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Review Article

From Oxidative Stress to Pain Modulation With Melatonin in Neonatal and Pediatric Care

Serafina Perrone, MD, PhD[1,#,*], Laura Cannavò, MD, PhD[2,#], Sofia Gerevini, MD[3], Luca Buttarelli, MD[3], Pietro Leuratti, MD[3], Elisa Caselli, MD[3], Chiara Petrolini, MD[1], Vincenzo Raitano, MD[1], Virginia Beretta, PhD[1], Silvia Carloni, PhD[4]

[1] *Neonatology Unit, Department of Medicine and Surgery, University of Parma, Pietro Barilla Children's Hospital, Parma, Italy*
[2] *Pediatric Intensive Care Unit, University Hospital of Verona, Verona, Italy*
[3] *Pediatric Unit, Pietro Barilla Children's Hospital, University of Parma, Parma, Italy*
[4] *Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy*

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ABSTRACT

Purpose: Pain and oxidative stress represent critical and frequently underrecognized challenges in neonatal and pediatric care. Their synergistic interaction may adversely affect neurodevelopmental outcomes and long-term quality of life. Melatonin, a neurohormone with well-established antioxidant properties, has also demonstrated analgesic and anti-inflammatory effects, making it an attractive therapeutic candidate in this vulnerable population.

This narrative review critically evaluates the available preclinical and clinical evidence on the analgesic role of melatonin in neonates and children. By integrating mechanistic insights with updated clinical data, the review aims to provide a unified perspective on melatonin as a multi-target analgesic, with particular emphasis on pain-modulating pathways that extend beyond its antioxidant activity.

Methods: Comprehensive literature search was performed using electronic databases, including PubMed and MEDLINE. The review considered peer-reviewed articles published in English that investigated melatonin's interaction with pain-related pathways in neonatal and pediatric populations. Both preclinical and clinical studies were analyzed, with particular focus on mechanisms involving MT2 receptor activation, suppression of neuroinflammation, modulation of GABAergic and opioid systems, and interference with *N*-methyl-D-aspartate receptor-mediated nociceptive signaling. Clinical evidence was reviewed with attention to analgesic efficacy, sedative properties, and safety in neonates and children. Studies with favorable, neutral, or inconclusive findings were considered if they were relevant to the review scope.

Findings: Melatonin exerts significant antinociceptive effects through both central and peripheral mechanisms, which are synergistic with its antioxidant action. These effects are mediated by MT2 receptor activation, modulation of inflammatory pathways (including NOD-like receptor protein 3 inflammasome inhibition), and regulation of central neurotransmission. Clinical studies in neonates and children indicate that melatonin is effective in reducing pain during invasive procedures and may serve as a sedative alternative in diagnostic and procedural settings. Across studies, melatonin demonstrated a favorable safety profile, including in preterm infants.

Implications: Melatonin emerges as a safe, pleiotropic, and multi-target analgesic in pediatric care. Further translational and clinical research is warranted to better define its role in condition-specific pain management, as well as to establish optimal dosing strategies and routes of administration in neonatal and pediatric populations.

Introduction

Neonates and children often experience pain associated with many clinical conditions, such as surgical interventions, invasive procedures, and chronic inflammatory diseases. Pain in this population is frequently under-recognized and undertreated, leading to increased physiological stress and adverse neurodevelopmental outcomes.[1]

Oxidative stress (OS) may potentially contribute to pain perception by sensitizing nociceptive pathways.[2] The vulnerability of neonates and children to oxidative damage and pain highlights the need for thera-

* Address correspondence to: Serafina Perrone, MD, PhD, Department of Medicine and Surgery, University of Parma, Via Gramsci 14, 43125 Parma, Italy.
E-mail address: Serafina.perrone@unipr.it (S. Perrone).
# These authors contributed equally and shared the first authorship.

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peutic strategies that exhibits potent free radical (FR) scavenging and analgesic properties.[3]

The unique biochemical properties of melatonin make it an attractive therapeutic agent in pediatric populations. Its potent antioxidant capacity allows it to neutralize reactive oxygen species (ROS) directly and to upregulate endogenous antioxidant enzymes (AOEs), providing multilevel protection against OS, which is especially relevant in neonates and children vulnerable to oxidative damage due to immature defense systems.[4,5]

Beyond antioxidation, melatonin exhibits significant anti-inflammatory and analgesic effects. It modulates inflammatory cytokine production, inhibits proinflammatory pathways, and interacts with melatonin receptors in the central and peripheral nervous systems to reduce pain perception. These dual properties address both oxidative injury and pain, common challenges in pediatric clinical care.[4]

Furthermore, melatonin is naturally present in human breast milk and crosses the placenta during pregnancy, indicating its physiological safety and involvement in early circadian rhythm development.[6] Its favorable safety profile, low toxicity, and minimal side effects support its use in vulnerable pediatric groups, including preterm infants and children with chronic inflammatory or painful conditions.[7]

Despite increasing interest in melatonin as a pain-modulating agent, no recent review has comprehensively integrated molecular and cellular mechanisms with clinical evidence in the context of neonatal and pediatric analgesia. The present article is a narrative review. It aims to bridge this gap by synthesizing and critically discussing preclinical and clinical data on melatonin as a multitarget analgesic in pediatric populations. To improve transparency, the literature search strategy, eligibility criteria, exclusion criteria, and qualitative approach used to select the most informative studies are summarized in the Methods section.

