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Review Article

Melatonin as a Neuroprotective Agent in Hypoxic Ischemic Encephalopathy: Is it Beneficial

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Abstract

Hypoxia-ischemic encephalopathy (HIE) is a global neonatal problem with 22% neonatal mortality worldwide. The incidence of neurological impairments in survivors is approximately 25%. Evidence from meta-analysis revealed that therapeutic hypothermia is beneficial in neonates with HIE to decrease mortality or major disability. Therapeutic hypothermia has become the standard of care in neonates with HIE in the developed world. However, almost 50% neonates are still having adverse outcome even after therapeutic hypothermia treatment. To further enhance the benefit effect of therapeutic hypothermia, melatonin is considered to be an adjuvant medication as it cross all physiological barriers including blood-brain barrier and placental barrier. Evidence from preclinical trials described neuroprotective role of melatonin in neonates with HIE. However, there is insufficient evidence in human neonates. In this review article, we discuss mechanism of action and evidence based neuroprotective role of melatonin in neonates with HIE.

Keywords: *Hypoxic-Ischemic Encephalopathy; Melatonin; Neuro-Protection*

Abbreviations

HIE: Hypoxic Ischemic Encephalopathy; NAA: N Acetyl Aspartate; NO: Nitric Oxide; NOS: Nitric Oxide Synthase; RCTs: Randomized Controlled Trials; SOD: Superoxide Dismutase; TH: Therapeutic Hypothermia

Introduction

Hypoxic-ischemic encephalopathy (HIE) is a global neonatal problem which significantly contributes to both morbidity and mortality. HIE occurs in 1 - 10 of per 1000 births, with a different incidence in different parts of the world [1]. HIE accounts for a 22% of neonatal mortality worldwide [1]. The incidence of neurological impairments in survivors is estimated to be of about 25% [2]. The rate of adverse neurodevelopmental outcomes varies with the severity of HIE. In the children under 3 years of age, this rate is nil for mild, approximate 32% for moderate and almost 100% for severe forms of HIE [3].

Therapeutic hypothermia (TH) is the most promising intervention as a neuroprotective agent in HIE. TH is becoming standard clinical care for moderate to severe neonatal encephalopathy in the developed world [4]. Although TH is beneficial in neonates with HIE to decrease mortality or major disability but benefit effect is very small with almost 50% of treated neonates having adverse outcomes (from 61% to 46%, 15% absolute reduction) [4,5]. Hence, various neuroprotective strategies like inhaled xenon, erythropoietin, melatonin, 2-iminobiotin, N-acetyl cysteine, stem cells, allopurinol and magnesium are in experimental stage to augment this beneficial effect [6]. Out of these strategies, melatonin has started to be considered as an attractive option to further minimize neurological sequelae from HI brain injury. In this review article, we discuss the evidence based role of melatonin as neuroprotective agent in neonates with HIE.

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenously produced indolamine originating from the pineal gland [7]. The main function of melatonin in all mammals is to transduce information concerning light-dark cycles to body physiology, particularly for organisation of body rhythms [8]. Melatonin has several other biological functions which include protection against oxidative stress, anti-inflammatory effect, anti-apoptotic action, blood pressure regulation and boost-up the immune function [9].

Possible mechanism of action

Melatonin exert their neuroprotective effect via anti-apoptotic, anti-inflammatory and antioxidant action. Melatonin mediate its anti-apoptotic action by decreased expression of pro-apoptotic proteins (Bad and Bax) [10,11], increased expression of anti-apoptotic proteins (Bcl-2, Bcl-xl) [10,12], reduced or blocked caspases enzyme activation [10-12], decreased cytochrome-c release [13], inhibited poly-ADP-ribose-polymerase cleavage [11] and decreased the number of TUNEL-positive cells/DNA breaks [11,13]. Melatonin also exerts anti-excitatory effects on neurons through the modulation of gamma-aminobutyric acid and glutamate receptors [14], which lead to decrease in cytosolic calcium concentrations [15].

Melatonin has strong antioxidant action. This action is mediated by decreased lipid peroxidation [16], decreased production of malondialdehyde [17], decreased protein oxidation [18], reduction of nitrite/nitrate levels [17] and maintaining the activity of antioxidative enzymes such as catalase [19].

Its anti-inflammatory effect mediated by down regulation of inflammatory mediators such as interleukin-6, interleukin-8, tumor necrosis factor-α [20], 5-lipoxygenase [21], cyclooxygenase [22], prostaglandin [23], inhibition of neuronal nitric oxide synthase (NOS) [24] and inducible NOS [25]. Inhibition of neuronal NOS provide protection from nitric oxide mediated mitochondrial impairment and cell damage [26]. Inhibition of inducible NOS prevent lipid peroxidation, shifts the glutathione redox state and boosts energy efficiency and ATP production in mitochondria [27] (Figure 1).

