Review

Effects of melatonin on the circadian functions of sleep-wake cycle, metabolism, hormonal regulation and immune activity: A recent review

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ABSTRACT

Rhythms following a period of approximately 24 hours are called circadian (from Latin circa diem, approximately one day) rhythms. These rhythms are observed in the activities of various vital body functions. Melatonin is considered as an important molecule participating in the formation of circadian rhythms of virtually all organisms. As a molecular regulator of the circadian clock, melatonin has various regulatory functions in both physiological and pathological conditions. Sleep-wake cycle depends on CSF melatonin levels, and melatonin also has a protective effect on the disrupted sleep-wake cycle in various pathological conditions. Melatonin ensures the proper function of vital metabolic pathways; therefore, it improves metabolism-related systems and protects them from damage. A bidirectional relationship between hormonal activity and melatonin ensures it having a healing effect on various reproductive disorders. Finally, melatonin can target inflammation pathways and various elements of immune system by changing their behavior and structure. In summary, melatonin has important effects on vital body functions mediated by its receptors, signaling pathways and clock genes, and has the capacity to protect and improve these functions under pathological conditions.

Key words: Melatonin, circadian genetics, metabolism, immunity, hormone, sleep-wake cycle

1. INTRODUCTION

The circadian rhythm links between the external environmental factors and the internal activities. This link is genetically encoded in a daily cycle to adapt to the environmental alterations. It contributes to the synchronization of neurological, cardiovascular, and metabolic activities in the physiological processes. At the molecular level, melatonin emerges to target transcription factors that involve in the translation and translocation of circadian genes. In this way, it regulates intracellular signaling pathways including DNA damage repair, cell proliferation, and immune response formation (1, 2). The genes of the circadian regulation, insulin secretion and glutamate signaling are widely expressed in the hindbrain, pituitary and hypothalamus. They are responsible for the formation of the rhythm (3). A variety of

physiological systems are under the control of different circadian rhythms, and the complex structure formed by all circadian rhythms is called the circadian clock network. The synchronization of the circadian clock network with the external environments is achieved by the central pacemaker, the suprachiasmatic nucleus (SCN) of the brain (4). Through the receptors in the SCN, the neurohormone melatonin regulates the circadian rhythm (5).

Melatonin, a regulator of the sleep-wake cycle, is an indolamine hormone primarily synthesized in the pineal gland (6). Its synthesis is light sensitive, and its synthesis increases in the dark while decreases in the presence of light (6). Besides its light-dependent regulation, melatonin synthesis is also regulated by the SCN (7). Melatonin can bind to the melatonin receptors on SCN neurons and affects the firing profiles of these neurons (8). In this way, melatonin, the endogenous synchronizer and a chrono biotic molecule, inputs dark information to the central clock and ensures its synchronization with the external environments (9). In parallel with its regulating effect on the central clock, melatonin has important effects on various peripheral circadian rhythms of the body (10, 11).

2. CIRCADIAN CLOCK

Based on the molecular mechanism of the circadian rhythm, several transcriptional-translational feedback loops that contain different positive and negative regulatory factors are intertwined with each other (12). The transcription factor, Circadian Locomotor Output Cycles Kaput (CLOCK) and the protein Brain and Muscle Arnt-like Protein-1 (BMAL1) play the central roles of the circadian clock. The heterodimerization of CLOCK and BMAL1 increases the expression of Cryptochrome (CRY) and Period (PER) transcription factors. In turn, CRY and PER inhibit the expression of BMAL and CLOCK genes, forming the first feedback loop (13). Also, CLOCK-BMAL heterodimerization stimulates the expression of REV-ERBα/b transcription factors and RORα/b/γ (Retinoid Acid related Orphan Receptors) receptors (14, 15). REV-ERBs work as direct negative regulators for BMAL1, while RORs work as direct positive regulators for BMAL1 (14, 15). RORs and REV-ERBs act on BMAL1 functionally competitive with each other, forming the secondary loop (14, 15). REV-ERBs are also involved in the regulation of the rhythmic control of ROR expression by suppressing the expression of NFIL3 (Nuclear Factor, Interleukin 3 regulated), which is an inhibitor of DBP (D-box Binding Protein), another transcriptional factor whose expression is stimulated by BMAL (16). In this way, the third transcription-translation cycle involved in REV-ERB and RORs is constructed in association with BMAL (16). With these three nested cycles, a time-of-day-dependent expression profile of clock genes is formed. Circadian regulation of many physiological functions occurs by the interaction of circadian genes directly or indirectly with various molecular mechanisms (2).

3. MELATONIN AND SLEEP-WAKE CYCLE

The sleep-wake cycle is one of the most important physiological states for mammals including humans. This cycle is regulated by homeostatic balance and circadian clocks. Orexin and hypocretin neuropeptides produced by neurons help to establish the sleep-wake balance. In addition, melanin-concentrating hormone (MCH) production by neurons in the lateral hypothalamic region also contributes to this balance (17). Sleep and wake times consist of S and C processes. These two processes are the main regulators of sleep-wake. The S process, which is referred to as the sleep homeostasis, consists of sleep pressure. The C process known as the circadian process creates a daily rhythm synchronized with the core temperature (18). Sleep contains stages of both non-rapid eye movement (NREM) and rapid eye movement (REM). In NREM sleep, the energy of the brain is conserved and the memory is consolidated.

Dreaming and cortical activation are seen in REM sleep. Control of the glutamatergic reticular formation neurons by cholinergic, aminergic and GABAergic neurons takes place in REM sleep. Sleep begins in the ventrolateral preoptic nucleus with endogenous chemical signals and circadian input from the anterior hypothalamus. Suppression of wake-promoting systems by adenosine, nitric oxide and GABAergic neurons is responsible for sleep initiation. Responding to external stimuli and acting consciously are called alertness. Circadian system damage, inconsistencies of the cycle and disrupted melatonin secretion will trigger sleep-wake disorders (19-21). The melatonin levels are important modulator of sleep. The opposite interaction of cortisol and melatonin affects sleep-wake. Thus, melatonergic agents can treat sleep disorders and modify the circadian rhythm. These agents are recommended for treatment of sleep disorders caused by various disorders including neurological and metabolic disorders (22).

