MINI REVIEW
Melatonin and viral infections

Abstract: The therapeutic effects of melatonin against viral infections, with emphasis on the Venezuelan equine encephalomyelitis (VEE), are reviewed. Melatonin has shown to prevent paralysis and death in mice infected with the encephalomyocarditis virus and to decrease viremia. Melatonin also postpones the onset of the disease produced by Semliki Forest virus inoculation and reduces the mortality of West Nile virus-infected mice stressed by either isolation or dexamethasone injection. An increase in the host resistance to the virus via a peripheral immunostimulatory activity is considered responsible for these effects. It has also been demonstrated that melatonin protects some strains of mink against Aleutian disease, and prevents the reduction of B- and T-cells as well as Th1 cytokine secretion in mice infected with leukemia retrovirus. In VEE-infected mice, melatonin postpones the onset of the disease and death for several days and reduces the mortality rate. This protective effect seems to be due to the increase in the production of interleukin-1β (IL-1β), as 100% of the infected mice treated with melatonin die when IL-1β is blocked with antimurine IL-1β antibodies. Although melatonin administration raises serum levels of tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), the mortality observed in neutralization experiments with the corresponding anticytokine antibodies, suggests that neither TNF-α nor IFN-γ are essential for the protective effect of melatonin on murine VEE virus infection. Melatonin treatment also enhances the efficiency of immunization against the VEE virus. Reactive oxygen species have been implicated in the dissemination of this virus, and their deleterious effects may be diminished by melatonin. This indole inhibits nitric oxide synthetase activity and it is a potent scavenger of nitric oxide, which also plays an important role in the spread of the VEE virus. In conclusion, the immunomodulatory, antioxidant, and neuroprotective effects of melatonin suggest that this indole must be considered as an additional therapeutic alternative to fight viral diseases.

Introduction

The pineal gland is a neurochemical transducer capable of perceiving basic environmental information and integrating it with the synthesis and release of a variety of agents of which melatonin (N-acetyl-5-methoxytryptamine) is the most thoroughly studied. This secretory product appears to be involved in synchronizing circadian and seasonal timing of several physiological and behavioral processes [1–4]. The melatonin rhythm is certainly an important efferent message from the suprachiasmatic nuclei signal for influencing the organism’s circadian system [5].

Melatonin is also a powerful and effective hydroxyl radical scavenger, which provides on-site protection against oxidative damage to cell components [6]. It also scavenges the peroxy radical generated during lipid peroxidation with an activity that is reportedly greater than that of vitamin E [7]. Additionally, in vivo it stimulates several antioxidative enzymes including glutathione peroxidase, thereby potentiating its antioxidant properties [6]. Thus, melatonin seems to be an essential component of the antioxidant defense system [8].

It has also been shown that melatonin has an important role in the immune function both under physiological and physiopathological conditions [9]. By binding to T-helper cells, the indole gives rise to a cascade of events leading to an increase in immune responsiveness [10, 11]. Several immune functions appear to be modulated by melatonin, including the antitumor defenses of the host [12] and cytotoxicity of natural killer cells [13]. The immune effects induced by melatonin seem to be mediated, at least in part, by endogenous opioid peptides [14]. Exogenous administration of melatonin enhances antibody production [14].

Melatonin binds to membrane receptors [15], which are coupled to two GTP-binding proteins, one sensitive to
pertussis toxin and the other to cholera toxin. Two subtypes of receptors have been described in mammals based on their kinetics and pharmacologic differences of 2-125I-melatonin binding [16] and on their molecular structure [17]. The intimate relationship of melatonin to the immune system is supported by the presence of melatonin receptors in immunocompetent cells [18]. Specific binding sites for the indoleamine have been described in different immune tissues from many species of birds and mammals [19], including human peripheral blood lymphocytes [20], monocytes [21], and granulocytes [22, 23]. Nuclear receptors for melatonin have also been described in human and murine immunocompetent cells [24–26].

**Effect of melatonin on viral infections**

The outbreaks of Venezuelan equine encephalomyelitis (VEE) infection in South America in 1995, West Nile encephalitis in the US in 2002, and the severe acute respiratory syndrome (SARS) in 2003, constitute health threats that need to be managed.

Venezuelan equine encephalomyelitis is an important human and equine disease caused by the VEE virus, a mosquito-borne virus of the family Togaviridae and genus Alphavirus [27]. Outbreaks occurred in northern South America from the 1920s to the 1970s with thousands of people, horses, and donkeys affected [28–30]. Mice infected with the virus show excitation and hypermotility followed by hypomotility, paralysis, coma, and death [31]. During the 1995 VEE epidemic more than 20 VEE-associated deaths were reported among adults and children in Venezuela and Colombia [32].

