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MELATONIN: A PLEIOTROPIC MOLECULE REGULATING INFLAMMATION

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ABSTRACT

Melatonin is a neurohormone produced by the pineal gland that regulates sleep and circadian functions. Melatonin also regulates inflammatory and immune processes acting as both an activator and inhibitor of these responses. Melatonin demonstrates endocrine, but also paracrine and autocrine effects in the leukocyte compartment: on one side, leukocytes respond to melatonin in a circadian fashion; on the other side, leukocytes are able to synthesize melatonin by themselves. With its endocrine and paracrine effects, melatonin differentially modulates pro-inflammatory enzymes, controls production of inflammatory mediators such as cytokines and leukotrienes and regulates the lifespan of leukocytes by interfering with apoptotic processes. Moreover, its potent anti-oxidant ability allows scavenging of oxidative stress in the inflamed tissues. The interesting timing of pro- and anti-inflammatory effects, such as those affecting lipoxygenase activity, suggests that melatonin might promote early phases of inflammation on one hand and contribute to its attenuation on the other hand, in order to avoid complications of chronic inflammation. This review aims at giving a comprehensive overview of the various inflammatory pathways regulated by this pleiotropic hormone.

KEY WORDS

Melatonin; inflammation; oxidative stress; reactive oxygen species; lipoxygenase; NF-κB

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1. Introduction

Inflammation is an essential response to tissue injuries induced by physical, chemical or biological insults. Acute and chronic inflammation are essential to restore homeostasis [1,2]. However, the mechanisms by which activated leukocytes fight germs and tumor cells or eliminate tissue debris in the inflamed area, lead to production of oxidants and/or of cytotoxic cytokines [2,3]. These processes are finely regulated, but may occasionally escape control and prolong the inflammatory response creating a self-perpetuating condition that produces further damage to the injured tissue. Since chronic inflammation is a major cause of many serious pathological conditions, and is considered by many scientists as a strong tumor promoter [4]. It is crucial to investigate novel pharmacological approaches that allow to reduce pathologic chronic inflammation without impairing the physiological inflammatory response, since many synthetic anti-inflammatory agents generate considerable side effects.

Pharmaceutical compounds modulating their action in subtle ways (which may be defined as smart compounds), more specifically of natural origins, able to control, regulate and defeat chronic inflammation, are within the focus of nowadays medicinal chemistry research. Endogenous molecules may be exogenously supplemented, thus increasing overall body levels, and changing timing or localization within the body with respect to their endogenous production.

Melatonin is very attractive from this point of view as it may act as a regulator of the inflammatory cell compartment with a potent anti-oxidant potential able to reduce the oxidative environment of chronic inflammation and to regulate leukocyte function and number [6,7].

We analyze here data describing the possible modulation of inflammatory functions with a focus on the anti-oxidant effects of melatonin on its ability to alter production of pro-inflammatory molecules *in vivo* and *in vitro*. Moreover, this review will cover the effects that melatonin exerts on the abundance of leukocytes *via* alteration of their proliferation and apoptosis.

2. Melatonin synthesis and its targets

Melatonin (N-acetyl-5-methoxytryptamine) is derived from tryptophan *via* enzymatic conversion reactions **(**see **Figure 1** for details**)** and is mainly produced by the pineal gland. Melatonin plasma concentration varies according to a circadian cycle and this cyclic production determines the periodic effects at a systemic level [8]. Melatonin is evolutionary conserved and exerts many regulatory functions by modulating cellular behavior *via* binding to specific receptors and intracellular targets (**Figure 2**)[9, 10].

Target cells express specific high affinity receptors, namely MT1/MT2, on plasma membrane [11], able to trigger intracellular signaling by adenylate cyclase or G-proteins (**Figure**

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2A), depending on the intra- and extra-cellular environment. Even though the mechanisms through which melatonin stimulates these signal transduction pathways have been extensively studied [12-21], little is known about the resulting cellular effects: MT1/MT2 receptors have been shown to mediate melatonin-dependent decrease of tumor cell proliferation [22] and anti-apoptotic effects [23-25]; MT1 seems to be responsible for the general response to photoperiodic signals [26].

High affinity nuclear receptors to melatonin have also been identified (**Figure 2B**). They belong to the RZR/ROR nuclear hormone receptor family; melatonin binds with high affinity to RZR-beta [27, 28]. Upon activation, this complex down-regulates the 5-lipoxygenase (LOX) whose promoter possesses an RZR-beta-repressor binding site [29]; subsequently, 5-LOX expression is reduced by melatonin in cells bearing this RZR-beta receptor [30].

Cells expressing either one of those receptors are targets of the hormonal effect of melatonin and respond to its nocturnal low nanomolar plasma levels. Additional binding to intracellular targets in the micromolar range, such as the enzymes hydroquinone [31] and calmodulin [32] (**Figure 2C**) have been reported. These interactions trigger signaling functions including microtubule regulation [33] and possibly phospholipase A2 activation [34]; since hydroquinone and calmodulin are ubiquitous, most cells are targets of melatonin; however, low affinity allows productive interaction only at concentrations much higher than those produced by the pineal gland, suggesting that other mechanisms of melatonin production or accumulation may take place.

In addition to the pineal gland, which is the major source of melatonin, other tissues like retina [35], gastrointestinal tract [36, 37] , skin [38] and leukocytes [6] (both in peripheral blood [39] and in bone marrow [40, 41]) synthesize melatonin. Melatonin produced by these non-endocrine organs is not regulated by circadian cycles but rather respond to other signals, exerting in fact a paracrine or autocrine effect [6]. Local melatonin levels may differ from those regulated by its neuro-endocrine functions; indeed, melatonin reaches the highest body concentration in the bone marrow (micromolar concentrations), exceeding plasma levels of at least two orders of magnitude [42]. This accumulation may result from the intrinsic melatonin synthesis machinery of the leukocyte.