The effect of melatonin on cognitive function in mice exposed to isoflurane was studied. Isoflurane can cause cognitive disorders by affecting the sleep-wake rhythm. Melatonin pretreatment in this condition resulted in improvement of cognitive function and normalization of circadian rhythm (23). The results indicate that melatonin may be an option for the treatment of anesthesia-induced cognitive dysfunction. The effect of melatonin on sleep disorders caused by propofol, another compound used for anesthesia, was also examined. In the study, melatonin pretreatment normalized the circadian factor expression (24). The postoperative sleep-wake disorders can also be corrected with melatonin treatment. Dialysis in patients with kidney diseases causes sleep disorders by disruption of the sleep-wake cycle and melatonin rhythm while the sleep quality and daytime activities were improved when patients were switched to nighttime hemodialysis (25) since nocturnal hemodialysis improved nighttime melatonin rhythm. Studies have been performed to investigate the influence of daytime, nighttime hemodialysis and peritoneal dialysis on sleep-wake cycle and melatonin rhythms. The results showed that sleep was impaired in all 3 groups of patients. Among them patients with daytime hemodialysis were most affected. Increased melatonin level was observed in patients with nocturnal hemodialysis, while this was not the case in patients with peritoneal dialysis (26) even the peritoneal dialysis was performed at night, the melatonin level was still relatively low. The lack of increase in melatonin may play a secondary role in the sleep-wake rhythm of these patients. In a study on diabetic patients, melatonin levels and sleep-wake cycles were investigated in patients with or without diabetic retinopathy (DR). The activity-rest intervals increased but, the nighttime melatonin level decreased in patients with DR (27). This may reflect the circadian rhythm disturbance in diabetic patients with DR. Disruption of melatonin production at night damages sleep-wake rhythm and this can also occur in breast cancer patients with chemotherapy. The first application of chemotherapy is more often to cause sleep-wake disorder in breast cancer patients (28). Repeated administration of chemotherapy may contribute to the progression of impaired nocturnal melatonin production, although administration of final cycles is less relevant to those with the disorder.

Sleep disturbance is common in orthopedic patients. Melatonin therapy was attempted to improve their sleep problem and, accordingly, the quality of their life; however, the clinical trial failed to achieve these goals (29). The patients with Williams-Beuren syndrome (WBS) also suffer sleep disorders. WBS is a neurodevelopmental disorder accompanied with behavioral problems caused from a microdeletion on chromosomic region 7q11.2. The relationship between behavior, memory, and sleep-wakefulness with melatonin was investigated in these patients. Low melatonin level may cause sleep disorders in individuals with WBS (30). There is a potential association between the low melatonin level and impaired memory and behavior in these patients. The sleep-wake cycle is also disrupted in neurodegenerative diseases including Smith-Magenis Syndrome (SMS), Parkinson's disease (PD) and Alzheimer's disease (AD). SMS is caused by a 17p11.2 deletion involving the Retinoic Acid Induced 1 (*RAI1*) gene. In this syndrome, circadian rhythm of melatonin

secretion is reversed. The high level of serum melatonin occures during the day instead of at night. This situation causes severe sleep disorders and the maladaptive daytime behaviors such as a general sleep-wake rhythm disorder (20, 32). As to the relationship between SMS, RAI1 and circadian rhythm the, heterozygous mutations in RAI1 which was found to cause impaired circadian rhythm (32). This can lead to decreased cognitive performance, abnormal sleep-wake cycles and abnormal eating patterns. Interestingly, clinical study showed that all SMS patients tested had significant sleep disorders with abnormal melatonin circardian rhythms (33). Changes in the metabolism, production, secretion and distribution of melatonin in these patients suggested the circadian rhythm disturbances. Accordingly, tasimelton, a melatonin alanog was used to treat the sleep disorders of SMS by significant improvment of the average sleep quality of patients (34). Tasimelton can be used as an effective and safe drug for SMS.

There is a causal relationship between neurodegenerative diseases and the sleep-wake cycle disorders (35). Modulation of the sleep-wake pattern may be a therapeutic target for these diseases. For example, the PD patients with dopaminergic therapy significantly increased their melatonin secretion. However, a delayed onset of sleep was found compared to the onset of melatonin in dim light (36), indicating the separation of circadian and sleep control of dopaminergic therapy in PD.

In addition to metabolic, orthopedic, or neurological diseases, the medical procedures of chemotherapy, dialysis and anesthesia also cause disruption of sleep-wake balance in humans. Sleep-wake balance is regulated by the melatonin levels. Study in melatonin's effects on sleep-wake disorders will provide basic knowledge to effectively treat these disorders with melatonin. The roles of melatonin on sleep-wake cycle and its potential mechanisms are summarized in Table 1 and Figure 1.

Table 1: Summaries of the roles of melatonin in sleep-wake cycle.

Study Type	Disease Model	Drug and Dose	Observation	Remarks	Ref.
In vivo	C57/BL-6J mice Isoflurane Anesthesia	Melatonin (10 mg/kg)	Isoflurane used for anesthesia causes cognitive disorders by affecting the sleep-wake rhythm. Melatonin pretreatment resulted in reversal of cognition and normalization of circadian rhythm.	Melatonin pretreatment may be an option for the treatment of anesthesia-induced cognitive dysfunction.	(23)
In vivo	Male Sprague Dawley (SD) rats Propofol Anesthesia	Melatonin (15 mg/kg)	Melatonin pretreatment could restore circadian rhythms altered by anesthesia, regulate circadian factor expression through post-translational modulation and inhibiting the over-synthesis of melatonin.	Postoperative sleep-wake disorders can be corrected with melatonin pretreatment.	(24)
Human Study	Daytime hemodialysis patients (n=13)	-	Sleep quality and daytime activities were improved when patients who received daytime hemodialysis were switched to nighttime hemodialysis.	Nocturnal hemodialysis can improve the sleep quality of patients by improving nighttime melatonin rhythm.	(25)
Human Study	Conventional daytime dialysis (n=20), Nocturnal hemodialysis (n=13), Automated peritoneal dialysis patients (n=6)	-	Nocturnal melatonin increase was normal in nocturnal hemodialysis patients. However, in daytime hemodialysis and automated peritoneal dialysis patients this rise was absent.	Although peritoneal dialysis was performed at night, the lack of increase in melatonin may play a secondary role in the sleep-wake disorders of these patients.	(26)
Human Study	Patients with diabetes (n=54) DR (n=25), Without DR (n=29)	-	Activity-rest intervals increase, and nighttime melatonin concentration decreases in diabetic patients with DR.	This may be an explanation for the circadian rhythm disturbance in diabetic patients with DR.	(27)
Human Study	Stage I-III postoperative female primary breast cancer patients	-	The first application of this chemotherapy is more effective in sleep-wake disorder in breast cancer patients.	Repeated administration of chemotherapy may contribute to the progression of impaired nocturnal melatonin production, although administration of final cycles is less relevant to those with the disorder.	(28)

Human Study	Orthopedic trauma patients (n=84)	Melatonin (5 mg)	Melatonin treatment applied in these patients did not change sleep quality, pain and quality of life.	Further study is required to increase the melatonin dosage.	(29)
Human Study	WBS patients (n=15), HC (n=20)	-	Low melatonin level may cause sleep disorders in individuals with WBS.	In this case, cognitive skills including memory and behavior may be negatively affected with melatonin level.	(30)
In vitro	(HEK) 293T cells	-	Heterozygous mutations in RAI1 and Rai1 were found to cause impaired circadian rhythm of melatonin	Impaired circadian rhythm of melatonin can cause decreased cognitive performance, abnormal sleep-wake cycles and eating patterns.	(32)
Human Study	SMS patients (n=28)	-	All SMS patients in the study were found to have significant sleep disorders in accordance with the criteria.	Changes in the metabolism, production, secretion and distribution of melatonin maycause sleep disorders .	(33).
Human Study	SMS patients	Tasimelton	Tasimelton significantly improved the average sleep quality in SMS patients	Tasimelton, a melatonin along, can be used as an effective and safe drug in SMS.	(34)
Human Study	PD patients (n=29) [untreated (n=13), treated with dopaminergic medicine (n=16)], HC (n=28)	-	PD patients treated with dopaminergic drugs increased melatonin production and a delayed onset of sleep vs the onset of melatonin in dim light compared to the non-treated PD.	This may indicate the separation of circadian and sleep control of dopaminergic therapy in PD.	(36)
GWAS	Sleep traits (n=12)	-	There is a causal relationship between neurodegenerative diseases and the sleep-wake cycle.	Modulation of the sleep-wake pattern by melatonin may be a therapeutic target for these diseases.	(35)

DR: Diabetic Retinopathy, HC: Healthy Control, PD: Parkinson's Disease, WBS: Williams-Beuren syndrome, GWAS: Genome-Wide Association Study.