West Nile encephalitis is a mosquito-borne viral disease caused by the West Nile virus (WNV), a flavivirus endemic in certain regions of Africa, Asia, and eastern Europe. Patients usually present with headache, fever, gastrointestinal symptoms, maculopapular rash, and lymphadenopathy. The disease may be fatal, especially in the elderly or in immunoparalyzed patients [9]. Meningitis and encephalitis may occur leading to death or leaving survivors with neurological deficits [33].

Semliki Forest virus (SFV) is an arbovirus of low pathogenicity in humans [34], but it causes fatal encephalitis in mice [35].

The isolation of a new coronavirus in the respiratory secretions of a patient with SARS and the subsequent demonstration or serological evidence of this virus in other patients with the disease suggest a causal relationship between this virus and SARS [36]. During the initial phase, the symptoms are similar to those of influenza. In all cases, body temperature is 38°C or higher and dry cough is constant, except when there is a superinfection. Dyspnea is frequent, and it occasionally leads to acute respiratory distress with hypoxemia and hypercapnia [37].

Viral infections can produce a generalized immunodepression [38, 39]. Evidence of the ability of melatonin to protect against viral encephalitis was provided by Maestrini et al. [40] who showed that it prevented paralysis and death in mice infected with encephalomyocarditis virus after acute stress. Ben-Nathan et al. [41] reported that administration of melatonin (500 μg/kg from 3 days before until 10 days after the SFV inoculation) reduced viremia and significantly postponed the onset of the disease by 7–10 days. Melatonin reduced the mortality of SFV (10 plaque-forming units, pfu)-inoculated mice from 100 to 60%. In those inoculated with a higher dose (100 pfu), melatonin reduced mortality by only 20%. In the surviving mice, anti-SFV antibodies were detected 22 days after virus inoculation. WNV infection of mice stressed by either isolation or dexamethasone injection induced mortality of 75 and 50%, respectively, which was reduced by melatonin administration to 31 and 25%, respectively [41].

Melatonin has also been found to protect both a wild-type or demi strain and demi/dark cross strain of mink from Aleutian disease which is produced by a persistent parvoviral infection that results in marked hypergammaglobulinemia and immune complex-mediated lesions of the liver, kidneys, lungs, and arteries [42].

Female C57BL/6 mice infected with the LP-BM5 leukemia retrovirus develop murine acquired immune-deficiency syndrome. This infection inhibits the release of Th1 cytokines, stimulates the secretion of Th2 cytokines, increases hepatic lipid peroxidation, and induces vitamin E deficiency. Zhang et al. [43] reported that treatment with dehydroepiandrosterone or melatonin, alone or in combination, prevented the reduction of B- and T-cell proliferation and the Th1 cytokine secretion caused by this retroviral infection. These hormones also suppressed the elevated production of Th2 cytokines, reduced hepatic lipid peroxidation, and prevented the loss of vitamin E.

**VEE virus infection and melatonin**

Melatonin administration protects mice infected with the VEE virus. When evaluated 6 days after virus inoculation (10 LD$_{50}$ per mouse) the mortality rates of mice treated with 250, 500, and 1000 μg melatonin per kilogram were 45, 40, and 16%, respectively, as compared with 100% in control-infected mice. In addition, melatonin treatment delayed the onset of the disease and prolonged the time to death in the treated mice. In surviving mice receiving the indoleamine, the VEE virus IgM antibody titers were highly elevated 7 wk after virus inoculation [44]. The administration of melatonin significantly decreased the virus levels in blood and brain as compared with the infected control mice. When mice were pretreated with melatonin for 10 days, a significant increase in the survival rate (73%) was observed when compared with the 60% obtained after the standard 3 days of pretreatment. In cultures of chicken embryo fibroblasts with serial dilutions of VEE virus, melatonin significantly reduced the degree of cell destruction. This finding contrasts with that reported by Ben-Nathan et al. [41] who found no effect of the indoleamine on SFV growth in tissue culture. However, our results do not preclude the possibility that melatonin alters the host resistance to the virus, rather than viral replication, via a peripheral immunostimulatory effect, as fibroblasts are capable of producing tumor necrosis factor-alpha (TNF-α) whose antiviral activity is known [45]. Melatonin did not stimulate the synthesis of interleukin-2 (IL-2) but increased the serum concentration of interferon-γ (IFN-γ). In infected mice, treated or non-treated with melatonin,
IFN-γ levels in serum were also augmented. A direct and/or an immune-based effect of melatonin on VEE viral replication within the brain was suggested by the fact that on day 5 after inoculation, the virus was not detected in the brain [44].

