The Antiapoptotic Effects of Melatonin in Neonatal Hypoxic-Ischemic Brain Injury and Adult Ischemic Stroke

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EDITORIAL

Melatonin (N-acetyl-5-methoxytryptamine) is a natural hormone secreted by the pineal gland. In clinical use for many years, melatonin is safe, well tolerated, has high efficacy, and easily crosses the blood-brain barrier [1,2]. Intensive research over the past decade (including our own) has indicated melatonin’s beneficial effects in minimizing the damage caused by newborn hypoxic-ischemic encephalopathy (HIE) and adult cerebral ischemia, as well as primary neuronal cell death by insult in experimental models of neonatal hypoxic-ischemic (H-I) brain injury and adult ischemic stroke. Reports show that melatonin has anti-apoptotic, anti-oxidant, and anti-inflammatory activity.

Apoptosis and necrosis are two types of cell death. The intrinsic apoptotic (mitochondrial cell death) pathways and the anti-apoptotic survival signal pathways play critical roles in neonatal H-I brain injury and adult ischemic stroke. This editorial focuses on the effects of melatonin on both pathways (which may be synchronous and complementary) and discusses the therapeutic potential of melatonin in neonatal H-I brain injury and adult ischemic stroke. The Rice-Vannucci model, combining unilateral carotid artery ligation with exposure to hypoxia in neonatal animal pups and middle cerebral artery occlusion (MCAO) in adult animals, has been widely used to study melatonin’s effects in neonatal H-I brain injury and adult ischemic stroke, respectively. Primary cortical neurons (PCNs), primary striatal neurons (PSNs), primary cerebellar granule neurons (PCGNs), and neural stem cells (NSCs) are the cells most commonly used in cellular models of neonatal H-I brain injury and adult ischemic stroke in the study of the antiapoptotic effects of melatonin.

MELATONIN TARGETS THE MITOCHONDRIAL CELL DEATH PATHWAYS

The highest levels of melatonin are found in the mitochondria [3], a known target of melatonin [4]. During the progression of neonatal H-I brain injury and adult ischemic stroke, once cytochrome c is released, it binds to Apaf-1 and dATP, which stimulates the activation of caspase-9, and then cleaves the key effector caspase-3. We reported that melatonin inhibits cytochrome c release in a cell-free purified mitochondrial system [5]. We and other laboratories also demonstrated that melatonin reduced the damage caused by cerebral ischemia in MCAO animal models, as well as cell death in PCNs, PSNs, and PCGNs caused by oxygen/glucose deprivation (OGD) or exposure to H₂O₂ or N-Methyl-D-aspartate through the inhibition of mitochondrial apoptogenic factor cytochrome c release [6-8]. Melatonin also inhibits other changes in mitochondrial factors such as the release of the additional apoptogenic factor apoptosis-inducing factor [4], the dissipation of Δψm [4,6,8], and mitochondrial transition pore opening in PSNs and/or PCNs after insult [4].

The release of mature IL-1β indicates caspase-1 activation, which we and other researchers have reported is an important and early event in neonatal H-I brain injury and adult ischemic stroke. Caspase-1 activator receptor interacting protein-2 (Rip2) stimulates caspase-1 to activate IL-1β, and Rip2 deficiency is neuroprotective against newborn H-I brain injury [9-11]. Melatonin inhibits OGD-induced caspase-1 activation and mature IL-1β release in PCNs [6]. Furthermore, melatonin inhibits downstream caspase-3 activation in OGD and/or H₂O₂-mediated PCN and PCGN cell death [6,8]. H-I-mediated newborn brain injury [11,12], and cerebral ischemia-induced MCAO animal models [6,13,14]. Caspase-3 is responsible for the cleavage of the DNA-repair enzyme poly (ADP-ribose) polymerase (PARP), while nuclear condensation and DNA fragmentation are induced, as shown by terminal deoxynucleotidyl transferase (dUTP)-mediated DNA nick-end labeling (TUNEL), nuclear staining, and DNA ladder. The administration of melatonin reduced levels of cleaved PARP [15], the amount of DNA fragments [4,8], and the number of TUNEL-positive cells in neonatal animals with H-I brain injury [16], MCAO animals with adult ischemic stroke [15].
and PCNs under insult [6].

**MELATONIN ACTIVATES THE ANTI-APOPTOTIC SURVIVAL SIGNAL PATHWAYS**

During the progression of neonatal H-I brain injury and adult ischemic stroke, melatonin activates survival signaling cascades, including the phosphoinositol-3 kinase/Akt pathway, the MAPK pathway, the Bcl-2 pathway, as well as the NF-κB pathway. Melatonin has been shown in animal models of MCAO to maintain the phosphorylated form of Akt and prevent injury-induced decrease in Akt activation and phosphorylation of mTOR and p70S6 kinases, and the subsequent decrease in S6 phosphorylation [13,17]. Melatonin also activates the JNK pathway [18] and inhibits apoptotic signals by preventing injury-induced decrease in phosphorylation of Raf-1, MEK1/2, and ERK1/2 and the downstream targets, including Bad and 90 ribosomal S6 kinase [15]. Additionally, melatonin’s promotion of NSC proliferation involved the increased phosphorylation of ERK1/2 in a model of neonatal H-I injury [19]. Melatonin effectively attenuated ischemic brain injury via the Bcl-2-related survival pathway by increasing the expression of Bcl-2 [20] and Bcl-xL [18]. Furthermore, melatonin increased the cellular content of IκBα (indicating the activation of NF-κB transcription factor) in primary neuronal cultures under sodium salt/reperfusion physiological conditions [21].

**FUTURE DIRECTIONS**

Therapeutic hypothermia is the only neuroprotective intervention that has translated to any clinical benefit for newborns with HIE, while the only FDA-approved treatment for adult ischemic stroke is tissue plasminogen activator. Melatonin is inexpensive, safe with low toxicity, and has been used in human trials in patients with stroke, amyotrophic lateral sclerosis, and other neurological disorders [1]. The combination of preclinical effectiveness and proven safety in humans, animals, and cultured cells recommends melatonin as a particularly promising candidate for clinical trials. Future research could be directed at identifying and developing therapeutic strategies to fight newborn HIE and adult ischemic stroke in the clinical setting using melatonin alone or in combination with other pharmacological interventions (e.g., tissue plasminogen activator) for adult ischemic stroke. Future studies should include optimizing the time and dose to inhibit the various apoptotic pathways, enhance survival pathways, and avoid adverse effects.

**ACKNOWLEDGEMENTS**

This work was supported by grant from the Bill & Melinda Gates Foundation (X.W.), the Muscular Dystrophy Association (X.W.), and the ALS Therapy Alliance (X.W.).

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