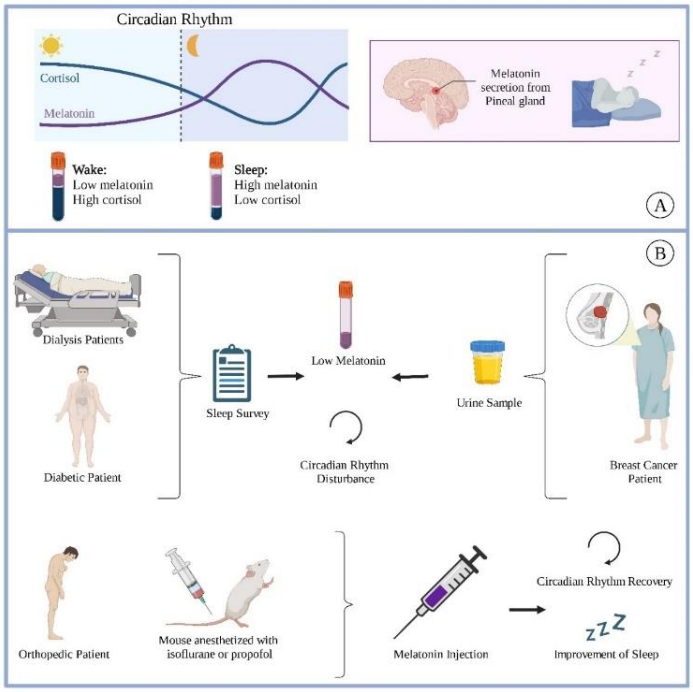

Fig. 1. Potential mechanisms of melatonin on sleep-wake cycle.

A) *Melatonin and cortisol have the reversed circadian rhythms. When awake, melatonin levels are low and cortisol levels are high. In the sleep state, the opposite is observed. In this way, the sleep-wake cycle is established.* ***B)*** *Sleep problems with low melatonin are common in a variety of diseases. In diabetic patients with retinopathy received daytime dialysis had the sleep disorder and low melatonin level. Melatonin supplementation improves circadian rhythm, sleep quality, and cognitive activity in orthopedic patients and mice anesthetized with isoflurane or propofol.*

4. MELATONIN AND METABOLISM

The circadian pattern of metabolism in organisms is developed to adapt the environmental alterations. Circadian rhythms regulate the activity of metabolic-related tissues and organs including the liver, adipose tissue, and digestive tract, where metabolic activity is dominant. (37, 38). The rhythmic release of insulin is regulated by the pancreatic clock while liver clock genes are involved in the stabilization of blood glucose level (39, 40). BMAL1 is known to have an effect on adiposity and body weight (41). Many gastrointestinal tract (GI) functions, such as the regulation of the integrity of the intestinal barrier, the rhythmic activities of digestive hormones and muscles, show circadian fluctuations. Disruption of the GI circadian clock leads to GI system vulnerable to many disorders. Peripheral clocks of metabolic activity can also be controlled by the hunger-satiety cycle, independent of the central clock's response to light (42). For example, NAD+ synthesis, which is an important molecule of the body's energy level, takes place in relation to circadian genes (43). Sirtuin family proteins, which are NAD+ dependent deacetylases, are important signaling molecules of metabolic pathways (44). In addition to being important energy sensor molecules, sirtuins also show a protective effect against metabolic stress (44). In particular, SIRT1 (silent mating type information regulation 2 homolog) rhythmically deacetylates the BMAL1 and PER2 clock genes, creating a feedback response on BMAL1:CLOCK activity (45). Due to the effect of SIRT1 on the basic genes of the circadian clock, metabolic activity is directly related to the circadian clock system. Since melatonin is a main regulator of the circadian clock network and the sirtuin family proteins are tightly related to metabolic clocks both have been the central focus of many studies which attempt to elucidate etiology of metabolic disorders (Table 1).

The effect of melatonin on liver clock genes was investigated in chicks whose circadian rhythm was changed by monochromatic light illumination. Improvement in the rhythm of plasma melatonin level with the improvement in light-dependent deterioration of clock gene expression has been reported in chicks exposed to different wavelengths of light, especially in green light (46). Melatonin may be an important mediator in the effect of monochromatic light on the circadian rhythm of clock genes in the liver. Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder (47). Cellular stress occurs as a result of impaired signaling due to the increased lipogenesis in NAFLD pathology (47). Because NAFLD is the primary cause of chronic liver disease, its relationship with melatonin has been closely studied. In a study using cell culture and animal model together, the effect of melatonin on the stress response genes of the liver in circadian disruption was investigated. In the cell culture, melatonin shifted the circadian oscillation of liver stress response genes Nrf2 and HO-1 towards physiological rhythm (48). In the animal model, a NAFDL-like clinical manifestation was induced by a high-fat and fructose diet. In this animal model, the rhythms of genes responsible for lipid regulation and oxidative stress defense exhibited similar pattern as cell culture after melatonin treatment (48). In an additional study, the effect of melatonin was examined in terms of regulation of gene expression in NAFDL, to compare healthy mice with SIRT1 +/- TG mice, melatonin suppressed the SIRT1 inhibitor miRNA-34a-5p and increased the expression of SIRT1 and reduced liver fat accumulation and ER stress (49).

Inflammation is another common biomarker of various liver diseases including NAFLD. The effect of melatonin on the sepsis-damaged liver and its relationship with the SIRT1/STAT3 pathway were investigated *in vivo*. Melatonin treatment ameliorated abnormal gluconeogenesis in damaged liver of mice via the SIRT1/STAT3 signaling pathway (50). The findings indicate that melatonin can be used to treat sepsis-related liver damage and abnormal glucose metabolism. The protective effect of melatonin on inflammation can also be observed in other metabolic organs excepting the liver. For example, the sepsis-associated small intestine damage in mice was protected with melatonin by improving the intestinal barrier with the activation of