As a matter of fact, melatonin finely regulates leukocyte function and number and contributes to the control of inflammatory tissue [43]. The effects of melatonin on leukocytes imply paracrine or autocrine responses that superimpose on the neuro-endocrine hormone response. In *vivo* studies with pinealectomized animals [44] allow to discriminate between hormonal and paracrine responses, whereas the study of melatonin synthesis and functions in cultured non-pineal cells [6] provides information of the autocrine effects of melatonin.

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3. Neuro-endocrine melatonin: systemic effects of pineal melatonin on immune system

It is an established notion that the nervous and endocrine systems can interact with the immune system in order to modulate its function [46]; indeed, many neurotransmitters, neuro-endocrine factors and hormones can alter immune function [47]. Also melatonin is able to modulate immune functions through its neuro-endocrine action. Melatonin acts as a regulator of circadian rhythms [48] in a hormone-like fashion by affecting target cells and by modulating other functions depending on the photoperiod including regulation of photoperiodic oscillations of the immune/inflammatory response [45, 48]. Leukocytes possess melatonin specific receptors including both MT1/MT2 and nuclear RZR, and this provides the molecular basis for the sensitivity of leukocytes to melatonin.

A strong indication of the effect of melatonin produced by the pineal gland on inflammatory cells *via* its endocrine activity was obtained in the Gerbil and in the Syrian Hamster. Here the artificial shortening of day lengths or alternatively melatonin injections induce an increase in thymus weight [49] and spleen hypertrophy [50], implying a melatonin-mediated photoperiodic effect on the immune system. Furthermore, it was demonstrated that melatonin controls diurnal and seasonal rhythms of leukocyte proliferation [51], of cytokine production [44, 45] and NK cell activity [52] in mammalian bone marrow cells. In other studies, the nocturnal peak of melatonin was associated with the proliferation peak of granulocytes and macrophage progenitor cells in mice [53].

Data from the studies of the effects of experimental pinealectomy further contribute to a better understanding of the underlying mechanisms. It was described that the removal of the pineal gland causes partial (and transient) impairment of immune response in rats [44, 53, 54]; moreover, NK activity and IL-2 production were described to be reduced in rats after pinealectomy [44]. Interestingly, melatonin administration in pinealectomized animals reverses the adverse effects on immune response. For example, Martins *et al.*, in an experimental model of allergic airway inflammation, showed that melatonin administration to pinealectomized rats restores the ability of cells to migrate from the bone marrow to the broncho-alveolar fluid, which was prevented after pineal gland removal, implying that neuro-endocrine melatonin is important in the control of cell recruitment from the bone marrow and the migration of those cells to the lung [54]

As the melatonin-dependent effects of pinealectomy on the immune response appear to be partial, this further indicates that other compartments can overcome or contribute to immunomodulatory effects triggered by melatonin.

4. Paracrine effects of melatonin on the white blood cell compartment

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Leucocytes possess the enzymatic machinery necessary to synthesize melatonin. Accordingly melatonin may act as a paracrine or autocrine regulator in leukocyte communication independent of the pineal gland. Indeed Jurkat T cells produce interleukine-2 (IL-2) as a result of endogenous melatonin signaling and Lardone *et al.* demonstrated that IL-2 production is hampered by specifically inhibiting melatonin signaling by the MT1/MT2 antagonist luzindole [55]. Recently it was discovered that in bone marrow, melatonin reaches micromolar concentrations, that is much higher than those found in the blood stream, suggesting that the paracrine/autocrine immuno-regulatory role of melatonin might be also mediated by binding to the low affinity targets; a putative signal transduction pathway triggered by melatonin binding to calmodulin (affinity = 63 μM [56]) leads to transient activation of phospholipase A2 and lipoxygenase activation [34] (**Figure 3**).

5. Effect of exogenous melatonin on inflammatory parameters

The potential use of melatonin as a pharmaceutical agent derives from numerous *in vitro* studies where melatonin doses exceeding the nocturnal plasma levels are required to exert clear effects. At supra-physiological concentrations, melatonin induces T-cell proliferation and up-regulation of pro-inflammatory cytokines [43, 57] . Exogenous melatonin administration increases the proliferative response of rat lymphocytes [58] , increases the number of NK cells [59, 60], stimulates the pro-inflammatory cytokines IL-1 and TNF-α [61,62] and enhances phagocytosis [63]. Melatonin exerts a concentration-dependent effect on the immune system. Indeed, increasing concentrations of melatonin induce T-cell proliferation in a dose-dependent way. In addition, it was demonstrated that pharmacological doses of melatonin inhibit INF-γ production in a range of 0.1 to 1mM [65]. In some systems, the modulation of apoptosis requires high melatonin doses [23, 24].

Whether or not these effects have a physiological significance, occurring as a paracrine or autocrine response, *i.e.*, in microenvironments with an elevated melatonin concentration like in the bone marrow, is presently investigated. In any case, these findings designate melatonin as a potential exogenous pharmacological modulator of the inflammatory response.

6. Melatonin, ROS and inflammation

Oxidative stress can be defined as the imbalance between cellular oxidant species like reactive oxygen species (ROS) production and antioxidant potential. ROS are involved in a variety of different cellular processes ranging from physiological to pathological responses. It is well known that ROS can promote cell survival, proliferation and differentiation at physiological levels, but also cell death by apoptosis or necrosis at higher levels [66]. Oxidative stress has a direct toxic

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effect on cells, which leads to lipid peroxidation, protein oxidation or DNA damage; it plays a causative or adjuvant role in almost all human pathologies, including cancer and neurodegeneration, and is involved in aging and chronic inflammatory pathologies. To cope with oxidative stress, a battery of enzymes able to scavenge ROS are up-regulated by intracellular signaling [67].