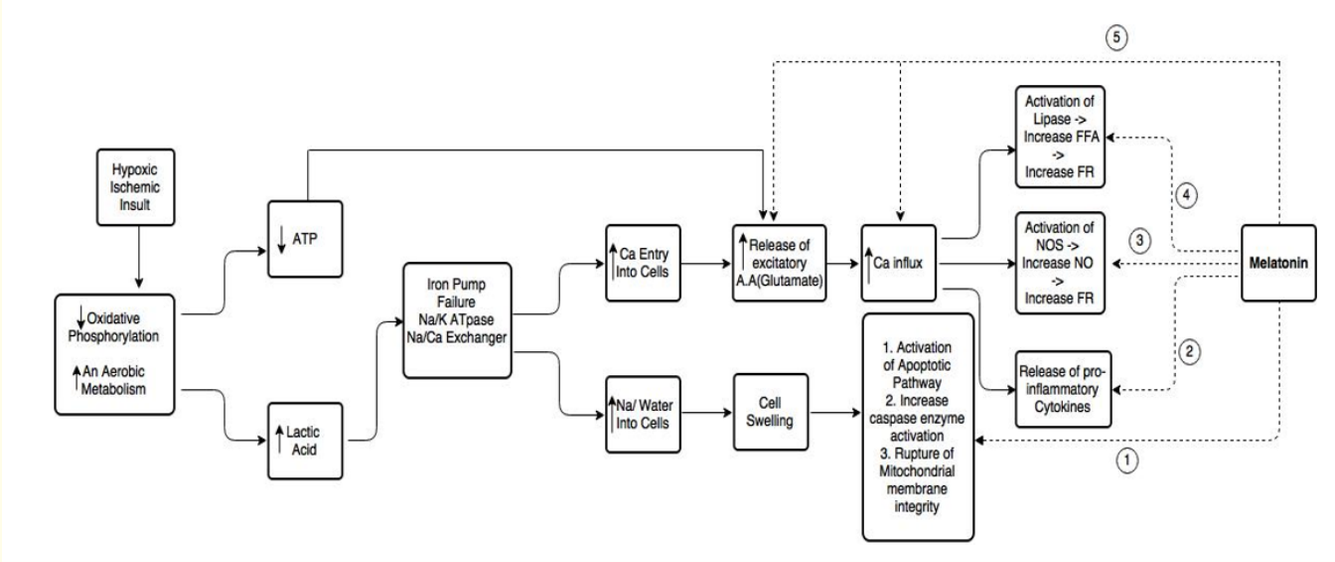

Figure 1: *Melatonin- Mechanism of action.*

1: Decreased expression of pro-apoptotic proteins (Bad and Bax), increased expression of anti-apoptotic proteins (Bcl-2, Bcl-xl), reduced or blocked caspases enzyme activation, decreased cytochrome-c release.

2: Down-regulation of inflammatory mediators such as interleukin-6, interleukin-8, tumor necrosis factor-α, 5-lipoxygenase, cyclooxygenase, prostaglandin.

3: Inhibition of neuronal nitric oxide synthase (NOS) and inducible NOS (Anti-oxidant action).

4: Inhibit lipid per oxidation (Anti-oxidant action).

5: Modulation of gamma-aminobutyric acid and glutamate receptors, which lead to decrease in cytosolic calcium concentrations.

Methods

Criteria for considering studies for this review

Types of studies: We included RCTs, quasi-randomized trials and pre-clinical trials in which melatonin has been given either alone or along with therapeutic hypothermia for the management of HIE.

Type of participants: We included all late preterm (≥ 34 weeks) and term neonates who fulfill the definition of HIE.

Type of interventions: Melatonin at any dosage or duration in asphyxiated neonates compared with placebo or no intervention.

Literature search

We performed search in Cochrane Central Register of Controlled Trials (CENTRAL), electronic database search of Pubmed and EMBASE. We also searched clinical trials registries for ongoing or recently completed trials (www.clinicaltrials.gov, www.controlled-trials.com, http://www.anzctr.org.au, http://ctri.nic.in/Clinicaltrials, http://www.who.int/ictrp/search/en/) and abstracts of conferences (American Pediatric Society, Society for Pediatric Research, and European Society for Pediatric Research).

Search methods for identification of studies

We searched MEDLINE and EMBASE by using following terms. No language restrictions were applied. Additional details were sought from authors as and when required. We searched all trials identified by reviewing the abstract. We also identified studies by citation tracking.