Melatonin Synthesis: Pineal and Extrapineal Sources

Melatonin (*N*-acetyl-5-methoxytryptamine) is primarily synthesized in the pineal gland via a multistep enzymatic pathway. The synthesis begins with the hydroxylation of tryptophan to 5-hydroxytryptophan, catalyzed by tryptophan hydroxylase, followed by decarboxylation to serotonin. Serotonin is then acetylated by arylalkylamine *N*-acetyltransferase (AANAT), forming *N*-acetylserotonin, which is subsequently methylated by acetylserotonin *O*-methyltransferase to produce melatonin. AANAT is the rate-limiting enzyme and is regulated by the light–dark cycle, with activity increasing significantly during nighttime.[8]

While the pineal gland is the central source of circulating melatonin, extrapineal synthesis occurs in several tissues, including the gastrointestinal tract (GIT), retina, skin, bone marrow, and placenta. Extrapineal melatonin synthesis is independent of the pineal gland. Indeed, studies have shown that even in pinealectomized animals, melatonin continues to be produced in peripheral tissues such as the GIT and placenta, indicating that local synthesis supports tissue-specific protective functions.[9] In the GIT, melatonin is synthesized by enterochromaffin cells from dietary tryptophan through a serotonin intermediate. Notably, GIT melatonin levels are up to 10 to 100 times higher than in plasma, and up to 400 times higher than pineal levels, suggesting local autocrine and paracrine roles.[10]

The placenta also produces melatonin. Serum melatonin levels have been shown to increase especially during the first trimester of pregnancy and to decrease dramatically after delivery, suggesting that melatonin may play a role in maintaining a normal pregnancy.[11] In addition, placental melatonin crosses into fetal circulation, playing an essential role in fetal development, protecting against OS, and contributing to the early formation of circadian rhythms.[12]

Neonates, especially those born preterm, exhibit limited endogenous melatonin production at birth. Endogenous melatonin synthesis gradually begins to develop over the first few months of life. Up to approximately the third month of life, infants largely depend on maternal melatonin, which is transferred via the placenta during gestation and later through breast milk, to regulate circadian rhythms and promote sleep. Studies have shown that melatonin levels in breast milk are significantly higher at night than during the day, aiding in the establishment of sleep–wake cycles in the newborn.[6]

Generally, urinary excretion of 6-sulfatoxymelatonin (aMT6s), a major metabolite of melatonin, significantly increases between weeks 6 and 24 of life, with a nocturnal peak occurring between 2:00 and 10:00 AM. However, in preterm infants, this developmental process is delayed by approximately 9 weeks compared to term infants, reflecting the immaturity of circadian centers and the pineal gland.[13] This delayed melatonin production in preterm neonates has been associated with higher incidence of sleep disturbances and infantile colic. The absence of sufficient melatonin during this critical developmental window may negatively impact neurological development and circadian rhythm regulation.[2]

OS plays a significant role in neonatal and pediatric morbidity, arising from an imbalance between the production of ROS and the body's antioxidant defenses. Neonates, especially preterm infants, are particularly vulnerable due to their immature antioxidant systems and increased exposure to oxidative challenges during the perinatal period.[9] Perinatal hypoxia-ischemia is a critical source of oxidative injury, contributing to brain damage and long-term neurodevelopmental deficits.[14–16]

The interaction between pain and OS is complex, but it appears that OS can sensitize nociceptive pathways, potentially exacerbating pain perception.[17] For instance, chronic inflammatory conditions common in pediatric patients, such as juvenile idiopathic arthritis and inflammatory bowel disease, contribute to sustained OS and pain, complicating clinical management and impairing quality of life.[3,7]

Mechanisms of Action

Melatonin is a multifunctional molecule that is present in all organisms. While one of the most well-known roles is in regulating circadian rhythms in vertebrates, melatonin is known to have a much more ancient and primary function: serving as a potent FR scavenger and antioxidant.[18]

Several features make melatonin an effective antioxidant, including its free-radical scavenging cascade and its ability to support AOE production. These aspects are summarized in the following paragraphs.

FR are highly reactive chemical species and major contributors to OS. By initiating chain reactions, they propagate molecular damage. One of the best-known radicals is the hydroxyl radical (OH), which contributes substantially to DNA and tissue injury induced by ionizing radiation. Other reactive species include reactive nitrogen species (RNS) and reactive sulfur species.[19]

Radical trapping is an important mechanism that counteracts OS. Type I antioxidants (free-radical scavengers) directly react with FR, producing less reactive species and thereby interrupting the chain reaction.[20]

Melatonin has antioxidant capacity not only because it directly interacts with ROS, but also because its metabolites continue to neutralize FR. This sequential activity is known as the free-radical scavenging cascade. Through this cascade, a single melatonin molecule can detoxify multiple ROS and RNS, thereby amplifying antioxidant efficiency and broadening the scavenging spectrum.

In some cases, melatonin metabolites have been shown to be more efficient antioxidants than melatonin itself. For example, AFMK (N^1-acetyl-N^2-formyl-5-methoxykynuramine) is a key molecule in melatonin metabolism, produced both through enzymatic degradation and through interactions with FR. An investigative study demonstrated AFMK as a powerful reducing agent, capable of donating two electrons to neutralize radicals—unlike classical small-molecule antioxidants, which typically donate only one. Furthermore, in cell culture, AFMK protected hippocampal neurons from OS induced by hydroxyl radicals, glutamate, and amyloid β peptides. In rat liver tissue, AFMK also prevented lipid peroxidation in a dose-dependent manner.[21]

Antioxidant molecules represent only one component of the cellular defense against OS. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are key AOEs that work in a coordinated manner to remove ROS. SOD catalyzes the conversion of the superoxide radical (O2−) into hydrogen peroxide (H2O2) and oxygen (O2), after which other enzymes convert H2O2 into water. Several studies have shown that melatonin can upregulate these enzymes. Early studies reported increased GPx mRNA levels in rat brain cortex after both acute and chronic melatonin treatment, as well as increased SOD and GPx levels in neuronal cell lines even after exposure to physiological serum concentrations of melatonin.[22,23] In 2015, skin grafts preincubated with melatonin showed a significantly smaller decline in AOE levels after UV-induced photodamage, supporting the hypothesis that melatonin may help preserve antioxidant defenses under OS conditions.[24] Similar increases in AOE levels were also described after melatonin treatment in mouse models of retinal degeneration.[25]

Melatonin has been investigated to be neuroprotective of neonatal hypoxic-ischemic brain injury. In a 2024 murine study, intravenous administration of melatonin (25 mg/kg) to rats subjected to hypoxic-ischemic injury resulted in significant reduction of brain loss and increased oligodendrogenesis through the mediation of the AMPK/mTOR signaling pathway, as shown by a marked improvement in motor function.[26] Furthermore, according to a 2025 study by Liu et al,[27] intraperitoneal melatonin in rats with ischemic brain injury seems to have a neuroprotective effect by reducing the brain edema through the PI3K/AKT/Nrf2 signaling pathway, as well.