Oxidative phenomena play a key role in signaling and management of the initial phase of inflammation. Activated neutrophils generate an oxidative burst that directs toxicity towards invading microbes [68]; ROS generation in the damaged tissue produce a concentration gradient that directs leukocyte recruitment to the site of tissue injury [69]. Moreover, ROS induce monocyte maturation [70] and promote adhesion to endothelial cells allowing diapedesis. Thus, beyond its anti-septic action, ROS generation can regulate leukocyte recruitment and maturation.

ROS generation also activates the intracellular inflammatory signaling pathways leading to the release of the inflammatory mediators, including cytokines produced by the activation of Redox-regulated transcription factors such as NF-κB [72,73]. Thus, ROS can promote and drive the inflammatory response in various tissues and can stimulate the signaling pathways triggered by inflammatory conditions [74].

If tissue healing is delayed or fails, inflammation may become chronic, increase tissue damage and induce the recruitment of additional leukocytes, producing a vicious cycle and leading to organ failure or eventually to cancer. This is characterized by chronic ROS production. An efficient anti-inflammatory therapeutic strategy requires anti-oxidants to stop this vicious cycle and to reduce the burden of inflammatory mediators at the inflammatory site.

Melatonin is a powerful anti-oxidant [75] as it has been shown to scavenge different types of free radicals *in vitro* [76], in body fluids [77] and in cells [78]. Furthermore, melatonin plays an important role in activating anti-oxidant defenses such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd) and glucose-6-phosphate dehydrogenase (G6PD) [79, 80].

Both effects allow melatonin to reduce the extent of ROS, improving oxidative related pathologies such as hypertension [81], atherosclerosis [82], cancer [83], ischemia [84] or neurodegenerative diseases [85] . Moreover it was suggested that melatonin prevents aging [86].

7. Effects of melatonin on the arachidonic acid metabolism

The inflammatory response starts with an insult that activates and recruits leukocytes to the inflamed tissue through recruitment of different molecular mediators; an important role in the early phase is played by phospholipase A2 (PLA2), which cleaves membrane phospholipids liberating membrane bound arachidonic acid (AA); arachidonic acid is then processed by cyclooxygenases

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(COX) and LOX to produce key inflammatory mediators such as prostaglandins and leukotrienes [87], respectively. After experimental inflammation induction *in vivo* and *ex vivo*, melatonin was shown to prevent or reduce the inflammatory-derived activation of PLA2 [88] , LOX [89] and COX [90]. The reduction of PLA2 [88] and LOX [89] up-regulation by melatonin occurs *via* non-oxidative mechanisms, implying the engagement of MT1/MT2 [88] or ROR/RZR [89] receptors; thus, many different actions of melatonin including receptor stimulation or radical scavenging seem to cooperate to achieve the same final goal, that is to interfere with the inflammatory cascade.

Moreover, recent evidence shows that melatonin activates PLA2 and 5-LOX [34] as a consequence to its binding to calmodulin [34, 91], leading to mobilization of arachidonic acid (AA) from phospholipids. Arachidonic acid then activates 5-LOX triggering production of endogenous monohydroxy-eicosatetraenoic acids (HETEs) [34]. Interestingly, the down-regulation of 5-LOX (and PLA2) requires the engagement of RZR (or MT1/MT2), receptors [88, 89] but its activation instead requires melatonin to bind to calmodulin (**Figure 3**). We can conclude that the stimulation by melatonin of different targets produces different effects, altogether suggesting a sophisticated regulation.

The activation of 5-LOX and PLA2 activity by melatonin is a transient phenomenon and extinguishes within 2-3 hrs, possibly because of the down-regulation of either 5-LOX or PLA2. These observations are consistent with the view that melatonin favors the early phases of inflammation on one side, in fact, LOX induces the production of leukotriene B, which promotes diapedesis by vessel permeabilization, and acts as a chemo-attractant [92, 93]. On the other side, melatonin reduces the inflammatory process when it proceeds towards the chronic phase.

Melatonin also inhibits the activity of the enzyme nitric oxide synthase (NOS) [94-96], which catalyzes the formation of NO•. In inflamed tissues, NO released after cytokine stimulation contributes to the development of micro-vessel damage and vascular hyporeactivity [97]. Thus, melatonin-related down-regulation of NOS could contribute to melatonin's prevention of inflammation.

It was reported by that melatonin inhibits iNOS expression *via* down-regulation of NF-κB [98] on one hand but is also able to activate NF-κB, thus regulating the expression of adhesion molecules on circulating leukocytes on the other hand [99, 100]. Thus, melatonin plays a multifaceted role depending largely on the targets involved.

8. Role of melatonin in leukocyte survival or apoptosis

Leukocytes have to perform briefly but efficiently in an oxidative environment that may cause mutations or cell death. It is nevertheless important that cells warrant a prolonged anti-

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bacterial effect in the injured area in spite of cellular injuries. To this purpose, the organism generated cellular resistance strategies contributing to extend leukocyte lifetime by avoiding or delaying cell death mechanisms.

Apoptosis plays an important role in inflammatory responses but also in the regulation of the maturation rate of B and T cells [101, 102]. In particular, melatonin was shown to regulate the apoptosis of B and T cells [103, 104]. Indeed, melatonin inhibits apoptosis of precursor B cells in mouse bone marrow [105]. Moreover, an anti-apoptotic effect on different leukocytes was also demonstrated [23, 102].

