



## The Influence of Melatonin on Immune System and Cancer

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### Summary

Melatonin has been shown to play a fundamental part in neuro immune modulation. Besides regulating the circadian rhythm it works as a natural antioxidant with immune stimulatory and anti-cancer properties. Melatonin is a regulator of hemopoiesis and modifies various cells and cytokines of the immune system. Melatonin elicits oncostatic properties in a variety of different tumor cells. A number of studies have documented that melatonin, given in combination with chemotherapy to patients with disseminated disease, increases the overall survival and reduces toxic side effects.

The incidence of people with cancer diagnosis is rising worldwide. At the same time the 5-year survival after chemotherapy in people with disseminated cancer is still very low [1]. The traditional treatment with chemotherapy is at high risk for inhibiting the immune system of the patients, which potentially increases the patient's risk for infections and lowers their chance of immune mediated tumor control [2].

The melatonin-mediated circadian rhythm is linked to diurnal changes of the immune system and thus to be an important regulator of specific immune functions which points to a circadian-clock-controlled immune system [3]. The circadian rhythm is regulated by different clock-genes with a master clock situated in the suprachiasmatic nucleus of the brain [3].

The amount of circulating cellular and humoral components such as hemopoietic cells hormones and cytokines varies through the day and exhibit circadian rhythms. At night there appears to be an increase in circulating hemopoietic stem cells, progenitor cells and mature leucocytes with CD8<sup>+</sup> T-lymphocytes being an exception [3]. At day time, there is initially an increase in the level of cortisol, adrenaline, nor adrenaline, tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-1-beta (IL1- $\beta$ ) [3]. Glucocorticoids exhibit a suppressive effect on the immune system. Melatonin/glucocorticoid-mediated changes of the immune system may contribute to an overall balanced functional homeostasis. Disruption of circadian rhythms in humans has been linked with an increasing risk of developing cardiovascular and neoplastic diseases [4].

The last decade has shown an increasing interest in melatonin, a pleiotropic molecule, which functions as sleep regulator, immune stimulant, antioxidant, is a modulator of apoptosis and has oncostatic properties [5-8]. Thus, women who work with night shift

work, and therefore exposed to light at night, have a suppressed level of melatonin and at the same time an increased risk of developing breast cancer [9].

During the last twenty years, studies have been conducted using melatonin as an adjuvant treatment concomitant with chemotherapy in patients with disseminated cancer. Many of these studies include randomised controlled trials (RCT) and are reviewed in several meta-analysis [10-12]. The studies unanimously found that melatonin increases the mean survival time and reduces the toxic side effects from chemotherapy. Melatonin significantly improves complete and partial remission as well as the one-year survival rate by around 50% [10]. These effects probably reflect the pleiotropic functions of melatonin as an immune stimulant and its protection from chemotherapeutic-induced bone marrow suppression, the increased efficacy of chemotherapy, reduce cellular immune levels of oxidative stress [13] as well as the oncostatic and anti-proliferative properties of melatonin [8] (Figure 1).

Melatonin displays low toxicity with no serious side effects reported with doses as high as 20 mg/day [10-12]. Post marketing studies of prolonged-release melatonin drugs have reported the most common side effects to be nausea, dizziness, restlessness and headache, while no symptoms occurred after withdrawal [14]. According to the Danish website [www.sundhed.dk](http://www.sundhed.dk), other side effects of melatonin can be drowsiness, confusion, fatigue, inhibition of ovulation and mood alterations.

### Melatonin and immune system

Melatonin exhibits a general stimulating effect on both the innate and adaptive immune system [6,15,16]. This has been demonstrated in different animal models [15]. Both surgical and functional pinealectomy correlates with weight reduction of the main immune organs such as lymph nodes, thymus and spleen. An almost total absence of circulating lymphocytes and a depletion of lymphoblasts were observed, whereas the major alteration in the spleen was the lack of typical germinal centres within the white pulp. Lymph nodes showed loss of follicles in the outer cortex and a reduction in the numbers of lymphocytes in paracortex. When melatonin was administered to pinealectomized animals the effects on the immune system was typically reversed [15].