Aim

The aim of this review is to synthesize the available preclinical and clinical literature on the use of melatonin as an analgesic agent in the pediatric population, with a focus on efficacy, safety, and underlying mechanisms of action. By integrating molecular insights with clinical evidence across different pain-related settings, this review provides evidence-informed clinical decision-making and to identify key knowledge gaps that warrant further investigation.

Methods

Literature Search

A narrative literature search was performed to identify preclinical and clinical studies exploring the role of melatonin in pain modulation within neonatal and pediatric populations. Electronic databases, including PubMed and MEDLINE, were searched using combinations of Medical Subject Headings (MeSH) terms and free-text keywords. Search terms included “melatonin,” “pain,” “nociception,” “procedural pain,” “surgery,” “neonates,” “newborns,” and “children.” Reference lists of relevant articles and previously published reviews were also manually screened to ensure comprehensive coverage of the available literature. Because this was a narrative review, the purpose of the search was not to generate a formally exhaustive dataset for quantitative synthesis, but rather to identify mechanistic and clinical studies most informative for the objectives of the review.

Eligibility Criteria

Studies were considered eligible if they were peer-reviewed articles published in English and investigated the interaction between melatonin and pain-related pathways in neonatal or pediatric populations. Both preclinical and clinical studies were included when they provided mechanistic, molecular, or clinically relevant insights consistent with the objectives of the review. Given the narrative scope of the article, no restrictions were applied regarding study design, intervention setting, or outcome measures.

Studies were excluded if they did not involve neonatal or pediatric subjects, were unrelated to pain or nociceptive processes, or lacked sufficient relevance to the mechanistic or clinical focus of the review. Non-English publications, conference abstracts without adequate methodological detail, and studies focused exclusively on adult populations without clear translational relevance were also excluded.

Study Selection

Study selection was performed by the authors through screening of titles, abstracts, and full texts to identify publications relevant to the aims of the review. Particular attention was given to the studies ultimately summarized in Tables I and II, which were selected because they were considered the most representative of the available mechanistic and clinical evidence in neonatal and pediatric settings. Emphasis was placed on studies contributing novel mechanistic insights, clinically meaningful observations, translational relevance, or practical implications for pediatric pain management. Key information regarding experimental models, clinical settings, proposed mechanisms of action, analgesic efficacy, safety profiles, dose ranges, and routes of administration was qualitatively extracted and synthesized.

Data Synthesis

Given the heterogeneity in study populations, experimental models, clinical contexts, and outcome measures, findings were synthesized using a descriptive and thematic approach. Particular attention was devoted to identifying converging mechanistic pathways, including receptor-mediated effects, modulation of neuroinflammation, and interactions with central neurotransmitter systems. Clinical evidence was discussed in relation to analgesic efficacy, sedative properties, and safety in neonatal and pediatric settings. Because of the narrative design, no formal risk-of-bias scoring system was applied; instead, the discussion qualitatively highlights the relative strength and translational relevance of the available evidence.

A quantitative meta-analysis was not performed, as the available data were not sufficiently homogeneous to allow meaningful statistical comparison.

Results

Analgesic Effects

Recent evidence supports melatonin’s role as a multi-target analgesic, effective in chronic pain syndromes as well as in acute and neuropathic models. Its therapeutic benefits have been reported across several pain conditions, including migraines, fibromyalgia, and irritable bowel syndrome.[28–30] In addition to normalizing circadian rhythms and improving sleep quality, which can indirectly contribute to pain reduction, melatonin also exerts direct analgesic actions through modulation of multiple neurotransmitter and neuroimmune pathways. Table I reports preclinical studies investigating the use of melatonin as analgesic agent.

Melatonin mediates its analgesic activity mainly via MT2 receptors, with MT1 playing a more limited role, which are widely distributed throughout the mammalian central nervous system.[28,29]

In 2014, Chen et al[31] conducted a study in a murine model of visceral pain, administering melatonin both orally and intraperitoneally, along with two novel MT1/MT2 receptor agonists (Neu-P11 and Neu-P12), demonstrating a dose-dependent reduction in pain responses. Several studies consistently indicate that MT2 receptors are the primary mediators of melatonin-induced analgesia.[29,30,32–34] In 2015, Lopez-Canul et al found that the MT2 receptor agonist UCM924 alleviated pain behaviors in rats following L5-L6 spinal nerve ligation. The mechanism involves modulation of the descending nociceptive system of the brain-

Table I
Preclinical studies investigating the use of melatonin as analgesic agent.