Population: infant, newborn"[MeSH Terms] OR ("infant"[All Fields] AND "newborn"[All Fields]) OR "newborn infant"[All Fields] OR "neonates"[All Fields].

Intervention: "Melatonin/blood"[MeSH Terms] OR "Melatonin "[All Fields] OR "Melatonin/therapeutic use [MeSH Term]".

Comparison: Clinical trials (MeSH) OR Controlled Clinical Trials (MeSH) OR randomized Controlled Trials (MeSH) OR Random Allocation (MeSH) OR Multicenter studies (MeSH) OR Control groups (MeSH) OR Evaluation studies (MeSH).

Outcome: "Hypoxia-ischemia Brain/Therapy"[MeSH Terms] OR "Hypoxia-ischemia Brain/drug therapy*"[MeSH Terms].

All these four sub-headings were combined by "AND".

Melatonin as neuroprotective

Evidence from preclinical trials

Uchida., *et al.* demonstrated melatonin has the potential to protect the neurons against an ischemic insult by reducing superoxide generation in rat hippocampal model [28]. Tutunculer., *et al.* described protective role of melatonin against brain injury by oxidative stress in neonatal rats which was explained by its direct scavenger activity and activation of catalase enzyme [19]. Signorini., *et al.* studied formation of oxidative damage mediators and effect of melatonin treatment in a model of HIE in seven day-old rats. Melatonin treated rats showed significantly low level of oxidative damage mediators and protect the brain after HI insult [29]. Carloni., *et al.* demonstrated long lasting benefits of melatonin in neonatal rats. Melatonin significantly improved the behavioral asymmetry and learning deficits induced by HI [30]. Koh., *et al.* described neuroprotective effect of melatonin by regulating NOS expression in ischemic brain of adult rats [31]. Wang., *et al*. demonstrated melatonin treatment decreased infarct size and improve neurological scores after ischemic insult in mice by inhibiting mitochondrial cytochrome C release [13].

Lekic., *et al.* demonstrated that melatonin reduced long-term brain atrophy, and return sensorimotor and cognitive function to near-normal levels in neonatal rats after hemorrhage-mediated free-radical brain injury [32]. Xu., *et al.* investigated the effects of melatonin on brain in neonatal rats with hypoxic-ischemic brain damage (HIBD) and concluded that melatonin reduced the histological injury in the brain. Melatonin also reduced HIBD induced cerebral edema and edema related proteins, such as AQP-4, ZO-1 and occluding [33].

Robertson., *et al*. conducted a RCT to evaluate neuroprotective effect of melatonin in a piglet model of perinatal asphyxia. After a quantified global HI insult, 17 newborn piglets were randomized to TH alone (33.5° C from 2 to 26 hours after resuscitation, n = 8) or TH plus intravenous melatonin (5 mg/kg/hours over 6 hours started at 10 min after resuscitation and repeated at 24 hours, n = 9).

Melatonin significantly reduced the HI-induced increase in the AUC lactate/N-acetyl aspartate and lactate/total creatine ratios in the deep grey matter compared to TH alone. Melatonin/TH group was also associated with increased levels of cerebral ATP, reduced caspase-3 in thalamus when compared to TH alone [34].

Thayyil., *et al.* conducted a meta-analysis of magnetic resonance spectroscopy (MRS) biomarkers in neonates with HIE and concluded that lactate/NAA ratio in deep grey matter seemed to be the most accurate MR biomarker for neuro-developmental outcome prediction [35]. NAA levels decrease with cerebral injury or impaired cerebral metabolism and lactate levels are increased [35].

Evidence from clinical trials

Based on evidence from preclinical trials, melatonin could represent a potential safe approach to perinatal brain damage in human neonates. Fulia., *et al.* measured product of lipid peroxidation, malondialdehyde, and the nitrite/nitrate levels in the serum of 20 neonates with HIE before and after treatment with the melatonin. Out of 20 neonates, 10 neonates received melatonin (total dose of 80 mg; 8 doses of 10 mg each separated by 2-hr intervals) with first dose was given within the first 6 hr of life and rest of the 10 neonates didn't receive melatonin (non-melatonin group). Blood samples were collected before and after (at 12 hours and 24 hours) melatonin administration. Serum malondialdehyde and nitrite/nitrate concentrations in neonates with HIE before treatment were significantly higher than those in neonates without HIE. There were significant reductions in malondialdehyde and nitrite/nitrate levels at both 12 and 24 hours in melatonin group neonates. There was 30% mortality in non-melatonin group within 72 hours of birth whereas no mortality was reported in melatonin group [17]. This study concluded that melatonin has protective role in neonates with HIE by their anti-oxidant action.