### Melatonin and innate immunity

Several in vivo-studies [6] have documented the influence

# Melatonin

## Oncostase

- Oxidative stress
- Angiogenesis
- Metastasis
- Antihormone
- Cellcycle arrest
- Apoptosis



## Immunomodulation

- T-cell and B-cell activity
- Monocyte activity
- NK-cell number and activity
- IFN- $\gamma$ , TNF- $\alpha$
- IL-2, IL-6, IL-12



Figure 1: Some of the beneficial effects of melatonin.

of melatonin on the immune system, under basal and immune suppressed conditions. Administration of melatonin for 7-14 days increased natural killer (NK)-cells and monocytes in the bone marrow [6] and stimulated hemopoiesis [6,16]. Human monocytes express both membrane and nuclear melatonin receptors [16]. In human monocytes and macrophages, melatonin stimulates the production of inflammatory cytokines as IL-1, IL-6, IL-12, and TNF- $\alpha$  while decreasing IL-10. Melatonin also stimulates the production of reactive oxygen species (ROS) in macrophages, which enhances their phagocytic potential [16,17].

The effect of melatonin on NK-cells is important because these cells are assumed to play a significant part in immune surveillance against early cancer development and viral infection [18]. Besides featuring a cancer killing effect, the NK-cells produce interferon (IFN)-gamma, which activates macrophages and other antigenic presenting cells, for processing of antigens including tumor antigens to the adaptive immune system. In this process, melatonin enhances the expression of major histocompatibility complex-II (MHC-II)-molecules on antigenic presenting cells [16]. Short-term administration of melatonin increases the IFN- $\gamma$  production from NK-cells, while long-term administration increases both the amount and the activity of circulating NK-cells [16].

### Melatonin and Adaptive Immunity

Melatonin enhances the production of thymus-derived peptides like thymosin- $\alpha_1$  and thymulin by an increase in prothymosin- $\alpha_1$  gene expression [19]. This contributes to an enhanced T-cell mediated immunity and differentiation.

A connection between the nuclear retinoid-related orphan (ROR)-receptor-alpha expression and IL-2 production has been observed. This implies regulation of cell differentiation, immunity and circadian rhythms as well as lipid- steroid- and glucose metabolism [5,7]. Administration of a melatonin receptor antagonist leads to a rise in cAMPa decrease in ROR- $\alpha$  expression, and thereby an inhibition of IL-2 production [17].

Melatonin protects CD4<sup>+</sup> T-lymphocytes [20] and monocytes [21] from apoptosis. This effect seems to be mediated by a protection of the mitochondria and activation of intracellular signal pathways [21].

The influence of melatonin on infectious and autoimmune diseases has been the focus for a number of studies. These studies show that melatonin administration can be beneficial for patients with infectious diseases by shorting disease duration and improve clinical outcome [6,22]. When it comes to melatonin and autoimmune diseases, the results are few and contradictory [6,22]. Here more research is required, and it is possible that melatonin might not be beneficial in this area.

### Melatonin and Cancer

Melatonin may increase effector T cells and decrease regulatory T cells (Tregs) infiltrating the tumors. Tregs have an inhibitory effect on anti-cancer immunity and some tumor cells are able to up regulate and recruit Tregs to block the antitumor effect of the cellular immune system [23]. Animal experiments have shown a melatonin-mediated down regulation of Tregs and its Foxp3 gene expression in tumor cells, which lead to their reduction in the tumor [24]. Similar results have been observed in cancer patients, where a melatonin-mediated decrease in Tregscorrelated stable disease [25].

A great number of in vitro studies have documented the oncostatic properties of melatonin in a wide variety of tumor cell lines, including, mammary-ovarian and prostate cancer, hepatocarcinomas, melanoma and colorectal cancer. With a few exceptions the general conclusion is that melatonin inhibits cell proliferation and induces apoptosis in most tumor cell lines [26]. The oncostatic activity of melatonin may synergize with novel cancer treatment, employing immune checkpoint inhibitory antibodies [13,26,30].