Melatonin also prolonged the survival of immunodepressed mice infected with the VEE virus [46]. This protection is approximately 50% of that provided to immunocompetent mice suggesting that this indoleamine requires, at least partially, the integrity of the immune system to induce its protective activity. The lack of effect of melatonin on the viral titers in the brain of immunodepressed animals contrasts with the finding that melatonin administration reduces VEE virus levels in the brain of immunocompetent mice [44] suggesting that the antiviral mechanisms stimulated by melatonin in immunocompetent mice are absent or diminished in the immunodepressed animals. The protection provided by melatonin against oxidative damage to cell components [6] could be responsible, at least in part, for the increase in the survival rate of the immunodepressed infected mice. In this regard, it was demonstrated that mice with targeted deletions in either their interferon-alpha-beta-receptor (IFNAR-1-/-) or interferon regulatory factor 2 (IRF-2-/-) genes were more susceptible than control mice to VEE infection. The IFNAR-1-/- mice exhibited accelerated VEE virus dissemination to serum and brain when compared with control mice. In IRF-2-/- mice inducible nitric oxide synthetase (iNOS) gene, induction was completely absent following VEE virus infection. When the role of cells involved in iNOS production was evaluated, it was found that primary microglial cell cultures were highly sensitive to VEE virus infection. Besides, this infection increased the levels of nitric oxide in resting microglial cultures, but decreased nitric oxide production in IFN-γ-stimulated microglia. These findings suggest that reactive nitrogen species play an important role in the spread of the VEE virus [47]. It is interesting to note that melatonin inhibits cerebellar NOS activity [48] and is a potent scavenger of nitric oxide in vitro [49]. In cultured murine macrophages, melatonin has been found to inhibit nitric oxide production due to a reduction in iNOS steady-state mRNA levels and iNOS protein expression [50].

In a recent study [51], it was found that melatonin stimulated the endogenous production of IFN-γ, IL-1β, and TNF-α but not of IL-2 and IL-4 in mice treated. It has been suggested that melatonin and IFN-γ create an immunoregulatory circuit responsible for the antiviral, antiproliferative, and immunomodulatory action of IFN-γ [52]. This cytokine increases serotonin and melatonin levels in lymphocytes and macrophages [53]. The early stimulation in the production of IFN-γ by melatonin shown by Valero et al. [51] suggests that previous treatment with this indoleamine increases the antiviral activity of IFN-γ, that could possibly control viral replication at the moment of the VEE virus inoculation.

In a mouse model, the kinetics of cytokine expression following infection with molecularly cloned VEE virus showed that there is a consistent gene expression pattern associated with a rapid increase of IFN-γ, IL-6, IL-10, IL-12, and TNF-α in the absence of elevations in either IL-2 or IL-4 during responses to both attenuated and virulent VEE virus [54]. The report of Valero et al. [51] corroborates and extends some of these findings.

Interleukin-1β and TNF-α appear to be more sensitive to melatonin administration than the other cytokines assayed. Both of them were elevated earlier, immediately after treatment, and during the VEE viral infection. Melatonin triggered the host IL-1β response, suggesting an important role in the primary defense against the VEE virus infection [51]. These results are in agreement with the demonstration that this indoleamine activates monocytes and induces their cytotoxic properties, along with the IL-1β secretion [55]. The monocytes/macrophages are not only sensitive to an external stimulation by melatonin, but they also synthesize it [52]; therefore, this agent increases the reactivity of monocytes against infections.

Although melatonin increased serum levels of TNF-α and IFN-γ, the average mortality obtained, during the neutralization experiments with the corresponding anticytokine antibodies, suggests that neither TNF-α nor IFN-γ are essential for the protective effect of melatonin observed in murine VEE virus infection [44, 51]. The fact that 100% of the infected mice treated with melatonin died when IL-1β was blocked with antimurine IL-1β antibodies, suggests that IL-1β induced by melatonin treatment is a target cytokine to promote the immune-enhanced state which produces the viral clearance, or help generate an earlier immune response against the VEE virus infection. The study of mice lacking functional genes for IL-1β or treatment of VEE-infected mice with IL-1β should provide further insight into its role in this infection.

The survival rate of mice infected with the VEE virus preincubated with melatonin, as well as the results of the studies in vitro with the same virus, suggest that this agent does not exert a direct effect on the VEE virus [51]. In fact, it has been suggested that the encephalitic phenomenon is due more to an increased immune response in the central nervous system (CNS) than to the VEE virus replication [56].

