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REVIEW

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Melatonin in relation to the antioxidative defense and immune systems: possible implications for cell and organ transplantation

Abstract Melatonin, a molecule synthesized and secreted by the mammalian (including human) pineal gland, has a variety of seemingly unrelated functions in organisms. In photoperiodically-dependent seasonal breeders, the changing melatonin signal imparts seasonal information to the species thereby regulating the annual cycle of reproduction [1]. Melatonin also is involved in a number of 24 h rhythms and is believed to be an important component of the circadian system [2, 3]. More recently, melatonin was found to relate to immune function [4] in organisms and to be an effective antioxidant [5]. As an antioxidant melatonin would appear to provide substantial protection against free radicals which are generated under a variety of experimental corrections, including ischemia/reperfusion injury [6, 7]. These latter two functions of melatonin, i.e., as an immune system modulator and as an antioxidant, both may have applicability to cell and organ transplantation [8–11].

Antioxidant effects of melatonin

Oxidative stress is a phrase used to describe cellular, tissue and organ damage inflicted as a consequence of toxic molecules that are persistently generated in organisms [12]. Many of the free radicals that are produced are a consequence of the utilization of oxygen (O_2 or dioxygen) by animals. While the bulk (>95%) of the O_2 inspired is utilized in the mitochondial respiratory chain in the production of energy in the form of ATP, up to 4% of the O_2 taken in via the lungs is converted to molecules or portions of molecules that have an unpaired electron in their outer orbital; these are classified as free radicals [13]. Because of their unpaired electron, free radicals are highly toxic as reflected in their very short half-lives (sometimes in the nanosecond range) (Table 1). While there are beneficial effects of radicals, e.g., the killing of bacteria by activated monocytes, etc., most of the interactions between radicals and molecules have a negative outcome. Of particular consequence is the damage that results to lipids, proteins and DNA as a result of free radical attack [14].

Fortunately, organisms are equipped with mechanisms to counter the destructive effects of free radicals; these processes are collectively referred to as the antioxidative defense system. This system includes a variety of direct free radical scavengers, metal chelators which reduce free radical generation, enzymes which metabolize radicals or their intermediates to non-toxic agents, and molecules which induce physical changes in the cell (e.g., stabilization of membranes) which help it resist oxidative processes. Any agent which prevents the damage inflicted by radicals is identified as an antioxidant [14].

Certain conditions stimulate the excessive production of free radicals in organisms thereby leading to increased oxidative damage. Most notably, physical or psychological stress, transient ischemia followed by reperfusion, hyperoxia, a variety of chemical toxins, and exposure to either ionizing or ultraviolet radiation all contribute to the burden of oxidative stress [12, 13]. Considering the role of oxidative damage in a very wide variety of diseases and disease processes, interest in molecules which neutralize free radicals has increased substantially in recent years.

Table 1 Estimated half-life of free radicals in vivo. The shorter the half-life, the more reactive and toxic is a radical. Using this and other criteria, the hydroxyl radical is considered the most toxic and it travels only a few Ångstroms before it reacts with another molecule. Oxygen is a diradical (it possesses two unpaired electrons) but it's reactivity is limited by what is referred to as spin restriction. Singlet oxygen is not strictly a free radical; its toxicity is a result of its high energy state.

Chemical Species	Symbol	Half-life (sec) at 37°C
Alkoxyl radical	RO•	1×10^{-6}
Hydroxyl radical	OH•	1×10^{-9}
Lipid peroxide	ROOH	$>10^{2}$
Molecular oxygen	O_2	$>10^{2}$
Peroxyl radical	ROO•	1×10^{-2}
Singlet oxygen	$^1O_2$	1×10^{-6}
Superoxide anion radical	O_2 –•	1×10^{-6}

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Free radicals are generated in all subcellular compartments and, as a consequence, molecular scavengers that have access to the interstices of the cell can afford maximal protection against these damaging agents. Once produced, because of their high reactivity, free radicals travel for very short distances (in many cases a few Ångstroms) before they interact with and damage molecules. While they indiscriminately damage all molecules, the destruction of large molecules (lipids, proteins and DNA) are most obvious and easiest to measure [14]. Also, because of the short distances they travel, unless a free radical scavenger is in very close proximity to where the radical is generated, it cannot prevent the damage the radical will inflict.

Some free radical scavengers are exclusively lipid soluble; an example is vitamin E (α-tocopherol). Vitamin E is usually considered, with justification, the premier lipid antioxidant because of it high reactivity with the peroxyl radical (LOO•) which is generated in lipid-rich cellular membranes [15]. However, vitamin E is ineffective in directly protecting cytosolic proteins and nuclear DNA from the oxidative hits of locally generated free radicals since the aqueous environments exclude vitamin E. Another limitation of vitamin E is the limited ability with which it traverses the blood-brain-barrier; thus, for vitamin E to be an effective neural lipid antioxidant in vivo, it must be administered in high doses for several days before it reaches concentrations in the brain sufficient to afford substantial protection against free-radical induced lipid peroxidation.