The mechanism behind these oncostatic properties is thoroughly described by Mediavilla et al. [26]. Briefly mentioned are some of the oncostatic actions of melatonin related to:

## Antioxidant Effects

Melatonin and its metabolites have the capability to both scavenging free radicals and radical related reactants. It detoxifies hydroxyl radical, superoxide anion radical, hydrogen peroxide, peroxyxynitrite anion and nitric oxide [13]. Melatonin also induces antioxidant enzymes as glutathione peroxidase and reductase, superoxide dismutase and catalase [13,26]. This reduces ROS-mediated DNA damage, which potentially is carcinogenic in all three steps of carcinogenesis (initiation, progression and metastasis [13,26].

## Modulation of Estrogen Receptor Expression

Melatonin shows anti-estrogenic properties and decreases the gene expression of estrogen-receptor-alpha (ER $\alpha$ ) and down regulates enzymes involved in the transformation of biological active estrogens [26].

## Modulation of Cell Cycle and Differentiation

Melatonin increases the duration of the cell cycle in various cancer cells by expanding the G1 phase and delaying the entrance of cells into the S phase, or arresting cells in G2/M. This reduces cell proliferation and extends the G0 phase, leading to cell differentiation. This could be explained in part by melatonin's capacity to up regulate the tumour suppressor gene p53 and the cyclin-dependent kinase inhibitor p21 [26].

## Inhibition of Telomerase Activity and Metastasis

Melatonin decreases telomerase activity in cancer cells and displays an anti-metastatic effect. This coincides with an increase in the expression of microfilaments and adhesion plaques, which reduce the invasiveness of cancer cells [27].

## Antiangiogenesis

Melatonin exerts direct anti-angiogenic effects through its inhibitory actions on tumor growth factors, such as IGF, EGF, VEGF and ET-1, which are strong mitogens and stimulate cancer cell-induced angiogenesis [24].

## Activation of Immune System

As previously mentioned melatonin stimulates the production of NK-cells, monocytes, and leucocytes and decreases Tregs. Activated Th1-cells are able to induce apoptosis through mechanisms such as FAS/FAS-ligand-interaction by upregulating FAS-expression on the surface of tumour cells [28].

## Anti-Proliferative and Pro-Apoptotic Effects

Melatonin decreases cell proliferation and induces apoptosis in cancer cells, including apoptosis-resistant hepatocarcinoma cells [29].

## Synergy with Chemotherapy

Melatonin increases chemo sensitivity in cancer cells [13] and exerts a synergistic anti-tumor effect with various antineoplastic drugs, by promoting the apoptotic pathways [30].

## Concluding Remarks and Clinical Perspectives

The beneficial effect and low toxicity of melatonin in cancer treatment has been widely documented in both *in vivo* and *in vitro* experimental investigations covering a large number of different neoplasias. Melatonin has been tested both as an adjuvant with chemotherapies but also as a supplement to palliative care. Melatonin definitely seems to improve overall survival and increase the rate of both partial remissions in cancer patients treated with chemotherapy and in patients in palliative care. Furthermore, the studies show that melatonin administration reduces the toxic side effects of radio- and chemotherapy on the bone marrow reducing lymphocytopenia, thrombocytopenia and anaemia. At the same time there has been reported a protective effects towards neurotoxicity, cardiotoxicity, fatigue and neoplastic cachexia [10-13,22,30].

Lissoni and his associates have been involved in essentially all clinical studies on melatonin in cancer treatment. Their results are supported by a great number of animal and *in vitro* studies. Although the number of patients in the reported RCT's are more than 700 [10-12], it is highly recommended that more controlled clinical trials are performed with a larger number of patients and a longer follow up period to establish the effects of melatonin as an adjuvant to cancer treatment.

Prednisolone is frequently given to cancer patients to mitigate the chemotherapeutic side effects but being a corticosteroid it suppresses the immune system further. In future chemotherapy based RCT prednisolone should be tested in the absence and presence of melatonin and a third arm should include chemo and melatonin only. In addition to primary outcome such as survival time, partial and complete remission, secondary outcomes should include evaluation of side effects and immunological and hematopoietic parameters.

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