

Melatonin: the Weapon Against Oxidative Stress and Nitrosative Stress in Neurovascular Diseases

Muhammad Masood Ahmed, Ji-Yun Huang, Rong-rong Tao and Feng Han*

Institute of Pharmacology, Toxicology and Biochemical Pharmaceutics, Zhejiang University, Hangzhou, China

Abstract

The multiple lines of evidence demonstrate that melatonin exerts powerful antioxidant actions by preventing and scavenging free radicals in a direct and indirect manner. Importantly, melatonin reduced post-ischemic oxidative/nitrosative damage to the afflicted neurovascular units and improved the preservation of blood-brain barrier permeability in various brain diseases. The information compiled here will serve as a comprehensive reference for the neurovascular protective actions of melatonin to date, and will hopefully help to design further experimental research and expand melatonin as neurovascular protective agent in neurovascular diseases.

Keywords: Melatonin; Nitrosative stress; Oxidative stress; Blood-brain barrier; Neurovascular protection

Abbreviations: AD: Alzheimer's disease; AFMK: N¹-acetyl-N²-formyl-5-methoxy-kynuramine; cAMP: cyclic adenosine monophosphate; Ca²⁺/CaM: Calcium/Calmodulin; cGMP: cyclic guanosine monophosphate; eNOS: endothelial nitric oxide synthase; GSH-Px: glutathione peroxidase; H₂O₂: hydrogen peroxide; HIOMT: hydroxyindole-O-methyl transferase; HtrA2-PED: HtrA serine peptidase 2; Keap1/Nrf2: Kelch-like ECH-associated protein 1/ nuclear factor (erythroid-derived 2)-like 2; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NMDA: N-methyl-D-aspartate; NO: nitric oxide; ONOO⁻: peroxynitrite anion; OGD: oxygen-glucose deprivation; PD: Parkinson's disease; RNS: reactive nitrogen species; ROS: reactive oxygen species; SNAT: serotonin N-acetyltransferase; VaD: vascular dementia; VCM's: vacuous chewing movements; ZO-1: zonula occludens-1

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is naturally occurring compound synthesized from serotonin in the pineal gland of mammals. It has been widely accepted that the primary physiological function of pineal melatonin secretion is to convey information about daily cycles of light and darkness to body physiology [1,2]. Beyond its physiological role in allowing the entrainment of the circadian rhythms, multiple lines of evidence demonstrate that melatonin also exerts a powerful antioxidant action by preventing and scavenging free radicals in a direct and indirect manner [3].

Cerebrovascular abnormalities contribute to the pathogenesis of ischemic stroke, Alzheimer's disease (AD), and vascular dementia (VaD) [1-4]. Stroke is an acute-onset cerebrovascular incident that can result in neurological deficits due to the impairment of cerebral circulation via microvascular injury [5-9]. Indeed, microvascular injury mechanisms may precede, exaggerate, or contribute to the progression of neurodegenerative disorders. The abnormal ion channel activations, intracellular overload with calcium and radical oxidative stresses participate in the pathological process of neurovascular damage [3,10,11].

The pharmacological effects of melatonin on neurovascular diseases have been currently supported by experimental and clinical data, including antioxidant, anti-inflammatory and anti-excitotoxic properties as well as prevent mitochondrial impairment, energy failure, and apoptosis in brain diseases. Notably, melatonin is highly effective

as calmodulin antagonist [12-15], a free radical scavenger [16-19] and has also been shown to exert neurovascular protective effects on brain pathologies [3]. This article summarizes the current understanding of the role and mechanisms of melatonin in neurovascular diseases.