Vitamin C (ascorbic acid) is also a widely accepted and used free radical scavenger and antioxidant [16]. Unlike vitamin E, vitamin C has ready access to the aqueous environments of the cell because it is soluble in aqueous media and, therefore, it readily protects cytosolic proteins and other molecules from oxidative damage. On the other hand, the fact that it is not lipid soluble limits its ability to directly protect against oxidative damage to membrane lipids. Furthermore, vitamin C is not exclusively an antioxidant. In cells vitamin C can actually generate free radicals, i.e., be a pro-oxidant, thereby increasing molecular damage. The ability of vitamin C to function as a pro-oxidant occurs especially when free iron is present within cells.

Although historically considered to be exclusively lipid soluble [17], melatonin has considerable aqueous solubility as well [18]. This being the case, it could be predicted that melatonin's antioxidative actions may be manifested throughout the cell. When subjected to test either in vitro or in vivo, melatonin proved to effectively limit lipid peroxidation [19], restrict free radical damage to protein [20], and greatly curtail ionizing radiation-induced damage to nuclear DNA [21]. The latter observations are consistent with radioimmunoassayable and immunocytochemical data which suggest relatively high concentrations of melatonin in the nuclei of cells after its peripheral administration [22]. Furthermore, unlike vitamin E, melatonin readily crosses the blood-brain-barrier so even when it is acutely administered it gets into the brain in sufficient concentrations to provide substantial protection against free radical damage. Thus, melatonin, like the combination of α-lipoid acid and its metabolite dihydrolipoate, may be considered a universally-acting antioxidant [23].

Melatonin, as a free radical scavenger, works via electron donation [24]. When, for example, it encounters the highly reactive •OH it renders an electron thereby detoxifying it. However, during an interaction between a non-radical species (in this case melatonin) and a radical (in this case the •OH), another radical must be produced. In the case of melatonin, electron donation leads to the production of an indolyl (or melatonyl) cation radical. Fortunately, the reactivity of this radical species is much lower than that of the •OH so there is a substantial net gain in reducing the potential of oxidative damage when melatonin detoxifies the •OH. Melatonin is reported to have a high capability of detoxifying the LOO• radical as well [25], although its efficacy in this regard is debated.

The indolyl cation radical that is produced when melatonin renders an electron is a species that has been only minimally investigated. It has been proposed that it may scavenge the superoxide anion radical (O_2 –•) to produce a molecule, N^1-acetyl-N^2-formyl-5-methoxykynuramine, which is excreted in the urine. If it does so, this would provide additional antioxidative protection since the O_2 –• itself has some toxicity but of even greater importance perhaps is that the O_2 –• is the precursor of the •OH. Therefore, any molecule that neutralizes the O_2 –• effectively reduces the generation of the highly toxic •OH.

The possibility that the indolyl cation radical can be converted back to melatonin is also a possibility. Not uncommonly antioxidants are regenerated; an example is vitamin E. When vitamin E reduces a radical it becomes the tocopherol radical which, at the lipid-aqueous interface of the cell, is restored to vitamin E when it is reduced by vitamin C [16]. Whether other electron donating molecules can restore the indolyl cation radical to melatonin is currently under consideration. Preliminary in vitro evidence suggests that melatonin may be recycled by some known antioxidants since they act synergistically in neutralizing free radicals. The specific mechanisms involved, however, requires additional clarification.

In all in vivo studies to date where it has been tested, melatonin has been shown to be an effective free radical scavenger and antioxidant [5, 26]. Melatonin administered by virtually any means, is readily absorbed through the gastrointestinal tract, through the skin and across mucous membranes and, even in very high doses it has not been shown to be toxic. Melatonin has been found as well to be protective against paraquat toxicity, excitatory neurotransmitter toxicity, ionizing radiation, ultraviolet light-induced cellular damage, and possibly against viral replication. Several of these functions may have relevance to organ transplantation patients where free radicals and toxic shock could be factors directly related to success of the procedure [8–10].

Besides its efficacy as a direct free radical scavenger, melatonin has another feature which would seemingly make it beneficial during and after surgical or transplantation procedures. The use of the indole in experimental studies has shown that it limits both viral [27, 28] and bac-

terial [29] infections and, additionally, it greatly attenuates bacterial lipopolysaccharide oxidative damage [30, 31]. Because of this latter property it has been proposed for use in combating endotoxic shock [32].