The mechanisms of the anti-apoptotic action of melatonin in leukocytes have been investigated. Studies in peripheral blood have suggested that melatonin inhibits apoptotic processes *via* its antioxidant properties [106], which is in line with many evidences showing that in some instances the apoptotic process occurs *via* oxidative signaling [107]. Alternative mechanisms have been proposed; in U937 monocytes, melatonin reduces apoptosis *via* intracellular signal transduction stimulated by interaction with MT1/MT2 plasma membrane receptors [23]. However, the majority of reports indicate that the anti-apoptotic of melatonin occurs by acting on the abundance of the two main members of the Bcl-2 family, namely the pro-apoptotic Bax and the anti-apoptotic Bcl-2 proteins [108].

Bcl-2 exerts its well-known anti-apoptotic effect by inhibiting the action of Bax [109], which induces apoptosis by forming pores in the outer mitochondrial membrane, permeabilizing it to the passage of pro-apoptotic factors such as cytochrome c [109]. The balance between Bax and Bcl-2 determines the propensity of cells to respond to a given insult by apoptosis or survival [109]. Melatonin is able to shift the balance towards a cell-protective state [108]. Indeed, many studies have demonstrated that its anti-apoptotic effect involves the down-regulation of Bax [110-113] and /or the up-regulation of Bcl-2 [110, 114, 115] that occurs *via* activation of NF-κB [115]. Besides altering their abundance, melatonin could alter Bax and Bcl-2 activity in other ways. Oxidative alterations cause Bax disulfide dimerization, and the resulting conformational changes are sufficient for its translocation to mitochondria independently of apoptosis [116]. However, melatonin maintains Bax in a monomeric state within mitochondria [108]. The inhibition of Bax activation may be due to melatonin's anti-oxidant ability, and/or to the simultaneous re-localization of Bcl-2 [108] to mitochondria, thus co-localizing with (and sequestering) Bax. Indeed, the persistence of Bax monomers in cells induced to apoptosis in the presence of melatonin results from the prevalence of a Bax-Bcl-2 heterodimer over the active Bax dimers within mitochondria, thus, blocking both cytochrome C release and apoptosis.

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It is interesting to note that melatonin has developed various independent strategies to inhibit the intrinsic pathway of apoptosis at the level of Bax activation, inducing a pro-survival pathway and by this way maintaining viability of cells at the inflammatory site.

9. Role of melatonin in maintaining and restoring human health

Melatonin controls the onset and progression of many human pathological states by multiple mechanisms asdescribed by studies reporting effects of clinical trials.

Alterations of circadian rhythms implying variations of the 24-h light/dark cycle, play a critical role in the development and progression of many diseases. Several mediators of the inflammatory response, including cytokines and hormones, have been observed to undergo significant diurnal plasma concentration variations [45-48]. Indeed, a mathematical model validated by experimental data and clinical observations, demonstrates the interplay between inflammation and circadian rhythms [117]. Melatonin is synthesized in the suprachiasmatic nucleus according to a circadian rhythm, thus causing diurnal oscillation of its blood levels. This regulation allows melatonin to coordinate circadian rhythms, including the sleep-wake cycle, thus possessing a beneficial and therapeutic effect as demonstrated by the reports of clinical trials with administration of melatonin [118].

Melatonin is widely used to treat various sleep disorders [119-130], including disorders associated with delayed phase sleep syndrome [119-121], periodic sleep disorders in blind patients [122-125] and sleep and behavioral disorders in children with severe brain damage [126-129]. In a randomized double-blind, placebo-controlled study [130], melatonin was shown to improve sleep efficiency and sleep latency [130] in cystic fibrosis (CF) patients, a chronic progressive disorder characterized by repeated episodes of respiratory distress and sleep disturbances [130].

However, most of the clinical trials involving melatonin exploit its anti-oxidant properties. Melatonin was shown to reduce nitrite levels in exhaled breath condensate in CF patients [130].

Sepsis is a severe condition associated with a significant imbalance of the intracellular redox state resulting from an increased production of oxidant species and a decrease in endogenous antioxidant defenses. In critically ill patients, sources of oxidative stress include the mitochondrial respiratory electron transport chain [131], the respiratory burst associated with neutrophil activation [68], and arachidonic acid metabolism [132]. Melatonin exerts well-documented protective effects against septic shock in both animals and humans. Melatonin was investigated in a prospective clinical trial regarding antioxidant therapy to inhibit the action of nitro-oxidative stress [133]. It was demonstrated that nitric oxide (NO) plays an important role in septic shock, an acute inflammatory response [134]. Indeed, melatonin improves the survival of mice with LPS-induced shock by

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reducing NO synthesis *via* its antioxidant and anti-apoptotic properties [135]. It was demonstrated that antioxidant treatment improves respiratory syncytial virus (RSV)-induced pulmonary inflammation. Interestingly, the administration of melatonin reverses the oxidative stress induced by RSV by reducing NO and hydroxyl radical (OH) levels and by increasing glutathione (GSH) level and SOD activity [136]. At the same time, melatonin inhibits the production of pro-inflammatory cytokines such as TNF-α improving RSV-induced lung inflammatory injury in mice.

Melatonin prevents multiple organ failure, circulatory failure, and mitochondrial damage in model of experimental sepsis [137], and reduces mortality in septic children together with lipid peroxidation as an index of inflammation [138]. Melatonin was shown to improve clinical outcome in terms of infection and organ failure in randomized clinical trials, thus being a promising antioxidant molecule in antioxidant therapy to combat sepsis [132-134,137].

Administration of melatonin to humans and animals at both physiological and pharmacological concentrations is essentially non-toxic; it can be easily synthesized in a pharmacologically pure form or extracted by natural sources; it possesses outstanding versatility in reducing oxidative stress and inflammation. Altogether, these features make of melatonin a potential therapeutic tool to improve human health.