Melatonin and Neurovascular Diseases

Studies show that Melatonin is synthesized by two enzymatic steps from serotonin. The first is the N-acetylation by serotonin N-acetyltransferase (SNAT) to yield N-acetylserotonin. The second step in melatonin synthesis is the transfer of a methyl group from S-adenosylmethionine to the 5-hydroxy group of N-acetylserotonin to yield melatonin and reaction catalyzed by the hydroxyindole-O-methyl transferase (HIOMT) [1,2,20,21]. Besides directly neutralizing a variety of reactive oxygen and reactive nitrogen species, melatonin is a potent free radical scavenger [22]. In specific reference to the brain, melatonin also has an advantage over some other antioxidants because it readily passes through the blood-brain barrier (BBB). Indeed, it has been shown by therapeutic trials that melatonin is effective in reducing oxidative damage and slowing the progression of neurovascular diseases [23-26].

Melatonin and brain ischemia

Brain stroke, is the third largest cause of mortality and is the single largest cause of adult disability [27]. Ischemic stroke, a significant cause of neurovascular dysfunction can result in cerebral hypoperfusion and leading to death of brain tissues within minutes to hours. The emerging epidemiological and clinical evidence indicates that oxidative and nitrosative stress is involved in the pathogenesis of ischemic complications [23,28].

Manev et al. demonstrated the direct evidence that endogenous

***Corresponding author:** Feng Han, Institute of Pharmacology, Toxicology and Biochemical Pharmaceutics, Zhejiang University, China, Tel: 86-571-8820-8402; Fax: 86-571-8820-8402; E-mail: changhuahan@zju.edu.cn

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melatonin may play a neuroprotective role and melatonin deficiency leads to increased brain vulnerability in focal brain ischemia model [28]. By using transient bilateral carotid artery ligation model, Guerrero et al. reported that melatonin prevents the increase in NO and cGMP production in brain is responsible for the protective effect of melatonin on neuronal structures during transient ischemia [29]. Moreover, melatonin protects against ischemia/reperfusion-induced damage to mitochondria in the fetal rat brain [30]. In *in vitro* oxygen-glucose deprivation (OGD) model, melatonin pretreatment significantly inhibited OGD-induced peroxynitrite formation and prevented a substantial imbalance in mitochondrial HtrA2-PED signaling as well as increase the endothelial cell survival rate after ischemia-like injury [14,31,32]. The BBB preserves the delicate homeostasis of the brain microenvironment through the maintenance of tight junctions between brain vascular endothelial cells. Brain edema formation and subsequently neuronal death due to loss of integrity of the BBB is a major consequence of cerebral ischemia [33-37]. Indeed, melatonin treatment is highly effective in decreasing the late increase in BBB permeability and the risk of tissue plasminogen activator in excitotoxicity or brain ischemia/reperfusion models [28,38,39]. Moreover, weak or irregular expression of zipper-like ZO-1 immunoreactivity was observed in OGD-treated cells compared with controls, and this decrease was markedly reduced by melatonin pretreatment [32].

Melatonin and Alzheimer's disease

Alzheimer's disease (AD) is characterized by progressive loss of cognition, loss of memory and other neurobehavioral manifestations. More than one third of AD patients exhibit variable cerebrovascular pathology and white matter injury, indicating the association of cerebrovascular risk factors with AD. In addition, oxidative stress, nitrosative stress and mitochondrial dysfunction have been implicated in the progression of AD.

In rodent model of AD, melatonin reduced plasma homocysteine and lipid levels, and the investigators suggested that the melatonin's antioxidant effects may have been responsible for these results [40-42]. Oxidative stress in AD is the result of decline in the production of endogenous melatonin and an imbalance in pro-oxidant/antioxidant homeostasis that leads to the overgeneration of toxic reactive oxygen species [43,44]. Melatonin prevents the death of neuroblastoma cells exposed to β -amyloid polypeptide [45-47]. Moreover, melatonin has exhibited neuroprotective and antioxidant properties against β -amyloid mediated oxidative injury *in vitro* [14,46,48], which may be associated with the inhibitory effect of melatonin on the formation of β -sheets and amyloid fibrils [49]. The unique feature of melatonin and its protective metabolites regarding AD is the ability to interact directly with the electron transport chain by increasing the electron flow as well as antagonizes formation of superoxide anions and β -amyloid toxicity [44,45,50,51]. Furthermore, there is evidence to suggest that melatonin administration to AD transgenic mice is associated with a reduction in a number of important disease markers, including β -amyloid levels, protein tyrosine nitration and reduced life expectancy [46,47,52].