Immunological effects of melatonin

The first evidence related to a possible immunological role of melatonin dates back in 1981 when it was reported that exposure of mice to continuous illumination or evening administration of beta-adrenergic blockers (both of which inhibit melatonin formation) were associated with depressed immune function [33]. Later studies demonstrated that melatonin augments both humoral and cell mediated immune responses [3]. While these effects of melatonin were not particularly noticeable under normal conditions, melatonin was highly effective under immunodepressed conditions such as those which follow acute stress, drug or corticosteroid treatment, viral diseases and aging [3, 34]. As an example, Wichmann et al. [11] recently showed that melatonin significantly attenuated depressed immune function which followed soft tissue trauma and hemorrhagic shock in mice. Furthermore, an increase of human leukocyte natural killer activity has been reported to occur upon chronic melatonin treatment [35]. Based on the results obtained in animal studies, melatonin has been administered in association with interleukin-2 (IL2)-treated cancer patients. This combination, i.e., melatonin + IL2, has been shown effective in controlling tumor growth even in patients who were unresponsive to IL2 alone and/or to conventional chemotherapy [36]. Most studies seem thus to support an immunoehancing role for melatonin, although the opposite effect, i.e., immunodepression also has been reported [37].

The ability of melatonin to counteract immunodepressive states and/or enhance immune functions seems to depend on its binding to specific receptors on T-helper lymphocytes [38]. Melatonin binding to these receptors results in an enhanced release of cytokines such as gamma-interferon, IL2 or opioid peptides [39]. Recent studies have shown that these melatonin-induced opioids (MIO) may also mediate an interesting hematopoietic action of melatonin. Hence, melatonin rescued the blood forming system from the toxic action of cancer chemotherapeutic agents administered to tumor bearing mice [40]. A scheme summarizing melatonin's influence on immune functions is shown in Fig. 1. While further studies suggested the involvement of interleukin-4 (IL4) in this effect, a more thorough investigation revealed that the putative IL4 was a group of opioids, i.e., two polypeptides with apparent molecular weights of 15 and 67 kDa which were recognized by anti-IL4, anti-common opioid sequence (Tyr-Gly-Gly-Phe) and anti-dynorphin antibodies. T-helper type 2 cells have been reported to release enkephalin-containing peptides; however, the molecular structure of these substances prevents opioid receptor-mediated effects [41]. On the contray, MIO exhibit at their amino terminal, the common opioid sequence which is essential for any opioid receptor-mediated effect and their action is naltrexone sensitive which is also indicative of an opioid effect (Fig. 1). These findings support the suggestion that MIO may belong to a new class of T-helper cell cytokines or opioid peptide family. Since melatonin may enhance gamma-interferon and IL2 production, but not IL4, it is possible that T-helper cells type 1 rather than type 2 are a target of melatonin.

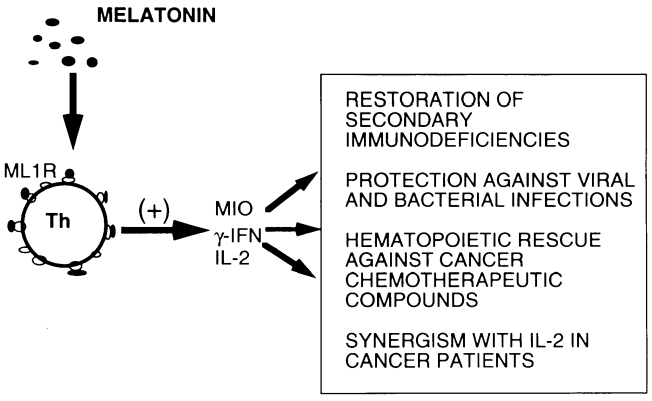

Fig. 1 Summary of the potential mechanisms and immunological actions of melatonin. Melatonin initially binds to type 1 receptors (ML1R) on T-helper lymphocytes (Th). This results in an increased production of opioid peptides (MIO), interleukin-2 (IL-2) and gamma-interferon (γ-IFN) which, in turn, presumably mediate the immunological effects summarized in the text

Concluding remarks

The present success of cell and organ transplantation is largely due to advances in immunosuppressive therapy aimed at avoiding graft rejection [42]. Melatonin might therefore not be useful because of its immuno-augmenting properties. Immunosuppressive therapy, however, is associated with an increased risk of infection and malignancy [42, 43] and here, perhaps, melatonin might be beneficial. As noted previously, melatonin is effective against viral [27, 28] and bacterial infections [29] and it protects against multiple organ dysfunction produced by the bacterial endotoxin lipopolysacchride [30, 31]. Additionally, melatonin has also been reported to have immunodepressive effects in certain situations. It is not obvious whether these seemingly opposite results depend on the concentration of melatonin used or the immune function end point measured. It has been shown that the administration of large pharmacological doses (>100 mg/kg BW) of melatonin depresses rather than enhances antibody production [3]. This may relate to the fact that, with such large doses, melatonin may be present on the receptors for long periods thereby down regulating them thus leading to immunosuppression. These immune system effects of melatonin, coupled with the antioxidant actions of the molecule [5], particularly since free radicals may play a significant role in graft rejection [8–10], suggest its potential utility in cases of cell and organ transplantation. Clearly, however, additional studies are required to define the potential efficacy

of melatonin in experimental conditions where organ or cell transplantation is involved. The virtual absence of toxicity of melatonin may help to make it a potentially useful adjunct therapy after transplantation.

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