SIRT1/3 genes under circadian control (51). The results support use of melatonin in the treatment of sepsis. The curative effect of melatonin on dextran sulfate sodium (DSS)-induced intestinal injury in the presence of circadian rhythm disturbance was observed in an animal model of inflammatory bowel disease (IBD). In the study, intestinal inflammation progresses more severely in the presence of circadian rhythm disorder (52) while melatonin has a healing effect by preventing the spread of HMGB1, a damage-related protein, in the intestinal tissue (53). A critical element of gut function is the gut microbiota. The metabolism of these microbiota may be regulated by melatonin. Indeed, melatonin suppressed gram negative bacteria via Toll-Like Receptor 4 (TLR4) and corrected the microbial dysbiosis and colon inflammation in IBD mouse model. In addition, an increase in goblet cells and an antimicrobial peptide, AMP, was observed in mice injected with melatonin (53). Based on these results, melatonin has the potential to treat IBD. Melatonin also has protective effect on organs that play a critical role in lipid metabolism. For example, the circadian rhythmicity of C57BL/6J mice was disrupted by constant light exposure, after melatonin treatment, the fat accumulation decreased and insulin sensitivity improved in these mice. It has also been reported that melatonin regulates the expression of circadian genes and lipid absorption (54). One of the most common and devastating consequences of disrupted lipid metabolism is obesity. Based on the effect of melatonin on lipid metabolism, its relationship with obesity has been extensively studied. The association of melatonin and obesity relates to lipolysis and the energy expenditure. Accelerated lipolysis and increased energy expenditure have been reported due to the increase in brown adipose tissue activity of mice following melatonin treatment. Accordingly, obesity-induced insulin resistance was successfully suppressed by melatonin administration (55). An increase in brown adipose tissue mass and its mitochondrial activities in ZDF mice after melatonin treatment supported the beneficial effects of melatonin on lipid metabolism (56). These results indicate the potentially therapeutic effects of melatonin in controlling human obesity and metabolic syndrome. In addition, melatonin triggered the conversion of white adipose tissue to brown adipose tissue in ZDF mice (57). Considering the low toxicity of melatonin, all these make this molecule be a strong candidate for the treatment of obesity.

In conclusion, melatonin and the clock genes are tightly associated with metabolism via SIRT1 pathway. The protective effects of melatonin on metabolic disorders are mediated by shifting the rhythm of circadian genes to physiological rhythm and/or via signaling pathways such as the SIRT1 pathway. These signaling pathways related to the metabolism are summarized in Table 2 and Figure 2,

Table 2. Summaries of the role of melatonin in metabolism.

Study Type	Experimental Model	Drug and Dose	Observation	Remarks	Ref.
In Vitro, In Vivo	HepG2 Cell Culture, C57BL/6J Mice.	Melatonin (100 um, 10mg/kg)	In cell culture, melatonin shifted the circadian oscillation of liver stress response genes Nrf2 and HO-1 towards physiological rhythm. In animal model, melatonin regulated the rhythms of genes responsible for lipid regulation and oxidative stress defense.	The effects of exogenous melatonin on the expression pattern of core clock genes and their associated genes point to the use of melatonin as a therapeutic agent for lifestyle diseases such as NAFLD.	(48)
In Vivo	C57BL/6J Mice	Melatonin (10 mg/kg)	Melatonin suppressed miRNA-34a-5p, which is a SIRT1 inhibitor, and increased the expression of SIRT1, resulting in decreased liver fat accumulation and ER stress.	The results indicate that melatonin directly inhibits miR-34a-5p and the abnormal metabolic pathways when the SIRT1 protein is fully present.	(49)

In Vivo	C57BL/6J Mice	Melatonin (50 mg/kg)	In mice, treatment with melatonin reversed the fat accumulation and increased the insulin resistance induced by the exposure to 24-h constant light	These findings provide a new explanation for the obesity-inducing effect of LAN exposure and suggest a possibility for obesity therapy	(54)
In Vivo	CLP-induced Sepsis Related Liver Damaged Male Sprague-Dewey rats	Melatonin (20 mg/kg)	Melatonin intraperitoneal injection improves abnormal gluconeogenesis in the damaged liver through SIRT1/STAT3 signaling pathway.	The findings indicate that melatonin can be used as a treatment for sepsis-related liver damage and abnormal glucose metabolism.	(50)
In Vivo	CLP/Endotoxeima Induced Sepsis Model	Melatonin (30 mg/kg)	Melatonin prevents intestinal damage by protecting the intestinal barrier with the activation of SIRT1/3 genes.	The results provide the necessary molecular data to support for the use of melatonin in the treatment of sepsis.	(51)
In Vivo	C57BL/6J	Melatonin (10 mg/kg)	The intestinal inflammation progresses more severely in the presence of circadian rhythm disorder and melatonin has a preventive effect by the spread of HMGB1, a damage-related protein, in the intestinal tissue.	Melatonin may be an alternative treatment, especially for IBD patients with circadian rhythm disorders.	(52)
In Vitro, In Vivo	TLR-4-deficient BALB/c mice, HT29 Cell Culture	Melatonin (10 mg/kg)	Melatonin ameliorates microbial dysbiosis and colon inflammation by suppressing gram-negative bacteria through TLR4 activation. An increase in goblet cells and an antimicrobial peptide, AMP, was observed in mice injected with melatonin.	Evidence shows that melatonin has the potential to treat IBD.	(53)
In Vivo	Arbor Acres Chickens (intact, pinealectomy or sham-operated) raised under white (WL), red (RL), green (GL) or blue light (BL) for 14 days.		Compared to WL, GL enhanced the mesor and amplitude of melatonin and all clock genes, whereas RL had the opposite effect. Pinealectomy decreased expression of liver clock genes with the reduction in plasma melatonin concentration.	Melatonin plays a key role in the effects of different light wavelength on clock gene rhythm in the chick liver.	(46)
In Vivo	Male C57BL/6J, FGF 21-/- Mouse	Melatonin (20 mg/kg)	Accelerated lipolysis was detected in brown adipose tissue of mice after melatonin treatment. Obesity-related insulin resistance was successfully suppressed by increased energy expenditure after melatonin treatment.	Melatonin may be used to control obesity given its low toxicity.	(57)
In Vivo	Zücker Diabetic Fatty Rats	Melatonin (10 mg/kg)	An increase in brown adipose tissue mass and its mitochondrial activities were observed after melatonin treatment.		(55)
In Vivo	Zücker Diabetic Fatty Rats	Melatonin (10 mg/kg)	Melatonin triggered browning of white adipose tissue.		(56)

Nrf2: Nuclear Factor Erythroid 2–Related Factor 2, SIRT: Silent Mating Type Information Regulation 2 Homolog, NAFLD: Non-Alcoholic Fatty Liver Disease, CLP: Cecal Ligation and Puncture STAT: Signal Transducer and Activator of Transcription ER: Endoplasmic Reticulum, LAN: Light at Night, IBD: Inflammatory Bowel Disease TLR: Toll-like Receptor.