Study	Species	Intervention (dose, route)	Outcome (O) and findings (F)	Source
Chen	Mice	Intraperitoneal or orally melatonin, Neu-P11, and Neu-P12 (melatonin receptor agonist; all 25, 50, and 100 mg/kg)	O: Orally and i.p. administered melatonin, Neu-P11, and Neu-P12 reduced pain responses in a dose-dependent manner. Neu-P12 was more effective and displayed longer duration of action compared to melatonin. The antinociceptive effects of Neu-P11 or Neu-P12 were antagonized by i.p. or i.c.v. administered naloxone F: All the agonists have reduced pain responses of visceral pain, Neu-P12 producing the most potent effect	31
Song	Rats	Intraperitoneal melatonin (10 mg/kg) or saline was administrated 10 min after morphine injection, for 14 days	O: Morphine-induced mechanical and thermal hyperalgesia were attenuated by co-administration of melatonin. The MPAE% (percent of maximal possible anti-nociceptive effect) was reduced approximately 60% in rats that received morphine treatment. However, following the treatment of morphine with melatonin, the MPAE% was reduced only about 30% F: Melatonin has potential to attenuate repetitive morphine-induced hyperalgesia and tolerance	45
Lin	Male mice	(1) Cuff + Vel group: i.p. injection of 100 mL/kg vehicle (1% ethanol/normal saline) (2) Cuff + Mel group: i.p. injection of 100 mg/kg Mel (3) Cuff + 8MP group: i.p. injection of 100 mg/kg 8MP (8-methoxy-2-propionamidotetralin, MT2 agonist) (4) Cuff + R + 4PP group: i.p. injection of 50 mg/kg ramelteon (MT1 and MT2 agonist) and 20 mg/kg 4PP (4-phenyl-2-propionamidotetralin, MT2 agonist)	O: Increased MT2 activation in Dorsal Root Ganglia DRG neurons of the cuff-implanted mice suppressed mechanical allodynia and thermal hyperalgesia Expression of neuroinflammatory cytokines could be modulated after MT2 activation by Mel or 8MP in the DRG neurons F: Both mechanical allodynia and heat hyperalgesia were relieved with exogenous Mel application in the DRG-friendly cuff models. Activation of MT2 by 8MP suppressed pain behaviors similar to Mel, while MT1 activation, using a combination of ramelteon and 4PP, did not produce a pronounced impact	30
Huang	Rats	Melatonine, orally, at a dose of 37.5, 75, or 150 mg/kg	O: Melatonin administration dose-dependently reduced neuropathic pain behavior, decreased glial and MAPKs activation, and diminished the release of pro-inflammatory cytokines in the ipsilateral cuneate nucleus after lysophosphatidylcholine (LPC)-induced median nerve demyelination neuropathy model F: Administration of melatonin, via its cognate MT_2 receptor, inhibited activation of glial MAPKs, production of pro-inflammatory cytokines, and development of demyelination-induced neuropathic pain behavior	33
Posa	Mice	//	O: In the Hot Plate Test and Formalin Test, mice with genetic inactivation of melatonin MT_1 ($MT_1^{-/-}$) display no differences compared to their wild-type littermates (CTL), whereas both $MT_2^{-/-}$ and $MT_1^{-/-}/MT_2^{-/-}$ mice showed a reduced thermal sensitivity and a decreased tonic nocifensive behavior F: Melatonin MT_2 receptor subtype is selectively involved in the regulation of pain responses	34
Liu	Mice	Microglia cells were pretreated with 200 μM melatonin for 30 min before morphine	O: There was a significant activation of the NLRP3 inflammasome in the prefrontal cortex and the peripheral blood of morphine-treated mice compared to control animals, which could be blocked by melatonin; co-administration of melatonin and low-dose morphine had better analgesia effects in the murine models of pain and led to a lower NLRP3 inflammasome activity in brain tissues F: Possible benefits in the co-administration of melatonin and morphine for the relief of severe pain	39
Wang	Male rats	Intraperitoneal melatonin injection	O: Melatonin has potent analgesic and anti-inflammatory effects in SNL-induced neuropathic pain via NF-κB/NLRP3 inflammasome signaling pathway F: The NF-κB/NLRP3/caspase-1 signaling cascade is a requisite for the neuroprotective effect of melatonin in neuropathic pain	37
Nacarkucuk	Rats	Intravenous melatonin (25 mg/kg)	O: To prove melatonin's short- and long-term neuroprotection and investigate its role on the AMPK (AMP-activated protein kinase)/mTOR (mammalian target of rapamycin) pathway following neonatal hypoxic–ischemic brain injury F: Melatonin treatment reduced brain area loss and promoted oligodendrogenesis with a clear improvement of motor function; melatonin-associated neuroprotection is regulated via the AMPK/mTOR/autophagy pathway	26
Liu	Rats	Intraperitoneal melatonin (5 mg/kg from Sigma)	O: To explore the neuroprotective effects of melatonin (Mel) administration on cerebral ischemia-reperfusion injury (CIRI) and elucidate its underlying mechanism in vivo to provide a theoretical foundation for the clinical application of Mel F: Mel administration exerts neuroprotective effects against CIRI by mitigating brain edema through upregulating the PI3K/AKT/Nrf2 signaling pathway, and then attenuating brain damage in CIRI rats	27
Wang	Mice	intraperitoneal melatonin (10 mg/kg) daily for 7 days	O: To investigate the effects of melatonin on neurovascular changes in rd1 mice, evaluating its therapeutic potential as an antioxidant for retinal degeneration F: Melatonin holds promise as a therapeutic approach for retinal degeneration by mitigating oxidative stress, thereby protecting photoreceptors and retinal vasculature	25

Table II
Clinical studies investigating the use of melatonin as analgesic agent.

Study	Target population	Intervention (dose, route)	Outcomes (O) and findings (F)	Source
Gitto	32-week gestation newborns admitted to NICU	Intravenous melatonin (10 mg/kg)	O: Serum levels of the pro-inflammatory cytokines IL-6, IL-8, and IL-12, similar before endotracheal intubation, were significantly lower at subsequent times because of melatonin treatment F: The use of melatonin as an adjunct analgesic therapy during procedural pain may be considered	[62]
Marseglia	<37 weeks pretermal babies with normal liver and kidney function tests	Oral dose of 0.5 mg/kg of melatonin, once a day in the morning, in the first week of life; the placebo group received 0.5 mL of 5% glucose solution	O: Nonprotein-bound iron NPBI advanced oxidation protein products AOPP did not show any statistically significant differences between the groups both at 24 and 48 h. At 48 h, the mean blood concentrations of F2-Isoprostanes were significantly lower in the MEL group than in the placebo group F: Early melatonin administration in preterm newborns reduces lipid peroxidation in the first days of life, showing a potential role to protect high-risk newborns	[63]
Behura	<34 weeks of PMA or BW < 2000 gr neonates	Oral melatonin (4 mg/kg)	O: The median PIPP score at 1′ and at 5′ between the 24% sucrose and the melatonin group were comparable. F: Oral melatonin is not inferior to oral 24% sucrose for pain management during ROP screening	[60]
Perrone	Newborn infants, gestational age >34 weeks, requiring surgery	Oral melatonin (0.5 mg/kg) in the morning, before surgery	O: The mean blood concentration of nonprotein-bound iron (NPBI), advanced oxidation protein products (AOPP), and F2-Isoprostanes (F2-IsoPs) significantly decreased from pre- to postoperative period in the melatonin group F: The administration of exogenous melatonin in newborn infants undergoing surgery reduces lipid and protein peroxidation in the postoperative period	[59]
Rahafard	3–6-year-old children	Oral melatonin (0.5 mg/kg maximum 5 mg) 30 min before venipuncture	O: The mean pain score and anxiety during venipuncture were lower in the melatonin group than in the control one F: Oral Melatonin before venipuncture reduces procedure-related pain	[61]
Gad	40 stable preterm infants <37-week gestational age	Oral melatonin (5 mg/kg) dissolved in 2 mL distilled water 30 min before cannula insertion	O: 60 min postcannula insertion, MDA levels were significantly higher in the PL group compared to the MT group ($P < 0.001$). The PL group had significantly higher PIPP-R scores and greater percentage of change in PIPP-R scores compared to the MT group, 5 min after cannula insertion F: Oral melatonin is a safe and effective therapy for preterm infants, showing promise in controlling neonatal pain with antioxidant effects	[53]
Perrone	Children between 3 and 5 years of age scheduled for elective surgery	Oral melatonin (0.5 mg/kg for a max 10 mg)	O: The reduction of oxidative stress and the modulation of circulating miR-34 and miR-124a, which target sirtuin 1 (SIRT1) activity F: A novel pathway underlying melatonin's antioxidant and analgesic effects mediated by SIRT1	[58]