10. Conclusions and perspectives

Commonly used anti-inflammatory agents hardy distinguish between physiological and pathological inflammation; the indiscriminate use of anti-inflammatory agents can lead to a de-regulation of the inflammatory response, with the risk to fail to fight pathogens or injuries. The differential pro- and anti-inflammatory roles of melatonin might promote, regulate, and counteract inflammation simultaneously.

Indeed, melatonin, as an anti-inflammatory molecule, could reduce oxidative tissue injuries by its anti-oxidant properties and selectively inhibit the late/chronic phases of the inflammatory response. Furthermore, melatonin could act on early phases of inflammation by stimulating pro-inflammatory mediators such as arachidonic acid and 5-HETE *via* activation of PLA2 and 5-LOX, respectively [34]. Interestingly, effects of 5-LOX extinguish within 2-3 h, since a down-regulation of the same enzymes is also triggered by melatonin as a later event induced by different mechanisms [88, 89].

Thus, melatonin might facilitate the promotion of the inflammatory response, at the same time limiting it by providing to its resolution *via* down-regulation of 5-LOX and/or PLA2. Altogether melatonin could then avoid complications of chronic inflammation.

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Freshly explanted blood monocytes require LOX-derived signals and also ROS to maximize *in vitro* activation and viability, two events that require the over-expression of Bcl-2 [70]. The role of LOX in maintaining cell viability is not unusual; 5-LOX expression is required to maintain viability of some tumor cells; in addition, in EBV-converted B lymphocytes, 5-LOX inhibition is a rapid and efficient mean of killing EBV+ cells [139].

It has been long debated that melatonin in some instances may exert a pro-apoptotic role [106]; the mechanisms and rationale of this effect are unclear. However, upon Bcl-2 silencing, melatonin acquires pro-apoptotic abilities [108], indicating that the very finely regulated changes in Bcl-2 levels during lymphocyte maturation may confer different susceptibility to melatonin, modulating a pro- or anti-apoptotic effect according to the intracellular amount of Bcl-2.

Thus, melatonin could promote acute inflammation recruiting leukocytes *via* an indirect pro-oxidant effect consisting of 5-LOX activation and promotion of inflammatory products, and prolonging the lifespan of the recruited leukocytes; it may also prevent chronic inflammation by its anti-oxidant ability. This includes a reduction of oxidative damage in the injured tissue as well as the inhibition of pro-inflammatory mediators; break the vicious cycle of oxidation/leukocyte recruitment, and promoting leukocyte apoptosis (**Figure 4**).

Altogether these investigations open new important avenues for the pharmacological use of melatonin in inflammation as the stimulation of different targets, combined with differential cellular sensitivities generates a variety of effects witnessing sophisticated regulatory mechanisms.

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Legends to figures

Figure 1: Biosynthesis of melatonin

Figure 2: Intracellular melatonin targets. Melatonin interacts with plasma membrane (MT1/MT2) and nuclear (ROR/RZR) receptors at high affinity. Additional intracellular targets at lower affinity are the enzymes calmodulin and hydroquinone. The affinity for melatonin and the relative reference is indicated.

Figure 3: Dual effects of melatonin on lipoxygenase. Melatonin both down-regulates lipoxygenase gene expression through RZR-beta (A) and activates 5-LOX *via* PLA2 activation *via* calmodulin-dependent signaling (B).

Figure 4: Pleiotropic effects of melatonin on different steps of inflammation. Summary of the multiple effects that melatonin exerts at different steps of the inflammatory response, indicating a pro-inflammatory role at an early phase, and an antagonist role at later phases. This evokes a smart behavior where melatonin may favor the inflammatory healing processes while contrasting pathologically chronic or deregulated inflammation, thus potentially being an ideal compound to treat inflammatory disturbances.