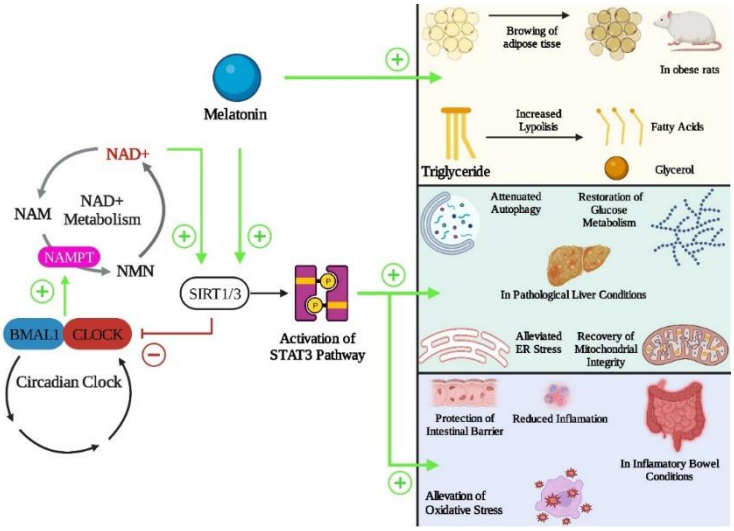

Fig. 2. Mechanism of melatonin on metabolic disorders.

Melatonin exerts its effect on metabolism mainly through secondary signaling pathways involved in the SIRT family. NAD+ dependent SIRT family of transcription factors are associated with circadian clocks. The BMAL1-CLOCK heterodimerization directly control the rate-limiting NAMPT enzyme in NAD+ metabolism. The expression of SIRT family shows a circadian rhythm. Melatonin shows various ameliorative effects on pathological conditions of metabolism-related systems after activation of SIRT1/3-mediated STAT3 signaling pathway. In addition, melatonin has a protective and healing effect against obesity by increasing the lipolysis of adipose tissue and energy expenditure.

5. MELATONIN AND HORMONAL REGULATION

Hormones are produced by the endocrine glands and are responsible for maintaining homeostasis. These chemicals are carried by body fluids and function in the distant target cells (58). These message molecules regulate synaptic and neuronal plasticity and metabolic activity in brain (59). The molecular circadian clock contributes to the regulation of hormonal and metabolic activity. Growth hormone, melatonin, cortisol, leptin, and ghrelin levels are affected by the light-dark cycle. These hormones are highly related to nutrition, sleep, and circadian rhythm. The relationship between circadian rhythms and the endocrine system is closely connected. Circadian disruptions affect glucose and lipid homeostasis and reverse melatonin rhythm. Sleep disorders that negatively affect hormonal regulation are associated with many diseases including obesity, diabetes, insulin insensitivity. Misconnection of them also increases the incidence of metabolic, endocrine and cardiovascular diseases (60, 61). Identifying the underlying causes and establishing effective therapies on the basis of circadian rhythm disruption would make a significant difference in the treatment of diseases (62). Discoveries in the molecular mechanism of the circadian clock are also seen as a therapeutic target in patients with endocrine disorders (63). Since melatonin is responsible for the modulation of this biological clock, modulation of the melatonin levels may establish hormonal balance in humans (64).

It was reported that cortisol and melatonin affected the day and night cycles in the feeding model of pigs while the circadian rhythms of leptin and ghrelin were less important than cortisol and melatonin in feeding behavior (65). The study may also apply to other living species such as humans, which have the similar endocrine and metabolic systems as pigs.

Growth hormone has close relation to nutrition. Adenylate cyclase (AC)/protein kinase A (PKA) and extracellular signal related to protein kinase (ERK)1/2 signaling pathways are involved in the secretion of growth hormone. Interestingly, the melatonin membrane receptors 1b (Mel1b) and Mel1c enabled the activation of ERK1/2 to regulate the secretion of growth hormone (66). Mel1b/AC/PKA/ERK1/2, Mel1c/ERK1/2 and intracellular and extracellular Ca^{2+} channel signaling pathways can induce growth hormone secretion.

Testosterone is synthesized from cholesterol derived from lipid droplets in the Leydig cells in the testicles. Melatonin is also an effective molecule on testosterone synthesis via lipolysis in lipid droplets in Leydig cells. Melatonin decreased cholesterol synthesis and testosterone level in Leydig cells (67). In this case, melatonin can reduce intracellular cholesterol content and testosterone synthesis by decreasing lipolysis of lipid droplets. The GATA-4 and SF-1 transcription factors were involved in the inhibitory effect of melatonin on testosterone synthesis. Downregulations in GATA-4 and SF-1 expressions contributed to the inhibitory activity of melatonin (68). There is an inverse relationship between these transcription factors and melatonin.

Leydig and Sertoli cells are involved in the production of androgens, which contribute to the production of sperm. In the in vitro study by culture of both Leydig cells and Sertoli cells which are responsible for the regulation of sperms growth, melatonin supplementation increased testosterone secretion. Melatonin regulated the expression of insulin-like growth factor-1 via melatonin receptor 1 (69). In addition, melatonin modulating receptor 1-induced suppression of estrogen synthesis to increase androgen production. Theca cells are also androgen-secreting cells that contribute to ovarian structure and function. Melatonin delayed aging in theca cells and promoted the synthesis of progesterone hormone (70). Melatonin also plays an important role in follicle development and ovarian steroid hormone secretion by theca cells. The contribution of melatonin to the release of oxytocin, which is an important hormone in females, was investigated. Activation of hypothalamic gonadotropin-releasing hormone receptors increases the release of oxytocin from the hypothalamo-neurohypophysial system. Administration of melatonin to this system significantly reduced oxytocin release. This inhibitory effect of melatonin is mediated by the cyclic adenosine monophosphate (cAMP) in intracellular signal transmission (71). In addition, other mechanisms of action may also contribute to the inhibitory effect of melatonin. The interaction of melatonin and its receptors with estrogen and thyroid hormones on ovarian functions in polycystic ovary syndrome (PCOS) was reported. This interaction may involve in the etiology of PCOS. The increase in melatonin level caused a decrease in follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels (72). The environmental pollutes can impact these reproductive hormones. For example, exposure to glyphosate, an herbicide used in agriculture, can cause pathological alterations in reproduction and endocrine system. This substance was also used to induce acute oocyte disruption and hormonal disorders in mice. Administration of melatonin increased oocyte quality and corrected hormone balance in mice exposed to glyphosate (73). Melatonin can be used for modulation of reproductive hormones.

The collateral mechanism of melatonin with chemotherapeutic drug, doxorubicin, was investigated through the TWIST1 transcription factor in breast cancer cells. Melatonin inhibited the initiation and progression of breast cancer by suppressing estrogen synthesis and the activation of the estrogen receptor in estrogen-dependent breast cancer cells, also downregulated TWIST1 in these cells (74). Therefore, melatonin can intensify the effect of doxorubicin in TWIST1 inhibition.

As a result, melatonin can effectively regulate the steroid hormones including androgen, testosterone, estrogen. Testosterone and progesterone synthesis can be altered through regulation of melatonin levels. In conclusion, melatonin can be used for therapeutic and

preventive purposes as well as controlling steroid hormones in breast cancer and these were summarized in the Table 3 and Figure 3.

Table 3: Summaries of the roles of melatonin in hormonal regulation.