Melatonin and Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disorder due to degeneration of dopaminergic neurons in the substantia nigra [53-56]. It has been reported regarding PD etiology that oxidative stress with reduced glutathione peroxidase while the level of the antioxidant enzyme manganese superoxide dismutase is high and not paralleled by glutathione levels [57,58]. The accumulating evidences

demonstrated that radical damage in lipids [59], proteins [60], and nucleic acids [61] of substantia nigra in parkinsonian patients. Studies show that dopaminergic neurons are susceptible to increase reactive oxygen species [62], while amines derive radical formation results in auto oxidation of dopamine. Recently it has been studied that mitochondrial dysfunction can lead to aggregation of α -synuclein might be a reason for neurodegeneration [23,63]. Further it has been reported that increase in iron level [64] catalyzes the fenton reaction [65] leading to metal induced lipid peroxidation [66] via hydroxyl radical generation. Animal models with altered dopaminergic function have been used to study efficacy of various therapeutic agents in the treatment of Parkinson's disease [67].

Antolin and his co-workers found that melatonin was effective in preventing neuronal cell death and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced damage to the substantia nigra in experimental parkinsonism [53,68]. MPTP and 6-OHDA models of PD showed that melatonin's antioxidant effect is able to counteract MPTP induced lipid peroxidation in striatum, hippocampal, and midbrain regions [5,69,70]. Melatonin inhibited neuronal excitation caused by NMDA activation in rat striatum [71]. The negative interaction between dopaminergic and adenosinergic system in striatum has been ameliorated by melatonin in ferric chloride induced model of experimental PD [72]. In addition, melatonin reversed vacuous chewing movements (VCM's) in rats, chronically treated with haloperidol as well as melatonin has role for the prevention and treatment of neuroleptic induced orofacial dyskinesia [73]. MPTP, through its metabolite MPP⁺ inhibits the complex I of substantia nigra of PD patients. Intriguingly, melatonin exerts antioxidant effect by increasing complex I and complex IV activities of mitochondrial electron transport chain [74,75].

Melatonin: A Weapon Against Oxidative and Nitrosative Stress

Free radicals are highly reactive molecules generated during cellular respiration and normal metabolism. However, imbalance between cellular production of free radicals and the ability of cells to defend against them is referred to as oxidative stress [76]. In addition, reactive nitrogen species are powerful oxidizing and nitrating agents for cell damage, including the nitrogen dioxide radical (NO₂·) and various non-free radicals, such as peroxynitrite (ONOO⁻) and its protonated form. The final products of oxidative and nitrosative stress are extremely aggressive oxidants, which is also a key factor of functional importance in driving neurovascular disease progression [32]. Brain is highly vulnerable to oxidative and nitrosative injury because of its high oxygen consumption, abundant lipid content, and the relative deficiency of antioxidant enzymes as compared with other tissues [27,77,78]. It has been indicated by evidence that the reactive nitrogen species contribute to early injury of neurovascular endothelial cells, which is also of key importance in neurovascular diseases [32,79]. Notably, melatonin elicits neurovascular protective effect via its antioxidant and free-radical-scavenging capacities in brain diseases [3,80].

Mechanism of melatonin against oxidative stress

A large body of evidence indicates that the scavenging potential of melatonin is mainly due to its antioxidative ability, may be defined as scavenging cascade reactions [22]. Melatonin showed its scavenging actions against peroxynitrite anion, singlet oxygen and nitric oxide, hydrogen peroxide as well as interacts with the highly toxic hydroxyl radical [16,17,81].