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Figure 1

Radogna et al. Fig. 1

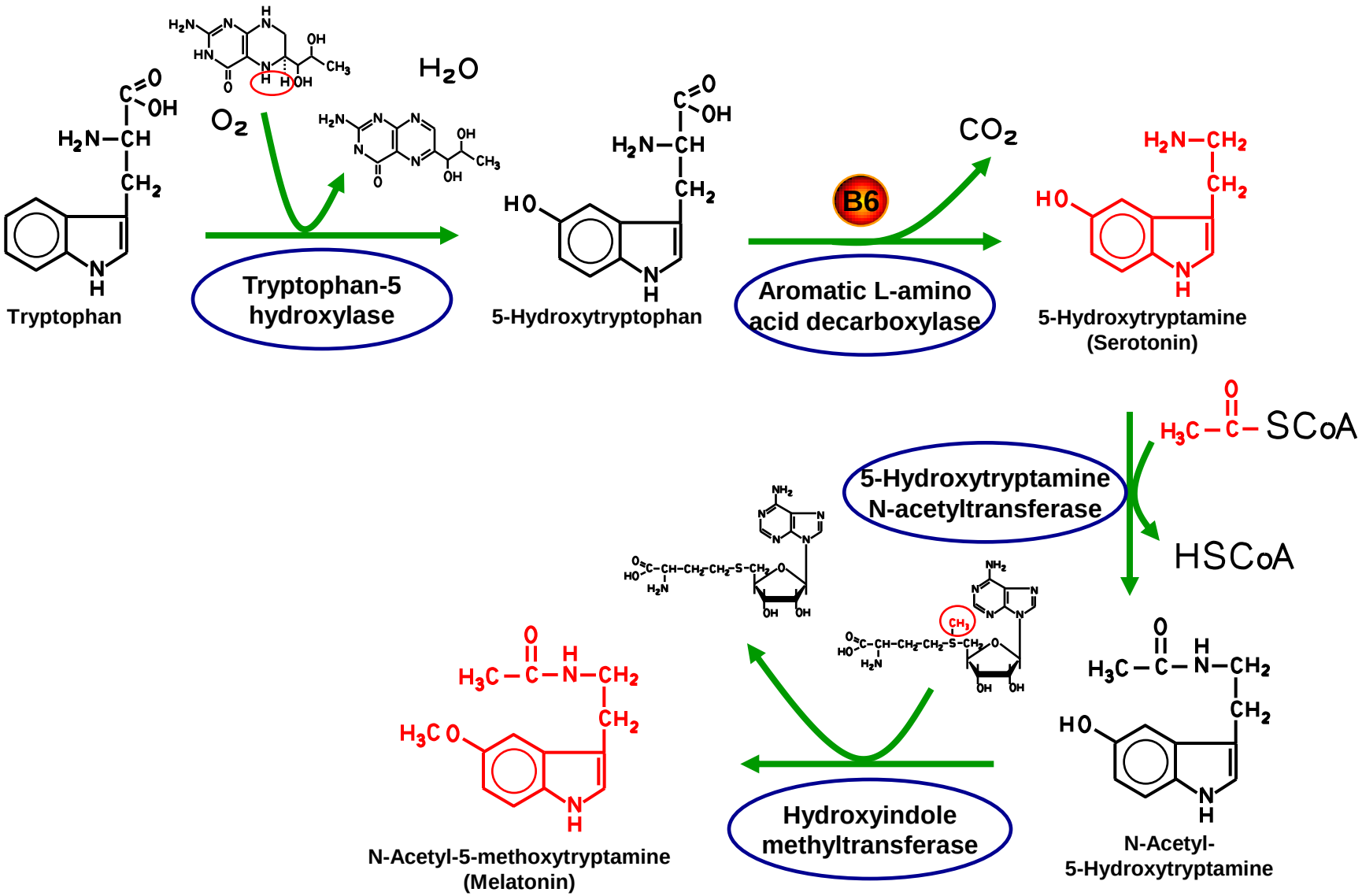
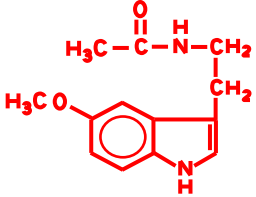


Figure 2

Radogna et al. Fig. 2

A.



Extracellular domain

NH2

MT1, MT2 (nM affinity [11])

Intracellular domain

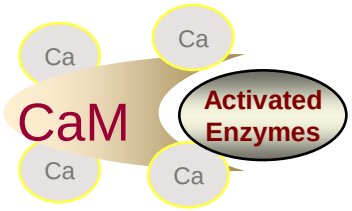
γ
β
Gα
i
AC

COOH

cAMP

Cytosol

C.



CALMODULIN (63 μM affinity [56])

B.

ROR/RZR (pM - nM affinity [27])