Study Type	Disease Model	Drug and Dose	Observation	Remarks	Ref.
In vitro	Human breast cancer cell lines MCF-7	Melatonin (1 nM) and Doxorubicin (1 µM)	Melatonin downregulated TWIST1 in estrogen-dependent breast cancer cells.	Melatonin can be used to increase the effect of doxorubicin in TWIST1 inhibition.	(74)
In vitro, In vivo	Sexually mature roosters	Melatonin (1 ng/mL)	Melatonin decreased cholesterol and testosterone syntheses in Leydig cells	Melatonin can reduce intracellular cholesterol content and testosterone synthesis to reduce lipolysis of lipid droplets.	(67)
In vitro, In vivo	C57BL/6J mice	Melatonin (0, 10^{-6}, 10^{-8}, 10^{-10} or 10^{-12} mol/l)	The inhibitory activity of melatonininvolves in downregulations in GATA-4 and SF-1 expressions	There is an inverse relationship between these transcription factors and melatonin.	(68)
In vitro, In vivo	Sheep Leydig cells	Melatonin (10^{-7} M)	Melatonin regulated the expression of insulin-like growth factor-1 via melatonin receptor 1.	Activation of melatonin receptor 1 induces suppression of estrogen synthesis to increase androgen production.	(69)
In vitro, In vivo	Sheep Theca Cells	Melatonin (0,10^{-10}, 10^{-8}, 10^{-6} and 10^{-4} M/L)	Melatonin delayed aging in theca cells and provided the synthesis of progesterone hormone.	The role of melatonin in follicle development and ovarian steroid hormone secretion in theca cells requires further study .	(70)
In vitro, In vivo	Wistar rats	Melatonin solution (5 ng/ml)	Melatonin significantly reduced oxytocin release.	This inhibitory effect of melatonin is mediated by the cAMP involved in intracellular signal transmission. In addition, other mechanisms may also involved.	(71)
In vitro, In vivo	Kunming mice (Gly 250 and 500 mg/kg)	Melatonin (15 mg/kg)	Administration of melatonin increased oocyte quality and maintained hormone balance in mice exposed to glyphosate.	Melatonin can be used for modulation of reproductive hormones.	(73)
In vivo	Animal Model (Pigs; Sus scrofa)	-	The circadian rhythms of leptin and ghrelin were found to be less important in feeding behavior than melatonin.	The study conducted on pigs may also apply to other species including humans, which have the similar endocrine and metabolic systems as pigs.	(65)
In vivo	Arbor Acre male broilers	-	Mel1b and Mel1c enabled the activation of ERK1/2 to regulate the secretion of growth hormone.	Mel1b/AC/PKA/ERK1/2, Mel1c/ERK1/2 and intracellular and extracellular Ca^{2+} channel signaling pathways can induce growth hormone secretion.	(66)
In vivo	Wistar rats	Melatonin (0.68 µg/µl)	The increase in melatonin level caused a decrease in FSH, LH and testosterone levels.	The interaction of melatonin receptors with estrogen and thyroid receptors may involve in the etiology of PCOS.	(72)

AC: Adenylate cyclase, cAMP: Cyclic adenosine monophosphate, ERK1/2: Extracellular signal-regulated kinases FSH: Follicle-Stimulating Hormone, LH: Luteinizing Hormone, Mel1b: Melatonin membrane receptor 1b PKA: protein kinase A Mel1c: Melatonin membrane receptor 1c, PCOS: Polycystic Ovary Syndrome

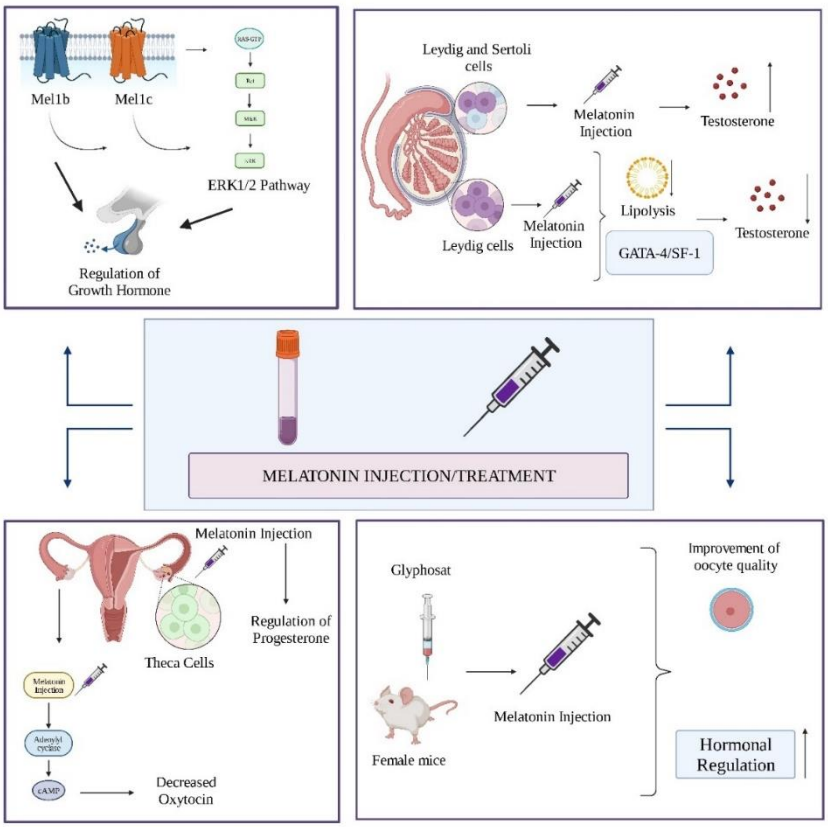

Fig. 3. Potential mechanisms of melatonin on reproductive hormone regulation.

Melatonin supplementation is effective on hormonal balance in humans. Activation of Mel1b and Mel1c receptors promotes the secretion of growth hormone via the ERK1/2 pathway. Exposure to glyphosate causes endocrine and neurological disorders. Melatonin treatment regulates oocyte quality and hormonal balance. In females, melatonin therapy increases progesterone. In addition, melatonin reduces the release of oxytocin through the cAMP pathway. supplementation of melatonin increases the level of testosterone in the environment of Sertoli and Leydig cells.

6. MELATONIN AND IMMUNE FUNCTION

To adapt to the alterations of the environments, the circadian control of the immune system is critical for organisms to meet different immune challenges at different times, seasons and climates. Indeed, many immune parameters differ depending on the time of day (75). For example, the capacity of leukocytes to infiltrate into tissues, their rate of differentiation, and their levels in the blood exhibit time-of-day variations (76, 77). Circadian control of the immune system is achieved by the interaction of clock genes with inflammation-related elements and signaling pathways. In particular, NLRP3 (NOD-, LRR and pyrin domain-containing protein 3) inflammasome and NF-kB (Nuclear factor kappa-light-chain-enhancer of activated B cells) transcription factor-related mechanisms are closely related to circadian genes. NLRP3 is an intracellular sensor that recognizes pathogens. The potentially pathogenic substances cause the NLRP3 inflammasome formation. After activation of NLRP3, programmed cell death occurs because of caspase 1-dependent release of proinflammatory cytokines (78). Numerous elements of innate and adaptive immune responses are controlled by the transcriptional regulator, NF-kB.