Aly., *et al.* studied effect of melatonin in 30 term neonates with HIE. All neonates received TH for a total of 72 hours. Neonates were randomized to TH alone or TH/melatonin group (n = 15 in each group; n = 15 healthy neonates as control). Melatonin was administered enterally in a doses of 10 mg/kg daily for total of five doses. Baseline superoxide dismutase (SOD), nitric oxide (NO) and melatonin concentrations were significantly high in both HIE groups when compared to the healthy control group ($p < 0.001$). At 5^{th} day, melatonin was significantly increased in both HIE group (TH group 20.6 ± 2.5 to 32.1 ± 3.5 pg/ml; $P < 0.001$and melatonin/TH group 21 ± 2.4 to 42.7 ± 5.1 pg/ml; $p < 0.001$) but the difference between baseline and follow-up of melatonin was significantly more in melatonin/TH group compared to TH alone group ($p < 0.001$). Similarly, NO was significantly decreased in both groups, but reduction was significantly more in melatonin/TH group ($p < 0.001$). SOD was significantly decreased in both groups ($p < 0.001$), but this reduction was significantly less in melatonin/TH group ($p = 0.004$). Survival without abnormalities at 6 months was significantly increased in the melatonin/hypothermia groups ($p < 0.001$) [7]. As per the evidence, there was increased SOD activity and elevated NO concentration in response to HI insult and their levels correlated with severity of brain damage [36,37]. Aly., *et al.* concluded that melatonin and TH both decreased NO concentration and melatonin augmented effect of TH. However, SOD reduction was less in melatonin/TH group [7]. Main limitations of the study was small sample size and too early neuro-developmental follow up at 6 month of age. Another recent study evaluated role of melatonin in the management of HIE in neonates with gestational age ≥ 34 weeks who fulfilled the definition of HIE and presented within 12 hours of birth. This study revealed that melatonin improved the survival rate in neonates with HIE [Melatonin group 87.5% (35/40) vs Standard treatment group 65% (26/40); $p = 0.03$] [38].

Effect of antenatal melatonin treatment

Melatonin can cross the all physiological barrier including placental barrier and blood-brain barrier. Thus, it is a novel idea to use melatonin prophylactically in fetuses who are at high risk of perinatal hypoxia [39]. Drury., *et al.* evaluated neuroprotective effect of prophylactic maternal low dose melatonin in preterm fetal sheep model after profound hypoxia. Maternal melatonin infusion was associated with faster recovery of fetal electroencephalogram (EEG), prolonged reduction in carotid blood flow, improved neuronal

survival in the caudate nucleus, improved numbers of mature oligodendrocytes, and reduced microglial activation in the white matter ($P < 0.05$) [40]. Miller., *et al.* showed that maternal prophylactic melatonin (1 mg total) given before 10-minute umbilical cord occlusion in fetal sheep model was associated with reduced oxygen free radicle formation, brain lipid peroxidation, neuronal death, microglial activation, and astrogliosis [16].

Wilkinson., *et al.* conducted a systematic review to assess the effect of antenatal melatonin as a neuroprotective agent in the fetus. There was no RCT for inclusion in this review. Although evidence from animals studies has supported a fetal neuroprotective role for melatonin when administered to the mother during pregnancy. However, there is no trial assessing fetal neuroprotective effect of melatonin in pregnant women till date [41]. There is one ongoing clinical trial to assess the effect of antenatal melatonin in mothers as a neuroprotective agent who are at risk of preterm birth between 24-28 weeks of gestation [42].

What is already known?	1. HIE stage 2 and 3 associated with adverse neurological outcomes. 2. Therapeutic hypothermia currently is becoming standard of care for moderate to severe HIE. 3. Despite beneficial effect of therapeutic hypothermia, almost 50% of treated neonates have adverse neurological outcomes.
What this review add?	1. Melatonin, due to its broad therapeutic range, is a feasible option to ameliorate the brain injury secondary to birth asphyxia. 2. Preclinical trials revealed neuroprotective effect of melatonin. No adverse effect was reported in animal studies. 3. Melatonin may be used as novel therapeutic adjunct in neonates with HIE to increase the neuroprotective effect of therapeutic hypothermia. However, current evidences are limited.

Table 1: *Summary of review.*

Conclusion

As per the evidence from preclinical trials, melatonin has neuroprotective role in neonates with HIE. However, there is insufficient clinical evidence to use melatonin as a neuroprotective agent in routine clinical practice in human neonates with HIE. Well-designed RCTs with relevant inclusion/exclusion criteria, adequate sample sizes and validated clinical outcome measures are needed in neonates with HIE. Future trials should also define optimal dose, duration, safety profile, short and long term neuroprotective effect of melatonin.

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