stem, specifically decreasing the activity of pronociceptive ON cells and enhancing antinociceptive OFF cells.[29]

In 2017, Lin et al immunohistochemically analyzed MT1 and MT2 expression in dorsal root ganglia neurons, demonstrating a marked upregulation of MT2 expression in the dorsal root ganglia of cuff-implanted mice. Conversely, melatonin application significantly inhibited peptidergic neuronal activation and reduced neuroinflammation in the dorsal root ganglia via both MT2-dependent and MT2-independent pathways.[30]

In 2024, Wang et al[32] induced neuropathic pain in mice through spared nerve injury and observed a dose-dependent alleviation of pain symptoms following both intraperitoneal and anterior cingulate cortex administration of melatonin. Furthermore, MT2 receptor expression was significantly upregulated in spared nerve injury mice, and this upregulation was reversed following melatonin administration, while MT1 receptor expression remained unchanged. The study revealed dual analgesic pathways involving both neurons and microglia cells. In neurons, melatonin reduced the excitability of anterior cingulate cortex pyramidal neurons and suppressed presynaptic excitatory input. Regarding microglia, two phenotypes were distinguished: the classically activated M1 phenotype and the alternatively activated M2 phenotype. The M1 phenotype exhibits high expression of pro-inflammatory cytokines, contributing to neuroinflammations and the exacerbation of chronic pain. In contrast, the M2 phenotype exhibits anti-inflammatory properties. Melatonin treatment was shown to reduce the release of the inflammatory factors while simultaneously promoting the polarization of microglia toward the M2 phenotype.[32]

In the 2020 study by Huang et al, it was observed that melatonin administration exerts a dose-dependent antinociceptive effect in models of acute inflammatory and neuropathic pain in rats in which demyelinating damage is induced with lysophosphatidylcholine. Oral administration of melatonin significantly reduced the activation of glial mitogen-activated protein kinases (MAP kinases) and the release of proinflammatory cytokines, preserving myelin integrity at the lesion site and preventing neuropathic pain. Therefore, it appears that melatonin exerts these actions through the suppression of intracellular Ca2+ levels in glia and the inhibition of MAP kinase phosphorylation mediated by MT2 receptors on the Schwann cell membrane.[33]

In the study by Posa et al, the nociceptive responses of mice with genetic inactivation of MT1 or MT2 receptors, or both, were tested in the hot plate test (paradigm of acute pain) and the formalin test (paradigm of tonic pain). It was found that genetic inactivation of MT2 receptors, but not MT1 receptors, produces a distinct effect on the nociceptive threshold, suggesting that the MT2 melatonin receptor subtype is selectively involved in the regulation of pain responses. MT2 receptors are expressed in the periaqueductal gray, an area of the brainstem of the descending antinociceptive pathways. Injection of MT2 partial agonists, as well as melatonin, into this area silences pronociceptive neurons and activates antinociceptive neurons in the rostral ventral medulla, similar to other classes of analgesic drugs.[34]

Growing evidence suggests that neuroinflammatory mediators are critical for analgesic tolerance and hyperalgesia.[35] Among them, the NOD-like receptor protein 3 (NLRP3) inflammasome, composed of the sensor NLRP3, caspase-1, and the adaptor molecule, is the most studied inflammasome.[36]

Melatonin analgesic action appears also to involve the suppression of the nuclear factor-kappa B (NF-κB)/NLRP3 inflammasome signaling pathway.[37,38] Wang et al demonstrated the role of inflammasome ac-

tivation and the release of proinflammatory cytokines in neuropathic pain, observing a correlation between hypersensitivity and increased NF-κB phosphorylation in spinal tissues of rats subjected to spinal nerve ligation. Intraperitoneal administration of melatonin, however, reduced the levels of molecular markers of inflammation and inhibited the development of pain hypersensitivity.[37]

Moreover, Liu et al have shown that chronic morphine exposure leads to excessive cellular ROS production and activation of the NLRP3 inflammasome in microglia. Melatonin, on the other hand, appears to reduce ROS and consequently inhibit the activation of the NLRP3 inflammasome in microglia, which is also responsible for morphine-induced analgesic tolerance. In this study, the authors used the acetic acid pain model and demonstrated that the combination of morphine and melatonin had a better analgesic effect than morphine alone.[39]

There is evidence suggesting that the central effects of melatonin may involve the facilitation of GABAergic transmission via GABA receptor modulation. The participation of GABA neurons in melatonin's effects on the brain has been subject of studies in the last decade. Research has reported increased GABA levels in rat brain following pinealectomy, and decreased levels after melatonin injection. Intracerebroventricular administration of melatonin, B-endorphin and melatonin plus B endorphin all increased benzodiazepine binding to GABA-A in a dose-dependent manner.[40] Melatonin injection has also been shown to increment significantly GABA turnover in the hypothalamus, due to a stimulatory effect on the GABA synthesizing enzyme glutamic acid decarboxylase.[40] More recently, a study investigated the effect of melatonin on GABA-induced currents in cultured rat hippocampal neurons.[41] Melatonin rapidly enhanced these currents in a dose-dependent manner and increased overall GABAergic inhibitory transmission. These effects were not blocked by melatonin receptor antagonist, suggesting that the enhancement did not occur via activation of melatonin membrane receptors. However, the results were inhibited by benzodiazepine antagonist flumazenil, indicating that melatonin may act as an allosteric modulator at the benzodiazepine binding site of the GABA-A receptors.[41]