NF-kB is involved in the control of survival, activation and development of innate immune cells and inflammatory T cells, and expression of various proinflammatory genes. In addition, both NF-kB and NLRP3 inflammasome are associated (79). Circadian genes play an active role in the control of these two pathways. The BMAL1-CLOCK heterodimer directly controls the activation of the NF-kB pathway (80). CRY 1 suppresses the activation of the NF-kB pathway (81). PER proteins, on the other hand, show both anti-inflammatory and pro-inflammatory effects (82). Even though activities of REV-ERB and RORα compete each other as a common feature, they create the anti-inflammatory responses by regulating NF-kB and NLRP3 (80, 83). Melatonin, on the other hand, can control the immune system by influencing the expression of clock genes mediated by its receptors or by directly affecting inflammation-related pathways. For example, deviations in clock rhythm and melatonin metabolism are seen in the condition of sepsis. In a study conducted with intensive care patients, a positive correlation was found between the melatonin metabolite, 6-sulfatoximelatonin, and proinflammatory cytokines (84). This observation suggests that melatonin may function in the immune response. Indeed, in a mouse model of sepsis, the NF-kB pathway was associated with the NLRP3 inflammasome and melatonin. In the study, it was reported that melatonin inhibited NF-kB and NLRP3 activation via RORα and SIRT1 (85). Similarly, the SIRT1-mediated anti-inflammatory effect of melatonin has also been reported in microglial-mediated inflammation in the CNS (86). The relationship between melatonin and NF-kB, and NLRP3 triangle was studied by comparing NLRP3 knockout and melatonin-treated mice. The results showed that melatonin treatment achieved similar protective effect as did the NLRP3 knockout mice on the pathways and clock genes affected (87). Melatonin can disrupt the communication between NF-kB and NLRP3 pathways by inhibiting NLRP3. The effect of melatonin on the immune system does not only occur by directly affecting the anti-inflammatory mechanisms. In animal model of polymicrobial sepsis, melatonin treatment increased mouse survival by increasing the capacity to form extracellular neutrophil trap (NET) (88). The antibacterial effects of melatonin-derived NETs could benefit sepsis patients.

Melatonin is also effective on adaptive immune response. The effect of melatonin on T/B cell differentiation was compared between healthy and pinealectomy mice. In the study, Th1, Th2 and Th17-related cytokines and T/B activation elements decreased after melatonin treatment (89). Although immune cells show the ability to synthesize melatonin on their own, the experiment may indicate that T/B differentiation is boringly related to the pineal gland. In the autoimmune patients with primary Sjogren's Syndrome (pSS), their serum melatonin levels were lower than that in healthy controls (90). It has also been reported that melatonin reduces the Th17 and ThCD4-CD8-mediated inflammatory response via RORα (90). Further examination of the effect of melatonin on the adaptive immune response may pave the way for using it as an immunomodulatory therapy. The function of macrophages was also affected by melatonin. The relationship between TLR (Toll-like receptor) 9 and melatonin was investigated in macrophage-mediated immune response. In the study, melatonin injection suppressed ERK 1/2 and AKT-mediated TLR9 activation (91). In addition to TLR9, melatonin is also known to regulate macrophage activity through TLR3 and TLR4. However, recent evidence shows that anti-inflammatory regulation of melatonin is not limited to TLR-mediated response. The anti-inflammatory effect of the melatonin-exosome interaction was reported on various cell types. The anti-inflammatory response capacity of melatonin-enhanced exosomes on the active THP-1 cells with proinflammatory cytokines was investigated. The results showed that melatonin-enhanced exosomes significantly stimulated THP-1-M2 macrophage differentiation compared to normal exosomes (92). The low toxicity and low immunogenicity of exosomes and their enhancement by melatonin make them the suitable candidates for treatment of inflammation. For example, melatonin-enhanced exosomes are also known to exert protective effects on the CNS including ischemia brain damage. The melatonin-enhanced exosomes significantly

reduced brain ischemia damage and triggered post-stroke recovery compared to normal exosomes (93). They also reduced inflammation-induced microglia and neuron death via NLR3P – NFkB pathways more effective than that of normal exosomes (93). The role of synaptic communication elements in melatonin-mediated anti-inflammatory response was also investigated. In the study by use of the BV2 cell tree, the interaction between melatonin and n7 acetylcholine receptors was studied. The result showed that melatonin improved autophagic flux and reduced ROS-producing NLRP3-induced inflammation, but its therapeutic effects were lost after inhibition of n7 acetylcholine receptors (94). The results suggested that melatonin exert its anti-inflammatory effect by increasing the expression of n7 receptors.

It appeares that melatonin regulates the circadian clock genes and/or exosomes by acting its receptors and the associated signaling pathways, therefore, initiates the strong anti-inflammatory responses and improves the immune system through genetic mechanisms. These mechanisms are summarized in Table 4 and Figure 4.

Table 4: Summaries of the roles of melatonin in immune function.

Study Type	Disease Model	Drug and Dose	Observation	Remarks	Ref.
Human Study	Septic ICU patients.		A positive correlation was found between the levels of melatonin metabolite and proinflammatory cytokines in intensive care patients.	The study provides basic clinical data on the role of melatonin in the immune system.	(84)
İn vivo	C57BL/6 CLP-induced sepsis Mice model	Melatonin (30 mg/kg)	Melatonin inhibited NF-kB and NLRP3 activation in relation with SIRT1 by acting through RORa.	Melatonin can modify communication between the NFKB and NLRP3 pathways.	(85)
In vitro	Murine BV2 Microglial Cells	Melatonin (100 µM)	Melatonin ameliorated microglia-mediated inflammation in the CNS through SIRT1.	Melatonin can be used in the treatment of CNS inflammation.	(86)
In Vivo	C57BL/6 CLP-induced sepsis Mice model	Melatonin (30 mg/kg)	Melatonin treatment showed similarities in inhibition of signaling pathways and clock genes as did by NLRP3 knockout mice.	Melatonin can be used as the anti-inflammatory drug to target NLRP3 inflammation.	(87)
In Vivo	C57BL/6 CLP-induced sepsis Mice model	Melatonin (10mg/kg)	NET-forming capacity of neutrophils and survival rate of mice were increased after melatonin injection.	The antibacterial effects of melatonin-enhanced NETs may also benefit human sepsis patients.	(88)
In Vivo	C57BL/6 Pinealectomy Mice	Melatonin (10 mg/kg, 20 mg/kg, 40 mg/kg)	Melatonin treatment, reduced the Th1, Th2 and Th17 related cytokines and T/B activation elements.	Although immune cells show the capacity to secrete melatonin independently of the pineal gland, T/B differentiation may be tightly associated with the pineal gland.	(89)
Human Study	Primary Sjogren's Syndrome		A significant decrease in serum melatonin levels was observed in pSS patients compared to healthy controls.	The effect of melatonin on the adaptive immune response suggest its immunomodulatory activity.	(90)
In Vivo	C57BL/6J Mice	Melatonin (5 mg/kg)	ERK 1/2 and ALT-mediated TLR9 activation was suppressed by melatonin.	Melatonin can target TLR9 as well as TLR3 and TLR4 to regulate the innate immunity	(91)
In Vitro	THP1 Cells, MHCS Cells, Fibroblasts.	Melatonin (10 µM), Exosome (10 µM)	Melatonin-enhanced exosomes induced M2 macrophage differentiation more intense than that of the normal exosomes.	Melatonin-enhanced exosomes with high safety profile can be used in the treatment of inflammatory-sensitive systems such as the CNS.	(92)
In Vivo	Sprague-Dawley	Exsozome (100 um/kg),	Melatonin-enhanced exosomes attenuated brain injury and induced post-stroke recovery	Melatonin-enhanced exosome may lead to the development of a	(93)

	pMCAO Stroke Model Rats	Melatonin (10 mg/kg)	more effeciency than the normal exosomes. Melatonin-enhanced exosomes reduced inflammatory microglia and neuron death via NLR3 – NFkB pathways.	popular treatment on neuronal injury.	
In Vitro	Murine BV2 LPS-Induced Inflamative Microglial Cells	Melatonin (10 nM)	Melatonin improved autophagic efflux and reduced NLRP3-dependent inflammation and these activities were blunted after inhibition of n7 acetylcholine receptors.	The complex interaction between 7n AChR and autophagy signaling may be modulated by melatonin.	(94)