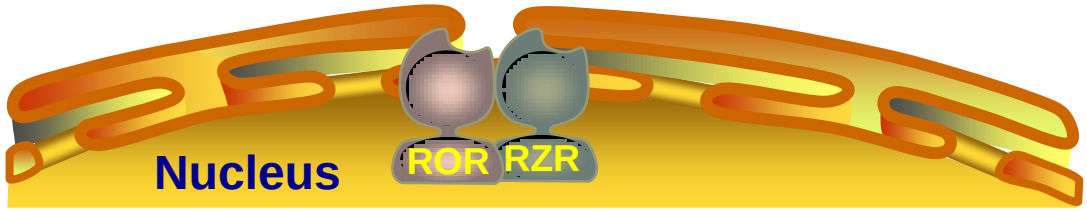


Figure 3

Radogna et al. Fig. 3

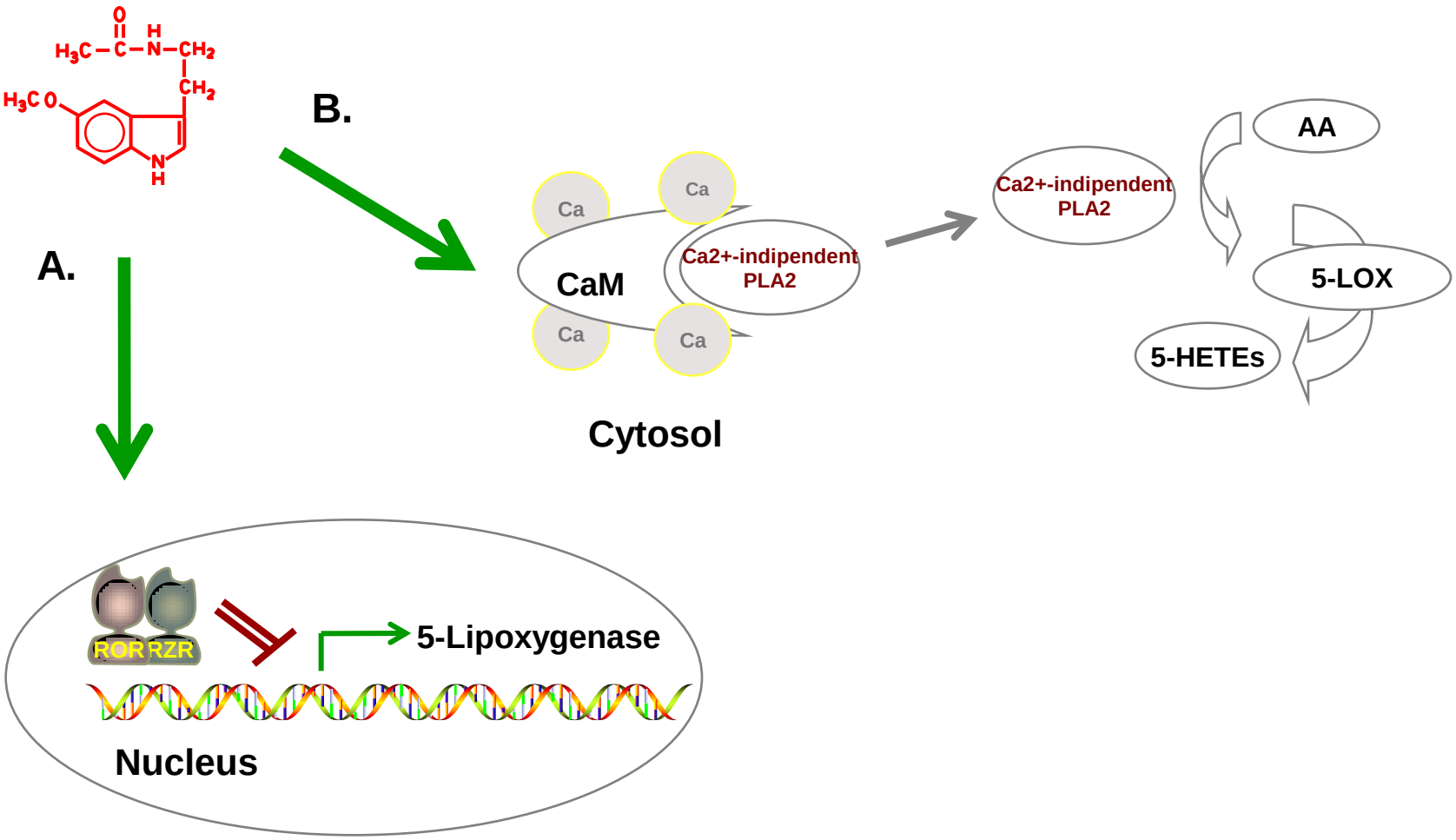
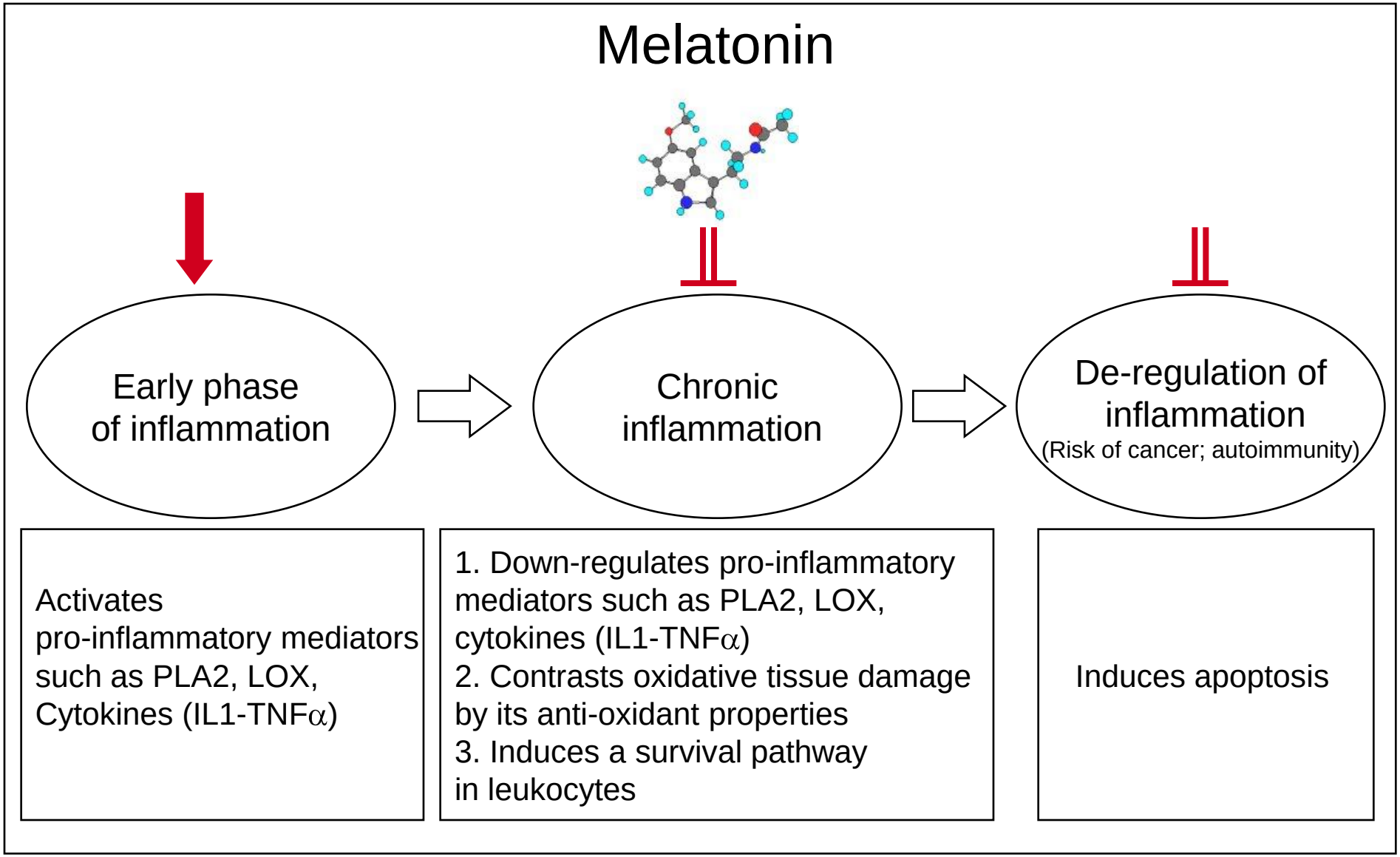