Melatonin appears also to exert its effect in supraspinal areas via the central opioid system, according to a study on visceral nociception and neurogenic inflammation rat models.[42] Opioids are used as analgesic agents for pain treatment of moderate to severe intensity, exerting their analgesic action by interacting with three major types of receptors: mu, delta, and kappa. Several observations show a significant interaction between melatonin and the opioid system in the brain.[39,43,44] Specifically, melatonin has been shown to prevent the onset of morphine-induced hyperalgesia and analgesic tolerance in rodent models. It is modulated, in part, by the inhibition of protein kinase C and downregulation of *N*-methyl-D-aspartate (NMDA) receptor activity, both of which are key molecular pathways to central sensitization and opioid tolerance.[45,46]

When administered to mice, melatonin has been shown to induce analgesia and enhance opioid antinociception on formalin-induced pain, while the administration of opioid receptor antagonist naloxone impaired the melatonin's analgesic response.[36] Melatonin, injected by intraperitoneal and intracerebroventricular routes, significantly enhanced the antinociceptive effects of delta opioid receptor agonist, but not those of mu opioid agonist. This indicates a receptor subtype-specific interaction, suggesting that melatonin may modulate opioid signaling through distinct mechanism depending on the receptor type.[37] Besides collateral effects, one major limitation of opioid usage is their tendency to produce tolerance. In an experimental study, conducted using the warm water tail-flick test to measure pain thresholds in mice, coadministration of melatonin prevented the development of antinociceptive tolerance to deltorphin-1, a delta opioid agonist. This attenuation of tolerance was then reversed by MT2 selective antagonist luzindole, proving the involvement of melatonin receptors in modulating opioid tolerance. However, melatonin did not affect the antinociceptive tolerance to endomorphine 1, a mu opioid agonist.[38]

The therapeutic effects of melatonin may also arise from its metabolism into the neurotransmitter serotonin, a newly recognized component of its pharmacological action. A 2016 study demonstrated that melatonin supports CYP2D-mediated synthesis of serotonin from 5-methoxytryptamine (5-MT), a melatonin metabolite. Following melatonin injection, the concentration of serotonin in various brain regions of mice was significantly increased. However, when animals were treated with pargyline (a MAO inhibitor, to prevent serotonin degradation), the effects of melatonin were no longer visible. This likely because the amount of serotonin synthetized from endogenous substrate and protected by MAO dominates the serotonin formed from melatonin metabolism. Interestingly, in pargyline rats treated with a partial lesion of the serotonergic system, melatonin still increased brain serotonin levels. This suggests that in conditions of serotonergic deficiency, melatonin-derived serotonin becomes more relevant. The CYP2D inhibitor propafenone prevented the melatonin-induced increased in serotonin levels. These findings indicate that melatonin support in vivo serotonin synthesis in the brain via CYP2D-catalyzed conversion of 5-MT.[47]

We have already seen how systemically administered melatonin has been reported to produce antinociceptive effects and to inhibit spinal nociceptive transmission in rats. Wind-up, a form of synaptic potentiation in the spinal cord, is a phenomenon evoked by repetitive stimulation of nociceptive C fibers and play a key role in the development and maintenance of chronic pain. An experimental study in anesthetized rats showed that intrathecal administration of melatonin induced a dose-dependent inhibition of the wind-up activity. This effect was prevented by the MT2 receptor antagonist luzindole. Since wind up is dependent on NMDA receptor activation, these results suggest that melatonin, acting via MT2 receptors, may also interfere with the NMDA mediated glutamatergic component of pain transmission.[48] In a different study investigating the link between pain and depression, Wistar-Kyoto rats (genetically predisposed to depressive behavior) with mechanical hyperalgesia induced by unilateral temporomandibular joint inflammation were found to have low plasma melatonin levels and downregulation of MT1 receptors, along with upregulation of the NMDA receptor NR1 subunit. In these rats, intracisternal administration of 6-cloromelatonin attenuated the mechanical hyperalgesia and depressive behaviors, while downregulating NR1 expression. Furthermore, melatonin dose dependently decreased NMDA-induced currents in substantia gelatinase neurons of the spinal cord dorsal horn.[49]

Figure depicts the principal analgesic mechanisms of melatonin, predominantly mediated by MT2 receptor activation, involving supraspinal, spinal, and peripheral pathways that converge to reduce neuroinflammation, central and peripheral sensitization, and opioid tolerance.

Clinical Evidence

Melatonin is recognized as a compound with an excellent safety profile, with studies reporting a very low incidence of adverse events, including those occurring in the long term. In the pediatric population, it has been widely administered, even in patients with comorbidities and concomitant pharmacological treatments, without evidence of clinically relevant side effects.[50,51] Considering the increased pain sensitivity and the frequent need for invasive procedures in preterm infants, melatonin has been investigated as a potential adjuvant analgesic in this particularly vulnerable group.[7,52,53] Table II reports clinical studies investigating the use of melatonin, as analgesic agent, in neonates and children.

Pain evaluation in neonates is generally performed using validated assessment tools, among which the Behavioral Indicators of Infant Pain and the Premature Infant Pain Profile (PIPP) are regarded as the most reliable.[54] Moreover, clinical evidence indicates a correlation between moderate-to-severe pain in preterm newborns (PIPP > 5) and reduced serum melatonin concentrations.[55]

Melatonin can be administered, depending on the indication, orally or intravenously, with the former being more subject to clinical studies and the latter to animal studies. As regards the enteral route, there are several commercially available formulations: capsules, immediate