In vitro studies, Stasica et al. [82,83] demonstrated that melatonin is a more efficient •OH scavenger than either glutathione or mannitol, melatonin reacts at a diffusion-controlled rate with the •OH [16,17]. Melatonin scavenged H₂O₂ efficiently but only one molecule, N¹-acetyl-N²-formyl-5-methoxy-kynuramine (AFMK) with molecular mass 264 resulted by the interaction with H₂O₂ during proposed metabolic pathway [84]. It has been reported that melatonin can detoxify O₂• to produce a kynuramine, a melatonyl radical which is formed when melatonin donates an electron, is recycled back to melatonin by both ascorbate or urate with equal efficiency [85-88]. In consequence, melatonin's ability to function as an anti-oxidant in virtually all parts of the cell and to protect membrane lipids, cytosolic proteins and nuclear DNA from oxidative damage [89]. The immunocytochemical data demonstrated that melatonin's immunoreactivity is certainly found in different subcellular compartments and plays a critical role as a bioenergetic agent [90,91]. The pathophysiological relevance of increased oxidative stress and the mitochondria-dependent apoptotic cascade has recently become a topic of interest. Importantly, melatonin can improve and maintain mitochondrial function during cellular degenerative processes via preventing protein oxidation, the mitochondrial permeability transition pore opening and the release of mitochondrial proteins [32,92].

Besides directly detoxifying a variety of reactive oxygen and reactive nitrogen species, other actions of melatonin, such as stimulation of antioxidative enzymes also improves its status as an antioxidant. Melatonin also induces or preserves the several intracellular antioxidant enzymes, including superoxide dismutase, glutathione peroxidase (GSH-Px), γ-glutamylcysteine synthetase as well as glutathione (GSH) [93,94].

Mechanism of melatonin against nitrosative stress

Accumulating evidence supports the importance of the nitroso-redox balance in the pathogenesis of neurovascular complications. The formation of highly reactive nitrogen-containing molecules mediates protein oxidation and nitration, mitochondrial dysfunction, caspase-dependent apoptotic cascade, DNA damage [38,88,95,96]. We and others have previously reported that presence of nitrosative stress in endothelial cells/microvessels can damage biomolecules and subsequently cause the breakdown of highly-structured cellular machinery in the brain [14,19]. The accumulating data demonstrate that neutralization of ONOO⁻ and/or pharmacological inhibition of nitrosative stress-related pathways will be therapeutically effective in a wide range of neurovascular diseases [32]. Importantly, melatonin scavenges a number of oxidants including the nitric acid, peroxynitrite (ONOO⁻) and peroxynitrous acid during pathological process of neurovascular diseases [15,19,97].

The excessive Ca²⁺ influx into neurons also causes abnormal activation of Ca²⁺ dependent enzymes such as phospholipase A₂, calpain and Ca²⁺/Calmodulin (CaM)-dependent enzymes [98-100]. Given that Ca²⁺/CaM-dependent enzymes are important for ONOO⁻ formation, and melatonin has been reported to modulate the Ca²⁺/CaM signaling pathway in various tissues [12,13]. In this regard, we have recently demonstrated that microvessel injury induced aberrant nitric oxide and peroxynitrite production in a CaM-dependent manner [15,32,96]. Additionally, recent reports have shown that melatonin can directly react and neutralize ONOO⁻ [19,18,97,101]. Several studies suggested melatonin may have a role in the biological regulation of