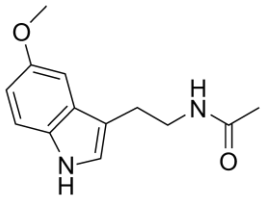
Figure 4

Radogna et al. Fig. 4



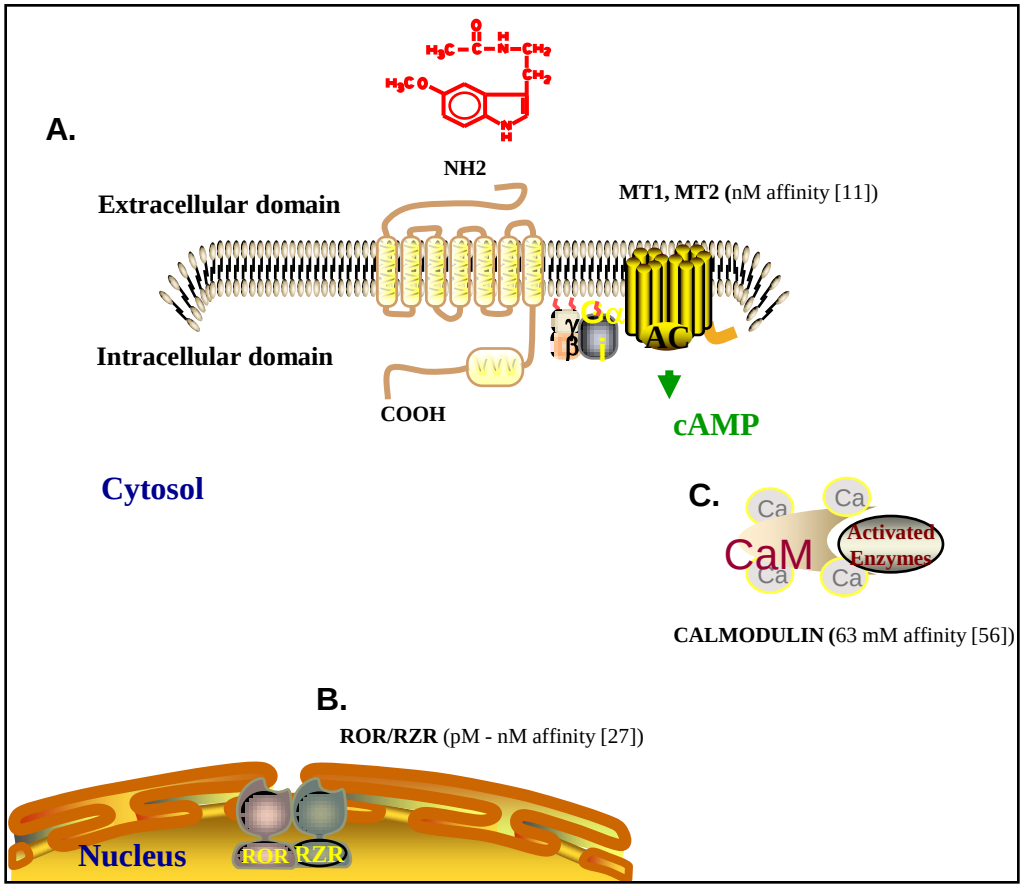
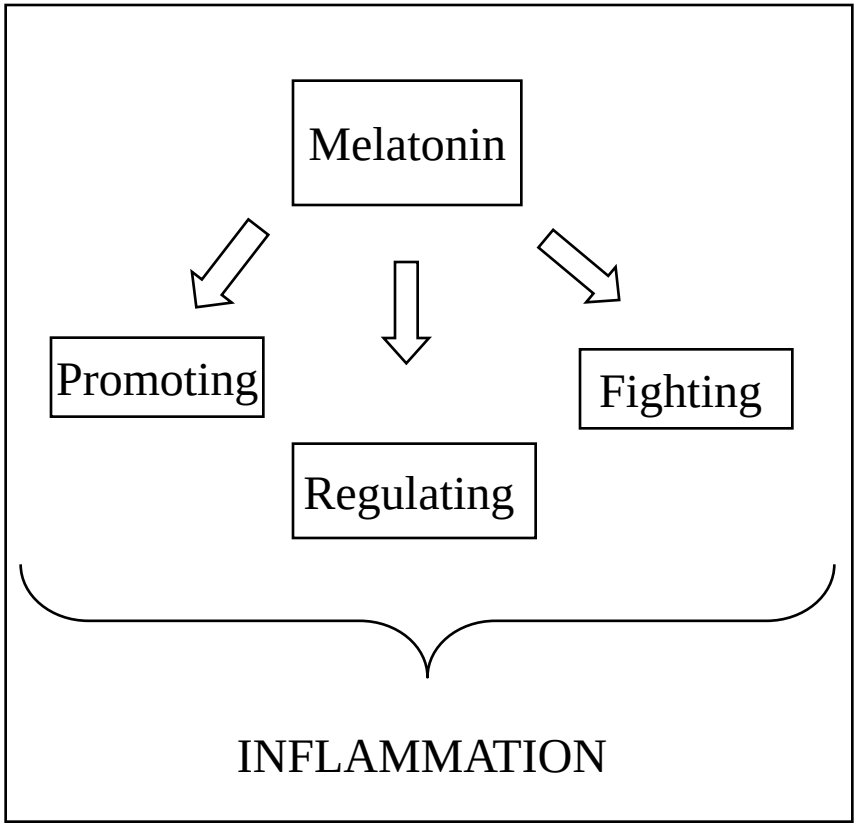
*Graphical Abstract

Molecular structure of melatonin



N-acetyl-5-methoxytryptamine

Intracellular melatonin targets.



Melatonin as a smart drug modulating inflammation.