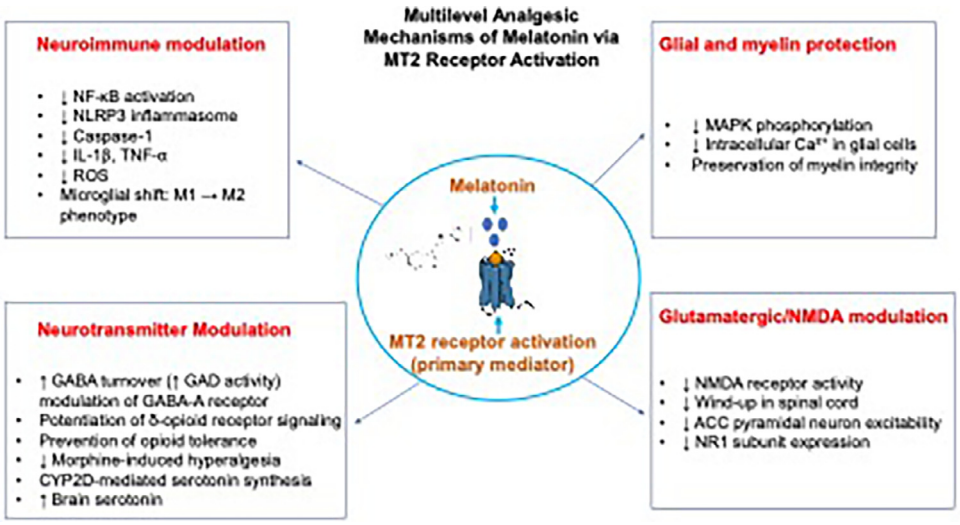

Figure. Melatonin exerts multilevel analgesic effects primarily through MT2 receptor activation. At supraspinal level, it enhances descending inhibition and potentiates opioid and GABAergic signaling. At spinal level, it inhibits NMDA-dependent wind-up and suppresses NF-κB/NLRP3-mediated neuroinflammation. Peripheral actions include modulation of dorsal root ganglia neurons and glial phenotype switching, ultimately reducing central and peripheral sensitization and limiting opioid tolerance. ACC = anterior cingulate cortex; NF-kB = nuclear factor-kappa B; NLRP3 = NOD-like receptor protein 3; NR1 = NMDA receptor subunit 1.

tablets, prolonged tablets, and oral solutions. The oral bioavailability of the molecule ranges from 1% to 56% with an average of 15%, depending on gender, age, and formulation. Other studies document oral bioavailability from 2.5% to 33%. Melatonin appears to have poor oral bioavailability because, in addition to poor enteral absorption, it has a metabolism characterized by a hepatic first-pass effect. Sex was defined as biological sex (male/female) recorded at birth.[50,56]

A pivotal study published in 2016 compared melatonin and midazolam as premedication in pediatric patients aged 5 to 14 years undergoing surgical procedures. The authors reported that oral administration of melatonin at a dose of 0.5 mg/kg, 40 minutes prior to anesthesia induction, significantly reduced the required dose of propofol, outperforming midazolam in this regard.[57] The benefits from the administration of oral melatonin in pediatric anesthesia before surgery may be related to its impact on modulating the Sirtuin 1 circulating levels, a key regulator of redox balance and inflammation.[58]

In the prospective, randomized, blinded study conducted in 2023 by Perrone et al, was evaluated the efficacy of melatonin supplementation on perioperative OS in newborns undergoing surgery. A single oral dose of melatonin (0.5 mg/kg) was administered to newborns before surgery, and they were compared with a control group. It was found that both in the preoperative and postoperative periods, melatonin concentrations were significantly higher in the treated group than in the untreated group, and blood concentrations of OS markers (nonprotein-bound iron, advanced oxidation protein products, and F2-isoprostanes) in the treated group decreased significantly from the preoperative to the postoperative period. This demonstrates that the administration of exogenous melatonin in newborns undergoing surgery reduces lipid and protein peroxidation in the postoperative period, showing a potential role in protecting patients from the consequences of OS.[59]

Furthermore, a recent clinical trial by Behura et al investigated the analgesic efficacy of melatonin during retinopathy of prematurity screening in preterm infants. The study reported that oral administration of melatonin at a dosage of 4 mg/kg in neonates younger than 34 weeks of postmenstrual age significantly reduced procedural pain, supporting its safety and effectiveness as an analgesic in neonatal ophthalmologic care.[60]

Melatonin has also been investigated as a potential pain and anxiety reliever in the pediatric population for procedural pain, ie, venipuncture. A 2024 double-blind randomized clinical trial highlighted how the administration of oral melatonin at a dose of 0.5 mg/kg, 30 minutes prior to venipuncture, significantly reduces procedure-related pain and anxiety in pediatric patients and may also be associated with improved venipuncture success rates.[61] Melatonin can be used in numerous contexts because of its anti-inflammatory, antioxidant, anticonvulsant, and circadian rhythm regulator effects. In adult patients, the dosage used to treat insomnia ranges from 3 to 10 mg, but in some diseases, such as amniotrophic lateral sclerosis, dosages as high as 300 mg/day are routinely used for neuroprotection. Regarding the pediatric population, the studies available in the literature are scarce, and the dosage of the drugs used in the various populations investigated varies according to the indication.[50]

In a study by Gitto et al, melatonin was used, for analgesic purposes, at a dose of 10 mg/kg intravenously along with vecuronium, atropine, and fentanyl in 30 premature infants (gestational age ≤32 weeks) during the orotracheal intubation procedure. This population was compared with the control group, finding a lower level of proinflammatory cytokines (IL-6, IL-8, IL-12) and higher level of anti-inflammatory cytokines (IL-10), in patients treated with melatonin. Clinically, no differences were found between the two groups in the Neonatal Infant Pain Scale, while the values recorded in the PIPP scale were significantly lower in melatonin-treated patients, demonstrating that the molecule might have benefits from an analgesic and anti-inflammatory point of view in preterm infants during painful procedures.[50,62] In addition, a study by Marseglia et al investigated the analgesic and anxiolytic use of melatonin at a dose of 0.5 mg/kg (maximum 5 mg) in 60 children aged 1 to 14 years before venipuncture, with findings of reduced procedure-related pain and anxiety in the melatonin-treated population compared with those treated with placebo.[63]

