacy as a therapeutic agent for SCI. Moreover, melatonin is an antioxidant and free radical scavenger and has been shown to have a neuroprotective effect following SCI [33,34]. Samantaray et al. [35] reported that melatonin reduced apoptotic death in neurons and in myelin-producing oligodendrocytes and promoted motor function following concussion injury in rats.
PHARMACOLOGICAL ROLE OF MELATONIN IN NEURAL PROLIFERATION AND DIFFERENTIATION

Melatonin and its metabolites possess several properties that may be responsible for its neuroprotective effects [36-38]. Melatonin has been reported to reduce lipid peroxidation levels following injury in animal models of SCI; this may be that because melatonin is a powerful antioxidant itself, and it stimulates other antioxidant enzymes, including catalase, glutathione reductase, peroxidase, and superoxide dismutase [39]. Moreover, melatonin has been reported to inhibit apoptosis in brain cells and other tissue [34,35,40]. In addition to its antioxidant and free radical scavenger activity, Moriya et al. [41] found that melatonin modulated proliferation and differentiation of neural stem cells in a concentration-dependent manner. The authors reported that melatonin suppressed epidermal growth factor and stimulated neural stem cell proliferation, including an increase in viable cells, DNA synthesis, and neurosphere formation, at pharmacological (1-100 uM) but not physiological (0.01-10 nM) concentrations, suggesting a possible role for melatonin in neurogenesis and potential for treating diseases such as cerebral infarction.

Previous studies using clonal neural cell lines have demonstrated beneficial effects of melatonin, such as promoting the growth and differentiation of neural blastomas and PC12 cells [42-45] and a protective action against rat pheochromocytoma (PC12 cell) death at pharmacological concentrations [43].

Previous studies have found that melatonin may play a role in increasing neural stem or progenitor cell proliferation in the dentate gyrus of rat pups [46] and in the neurogenesis of glial cell-derived neurotrophic factor (GDNF) and brain derived neurotrophic factor (BDNF) [47,48]. Kong et al. [49] demonstrated several beneficial effects of melatonin in a cell culture study. The authors showed that melatonin could promote the viability of neural stem cells derived from the rat ventral midbrain, induce the differentiation of neural stem cells into dopaminergic neurons, increase the production of GDNF and BDNF, and decrease astrocyte production.

EFFECT OF MELATONIN PLUS EXERCISE ON NEURAL CELL SENESCENCE IN SCI

Cellular senescence is characterized by the altered expression of cell-cycle proteins, particularly the up-regulation of cyclin-dependent kinase inhibitors such as p16 and p21, and the accumulation of senescence-associated $\beta$-galactosidase [50]. Cellular senescence is different from transient quiescence or differentiation. We found that mRNA expression of p53 and p16 decreased following SCI, whereas p21 mRNA expression increased. We considered this to be a result of transient cell cycle arrest rather than permanent cellular senescence. Cell cycle-related proteins have been shown to be markedly up-regulated following trauma or ischemia in the central nervous system [51-53]. Immunostaining for p53, Bcl-2, and Bax, or performing the TUNEL assay, revealed that apoptosis-related molecules were expressed in the damaged area 3 days after SCI [54].

P53 is involved in intracellular signal transduction and turns on hundreds of genes in response to cellular stress. The molecule is involved in apoptosis, autophagy, and cell cycle arrest [55]. Aging continually increases p53 expression in normal cells, leading to oxidative stress and DNA damage-mediated apoptosis [56]. A previous study found a significant increase in p53 following SCI that persisted for 28 days and was not affected by treatment with melatonin alone or in combination with physical exercise [31]. In contrast, melatonin has been reported to inhibit the proliferation of MCF-7 cells and induce $p21^{Waf1}$-mediated cell cycle arrest [57]. Cytoplasmic $p21^{Cip1/Wan1}$, a negative regulator of cell-cycle transition, facilitates neurite outgrowth from neurons. Following delivery of the cytoplasmic $p21^{Cip1/Wan1}$ TAT-fusion protein to the injured spinal cord in hemisectioned rats, exogenous melatonin was found to significantly stimulate axonal regeneration and recovery of hind limb function by inhibiting cavity formation in the damaged region [58]. p21 induction inhibited cell death signals under the cellular-stress condition, resulting in recovery of damage. Prior to the cell cycle transition from G1 to the S phase, either the p53-mediated G1 phase arrest or the cell death program is activated, p53 activates p21 expression to induce G1 arrest. A mutant p53 does not activate p21, and the “stop cell” signal is not available [59]. In the present
study, we found that treatment with melatonin alone and in combination with physical training increased the expression of p21 and p16 by day 28 following SCI.

Melatonin, which can induce or suppress apoptosis, regulates the complex cell death machinery in normal cells [60]. Exogenous melatonin has been shown to enhance or promote apoptosis by activating c-jun N-terminal kinase signaling and the p53/p21 system in tumor cells [61,62] and by inducing cell death and cell cycle arrest [15].

Most previous studies have focused on the cell death program, particularly apoptosis, induced by neuronal injury, and investigations have been limited to neurons, oligodendrocytes, and microglial cells [63]. A loss of oligodendrocytes in the white matter tracts has been found to persist for several weeks after SCI and may contribute to progressive post-injury damage such as demyelination [64,65]. Physical exercise is often used to retrain locomotor activity or promote neuronal plasticity in the spinal cord [66]. Exercise has been reported to significantly decrease several microRNAs in the apoptosis pathway, suggesting that physical activity exerts a neuroprotective effect following SCI [67,68]. Inhibition of the cell cycle progression has been shown to prevent neuronal cell death in post-mitotic neurons and to reduce scar formation and inflammation in mitotic cells including astrocytes and microglia [69]. Peroxiredoxins (Prxs) were regarded as yeast thiol-specific antioxidants (TSA) in a previous study; cysteiny1 residues react with peroxide synthesized during normal metabolism, and it is highly conserved from yeast to human [66]. Prx3 is a unique enzyme and acts as a mitochondrial antioxidant that protects neurons from cell death [70]. Prx2 plays a protective role against oxidative damage caused by ROS. Thus, our finding that Prxs expression differs among interventions warrants further study.

**CONCLUSION**

Melatonin modulates circadian rhythmicity (acting as a serotonin antagonist), participates in transmembrane transport of ions and electrolytes, and may be involved in neuronal proliferation and differentiation in the central and peripheral nervous systems. Combined treatment with melatonin and therapeutic exercise has been shown to influence neural regeneration in animal models, although supraphysiological doses are required. The preventive or curative effect of the combined therapy is the result of an increase in the blood supply surrounding neural tissues, stimulation of mitosis, and a strong antioxidative effect, as previously demonstrated. Thus, melatonin alone or in combination with exercise is a potential treatment for the acute and post-acute stages of SCI.

Melatonin plus exercise therapy for SCI has a synergistic effect on motor function and is an effective prophylactic against secondary damage caused by neuronal cell death and changes in morphology that occur through proliferation and differentiation of neuronal cells. Thus, we recommend that clinicians be familiar with the optimal time frame for administration of melatonin in the clinical setting. The melatonin-induced alterations in important transcription factors found in recent studies may provide new insights into melatonin regulation of neural function. Further studies are needed to confirm the diverse results and to clarify the mechanisms underlying this process.

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