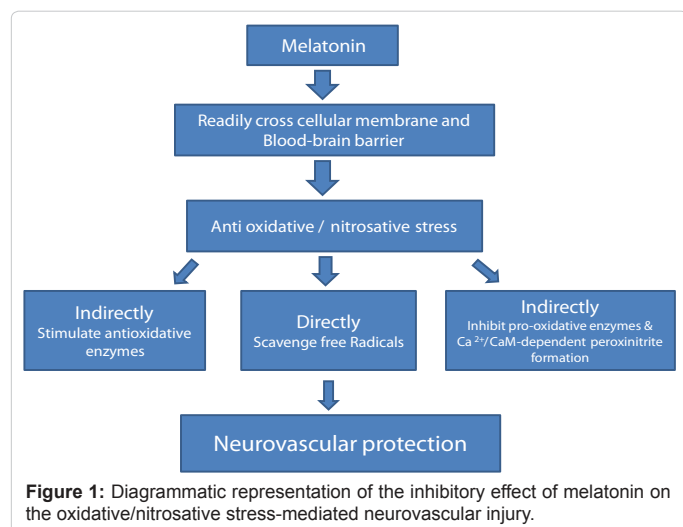
cerebrovascular disease probably *via* its effect on the nitric oxide [29,96].

The melatonin significantly inhibited OGD-induced ONOO⁻ formation and caused a substantial imbalance in HtrA2-PED signaling as well as melatonin increased the survival rate of endothelial cells after ischemic-like injury [32]. Several studies suggested that the inhibitory effect of melatonin on nitric oxide synthase activity might be produced through a high affinity CaM-melatonin binding [12,13,32,102]. Pharmacological repression of ONOO⁻ formation by melatonin partially inhibited ischemia-induced protein tyrosine nitration of Keap1 as well as disturbance of Keap1/Nrf2 signaling in endothelial cells [103]. In addition, our group has found that activation of autophagy by OGD was partially inhibited in melatonin-treated cells, suggesting a critical role for nitrosative stress in the induction of autophagy by ischemic stress [96]. It has also been observed that the melatonin treatment effectively inhibited OGD-induced cathepsin B activation, which indicates that an interaction exists between the autophagy-lysosome pathway and nitrosative stress [96]. It is also notable that melatonin administration prevented rigidity in the mitochondrial membrane and decrease age-related autophagy-lysosomal alterations [104]. Consistent with the suppression of autophagic signaling in eNOS knock-down cells, activation of the either autophagic or lysosome process by OGD was partially blocked in the melatonin-treated endothelial cells, suggesting a critical role for nitrosative stress in the induction of mitochondrial damage and autophagy process [14,32]. Thus, ischemic insult promotes the ONOO⁻ pathway and activates lysosomal signaling and that such activation can be inhibited by melatonin pretreatment. Nagai et al. [30] demonstrated that melatonin protects against ischemia/reperfusion-induced damage to mitochondria in the fetal rat brain. Most importantly, melatonin reduced tight junction protein breakdown in a brain ischemia model in close association with concomitant inhibition of nitrosative stress and protein tyrosine nitration [14,32,105]. Although the identities of target proteins that may be regulated by ONOO⁻ remain to be defined in future studies, data derived from the present study raise the possibility that ONOO⁻ targeting by melatonin may be a new strategy to prevent or treat neurovascular diseases.

Concluding Remarks

Although our knowledge about the crucial role of melatonin on various neurodegenerative disorders has advanced considerably in recent years, but several important issues remain to be resolved. For example, 1) It remains to be ascertained the role of MT1 or MT2 receptors on melatonin-mediated neurovascular protection. 2) There may be different dose requirements for different phase of neurovascular pathological conditions, understanding the appropriate dosage of melatonin will provide new insight to develop better neurovascular protective drugs. 3) There is also increasing interest in studying co-administration of melatonin with other therapeutic agents that may be even more effective in extending the therapeutic time window for neurovascular diseases.

To conclude, melatonin is a highly effective calmodulin antagonist and anti-oxidant, which directly scavenges a variety of oxygen- and nitrogen-based reactants, preserves the integrity of the mitochondria, stimulates antioxidative enzymes; eventually elicit its neurovascular protective effect (Figure 1). Finally, one might expect that the body of direct mechanistic and clinical data on melatonin and its derivatives



targeting the oxidative/nitrosative stress in the pathogenesis of neurovascular diseases may afford clinicians a new therapeutic armamentarium to treat patients with neurovascular disorders.

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