Melatonin can also be used for diagnostic procedures where sedation is required, such as magnetic resonance imaging. Several studies in the literature have used melatonin in patients from 3 months to 6 years of age at dosages from 3 to 20 mg, depending on weight and age, and concluded that melatonin is a good alternative to general anesthesia, particularly in patients less than 3 years of age.[50,63] Melatonin can also be used to perform electroencephalography during sleep. This is a difficult test to perform in the pediatric population, particularly those with neurodevelopmental delay, where sleep is usually achieved by deprivation or sleep-inducing drugs. Indeed, in the study by Wasserman et al, melatonin (at a dose of 2.5 or 5 mg depending on age) was used in 68 patients who were to undergo electroencephalography during sleep without finding any differences in sleep macrostructure compared with the deprivation method, concluding that melatonin is a good alternative.[63]

Melatonin can also be used with good efficacy in the treatment of sleep disorders in the pediatric population, particularly in children with neurodevelopmental disorders.[64]

Discussion

This review highlights melatonin as a biologically plausible and clinically promising modulator of pain, particularly in pediatric and neona-

tal settings, where safe and noninvasive analgesic strategies are urgently needed. The convergence of preclinical and early clinical findings suggests that melatonin acts through multiple, partially overlapping mechanisms that extend beyond circadian regulation and sleep promotion, encompassing neuroimmune modulation, synaptic plasticity, and neurotransmitter signaling.

A central theme emerging from experimental studies is the predominant role of MT2 receptors in mediating melatonin-induced antinociception, coupled with a suppression of neuroinflammatory signaling pathways, including NF-κB and NLRP3 inflammasome activation.[28,29,37,38] These mechanisms are especially relevant in developmental contexts, where glial activation, central sensitization, and inflammatory priming may disproportionately influence pain perception. However, most mechanistic insights are derived from animal models, and how these pathways are regulated across different stages of neurodevelopment remains largely unexplored.[41,42]

Clinical evidence, although still limited, supports melatonin's favorable safety profile and its potential utility as an adjuvant in procedural pain and perioperative care. Reductions in pain scores, inflammatory markers, and sedative requirements suggest that melatonin may enhance analgesic efficacy while minimizing pharmacological burden, an aspect of particular importance in preterm infants and young children.[57,58,59] Nevertheless, the heterogeneity of study designs, dosing regimens, and routes of administration precludes firm conclusions regarding optimal therapeutic protocols. From a practical bedside perspective, the currently reported doses should not be considered interchangeable. Lower oral doses (eg, 0.5 mg/kg) have mainly been studied for premedication, venipuncture, or perioperative support, whereas higher doses or intravenous administration have generally been used in critically ill preterm infants or in settings where anti-inflammatory and antioxidant effects were also being targeted.[50,57,59,62,63] This means that dose selection in clinical practice cannot yet be extrapolated across indications, age groups, or routes of administration without caution.

In particular, the wide interindividual variability in oral bioavailability may lead to substantial differences in systemic exposure, even when similar weight-based doses are administered.[65] This variability is likely driven by differences in gastrointestinal absorption, formulation-dependent release, and the extent of hepatic first-pass metabolism.[66] These aspects are further complicated in neonatal populations, where drug metabolism is developmentally regulated. Neonates, and especially preterm infants, exhibit immature hepatic enzyme systems, including cytochrome P450 isoforms involved in melatonin metabolism (eg, CYP1A2), as well as reduced renal clearance.[67] As a consequence, melatonin may exhibit a prolonged half-life and higher plasma concentrations in this population than in older children.[68] While this could potentially enhance therapeutic efficacy, it may also increase variability in clinical response and raise important considerations regarding optimal dosing intervals and accumulation with repeated administration.

A major translational challenge lies in the incomplete characterization of melatonin pharmacokinetics and pharmacodynamics in pediatric populations. Factors such as age-dependent metabolism, variable oral bioavailability, hepatic first-pass effects, and circadian influences may substantially affect therapeutic responses. The wide reported range of oral bioavailability suggests that identical oral doses may yield very different systemic exposures, particularly in neonates whose absorption, hepatic metabolism, and clearance are developmentally immature.[50,56] As a consequence, apparent differences in efficacy between studies may partly reflect pharmacokinetic variability rather than true pharmacodynamic inconsistency. This issue is especially relevant when translating findings from older children to preterm infants, in whom slower metabolism and different body-water composition may alter both onset and duration of action. These variables are rarely standardized or directly measured in existing studies, limiting the comparability and generalizability of clinical findings.

Future research should therefore prioritize adequately powered, age-stratified randomized controlled trials integrating standardized pain assessment tools and biomarker-based endpoints. These studies should also clarify the clinical implications of melatonin-opioid interactions. Although preclinical findings suggest potential opioid-sparing effects and attenuation of tolerance, current evidence does not yet justify assuming the same magnitude of benefit in routine pediatric practice, especially for commonly used mu-opioid agonists such as morphine and fentanyl.[39,43–46] At present, the most clinically relevant interpretation is that melatonin may represent an adjunct rather than a replacement for opioid analgesia, with possible value in reducing inflammatory amplification of pain or analgesic requirements in selected settings. Longitudinal investigations are also warranted to evaluate the impact of repeated melatonin exposure on neurodevelopment, circadian organization, and long-term pain sensitivity. A translational framework combining clinical outcomes with mechanistic biomarkers of neuroinflammation and synaptic plasticity may be particularly valuable in defining melatonin's precise role within multimodal pediatric analgesia.

In conclusion, melatonin represents a low-risk, biologically versatile candidate for adjunctive pain management in children and neonates. While current evidence is encouraging, it remains uneven and is based on studies that vary substantially in design and quality. In addition, within the pediatric and neonatal scope of this review, studies showing a clear absence of analgesic or antioxidant effect are limited, which may reflect both the early stage of the field and selective concentration of the literature on promising settings. For this reason, melatonin should still be viewed as a promising but not yet definitive option, and rigorous translational and clinical studies are essential to move from empirical use toward evidence-based integration into pediatric pain and sedation protocols.

Author Contributions

S.P.: conceptualization; supervision; visualization; writing—original draft; writing—review and editing. L.C.: supervision; project administration; writing—review and editing. S.G., L.B., P.L., E.C., C.P.: writing—original draft; writing—review and editing. V.R.: visualization; supervision; writing—original draft; writing—review and editing. V.B.: visualization; writing—review and editing. S.C.: supervision; visualization; writing—review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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