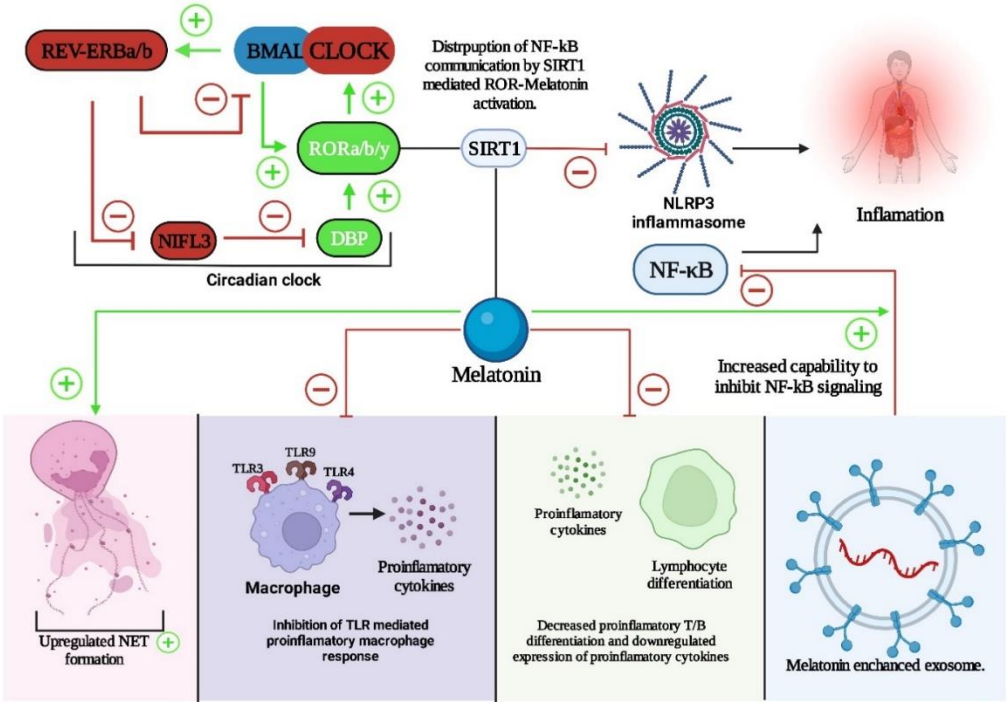

Fig. 4: Mechanisms and actions of melatonin on immune function.

The anti-inflammatory effect of melatonin, especially in the sepsis, is mediated by the activation of sirt1 through Ror receptors. After sirt1 activation, the communication between the NLRP3 inflammasome and the NF-kB transcription factor, which are the two most important elements of the inflammatory response, is blunted by NLRP3 inhibition. However, in some conditions, inhibiting the inflammatory response may be ineffective because the inflammatory pathogen is still present. On the other hand, melatonin also has a strengthening effect on the immune system elements. In addition, melatonin may contribute to the anti-inflammatory response by increasing the capacity of exosomes to inhibit the NF-kB pathway.

7. CONCLUSION

Circadian rhythms are of great importance for orgasms to adapt the frequent environmental alterations. Circadian rhythms occur in almost all organisms, which are generated by a collection of clock genes. The molecular foundations of clock genes depend on alterations in mutually dependent transcriptional-translational feedback loops. The complex structure formed by the circadian clocks in different physiological systems is called the circadian clock network. The central clock located inS CN of the brain synchronizes different peripheral circadian clocks. Melatonin as a light/dark message molecule participates in the regulation of circadian system by adjusting the biological rhythm to fit the environmental light/dark cycle as

the light-dependent input. Therefore, in most of the cases, melatonin has the capacity to restore disrupted rhythms of the local or central clocks to their appropriate physiological ones.

The precise regulation of the sleep-wake cycle is one of the most crucial elements in guaranteeing the proper biological functions of human body. Melatonin level is one of the key factors in the control of the sleep-wake cycle. Additionally, melatonin has the strong potential to shield the sleep-wake cycle from pathological circumstances. Melatonin is currently used as a medicine for individuals with sleep disorders. It can protect circadian genes against disrupt sleep-wake disorders caused by anesthetic drugs or various diseases.

Metabolism is the set of chemical processes necessary to maintain life. Circadian rhythms are directly associated with metabolism in all organisms. Melatonin affects metabolism mostly through the SIRT family members, which have a significant impact on energy metabolism. Melatonin can upregulate SIRT family expression to inhibit inflammatory response since the anti-inflammation pathways are directly related to the SIRT family, or through SIRT-mediated regulation of key clock genes. Apart from targeting the SIRT family, melatonin is able to protect metabolic system by acting as a powerful free radical scavenger. The regulatory effect of melatonin on metabolic systems is attributed to its impacts on genetic regulation, anti-inflammatory effects, and antioxidant activities.

One of the most important information transmission systems is hormones. Hormonal system is heavily regulated by circadian rhythms and a lot of hormones express circadian dependent manner. Melatonin exhibits mutual relationships with many of these hormones.

Successful and efficient protection against pathogens requires circadian control of the immune system. Alterations of circadian rhythm influence immune functions including plasma leukocyte counts and the activity and morphology of different immune system components. Numerous immune system components possess the ability to synthesize melatonin, which is one of the characteristics that makes the immune system's circadian feature unique. Although melatonin has a variety of effects on the immune system via its receptors, the main mechanism is via its direct regulation of NLRP3 and NF-kB, which are two important inflammation-related pathways. Based on the evidence mentioned above as well as the suitable safety margin of melatonin, we suggest that melatonin can be used as the therapeutic agent for a spectrum of diseases including sleep-wake disruption, autoimmune disease, cancer and metabolic disorders.

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AUTHORSHIP

B.S. contributed to the drafting, conception and design of the manuscript and prepared figures. F.S.A. contributed to the drafting, conception and design of the manuscript and prepared figures. E.A. edited the manuscript and contributed to drafting, conception and design of the manuscript, data analyzing and interpretation. All authors contributed equally to the writing of this paper.

CONFLICT OF INTEREST

Author of this article has not declared any competing interest.

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