The VEE virus infection also produced a striking increase in the levels of TNF-α and IL-1β in the brain of mice. Melatonin stimulated the endogenous production of IL-1β and reduced the concentration of TNF-α in the brain of these infected mice [57]. Elevations of IL-β levels in the brain have been detected in several viral infections of the CNS, such as those produced by the human immunodeficiency virus [58], simian immunodeficiency virus [59], SFV [60], rabies virus [61], and Sindbis virus [62]. However, these studies did not directly examine whether IL-1β plays a protective or deleterious role in CNS viral infections. Liang et al. [63] compared the natural histories of deficient and wild-type 129 SV (ev) mice infected with a neurovirulent viral strain, neuroadapted Sindbis virus. Their results suggest that IL-1β deficiency is protective against fatal Sindbis virus infection. In contrast, mice deficient in IL-1β have increased susceptibility to influenza virus [64], and in poxvirus animal models the viral induction of this cytokine is also beneficial for the host [65].

The increase in IL-1β levels detected in blood [51] and brain [57] of VEE virus-infected mice after melatonin...
treatment plays a protective role to the infected animals, as shown by the effects of the blockade of IL-1β with antimurine IL-1β antibodies [51]. The mechanism of this effect is unknown, but it is possible to envision that during the VEE viral infection certain degenerating neurons that contain substance P might release this neuropeptide, which subsequently induces the release of IL-1β and TNF-α from monocytes [66], which can then infiltrate the CNS from the blood [67]. Microglia proliferate and phagocyte local neuronal debris, resulting in microglial synthesis of IL-1β [67, 68], which in turn induces the expression of adhesion molecules on local endothelium, favoring transmigration of lymphocytes and monocytes [69]. The increase in IL-1β levels produced by melatonin would provide neuronal support and protection by inducing nerve growth factor secretion by astrocytes [70], thereby supplying a trophic factor for many neuronal cell types in times of stress, such as that produced by the VEE virus infection.

TNF-α seems to be upregulated in a wide range of CNS disorders [71, 72] and has been implicated in both neuronal death and survival [73–76]. It plays an important role in the initiation of inflammation and the resulting pathological effects and can stimulate different cell types to produce several cytokines including IL-1β and TNF-α itself [77, 78].

In VEE virus infection it is presumed that upon stimulation by the increased brain levels of TNF-α, astrocytes produce colony-stimulating factors that can augment inflammatory responses because of their leukocyte chemo tactic properties, which would promote migration of granulocytes and macrophages to inflammatory sites within the CNS [79]. Astrocyte-derived TNF-α can induce intercellular adhesion molecules on neighboring endothelial cells [80], alter blood-brain barrier permeability, and promote inflammatory infiltration into the CNS [81]. Therefore, it is reasonable to assume that the highly significant decrease in the concentration of brain TNF-α produced by melatonin in mice infected with the VEE virus [57] is aimed at diminishing the inflammatory response in the CNS and at correcting the alteration in blood-brain barrier permeability. Thus, the indoleamine would help to protect the brain of the infected mice.

High intensity light and VEE virus infection

In VEE-infected mice exposed to a high intensity light (HIL) of 2500 lux with a 12 h light:12 h dark photoperiod, both nocturnal and diurnal serum levels of melatonin were constantly elevated and the survival rate increased from 6 to 13 days after virus inoculation. Melatonin concentrations remained high throughout the day and night, breaking the established pattern observed in the 400 lux control mice, characterized by high levels of melatonin at night and low levels during the day [82]. These results confirm that the intensity of illumination during the light period affects the metabolism of pineal indoles in rodents [83]. The HIL seems to be responsible for the rise in melatonin production and the associated increase in the survival rate of the infected mice.

Exposure to 2500 lux also increased significantly the levels of melatonin in the olfactory bulb of VEE-infected mice [84]. This rise in melatonin might be one of the mechanisms of defense against the viral attack as VEE virus enters the brain first via olfactory pathways [85].

Melatonin enhances immunization against VEE virus

The immunoenhancing properties of melatonin treatment were evident in mice vaccinated with the VEE virus TC-83. Blood IgM titers in mice treated with multiple doses of melatonin (1 mg/kg) were significantly higher than those of the control group, at day 14 after vaccination. When treated with multiple doses of melatonin (5 mg/kg) the antibody titers rose significantly after the first week of immunization [86]. It is known that IL-10 induces activated B cells to secrete large amounts of IgA, IgG, and IgM antibodies [87]. The increase in IL-10 serum levels detected in mice vaccinated with TC-83 and treated with 1 and 5 mg/kg of melatonin, coincided with the rise in antibody titers displayed by these groups of mice at weeks 1 and 2 postimmunization. However, the virus levels in the brains of mice challenged with live VEE virus at day 21 postimmunization were significantly reduced. These results suggest that melatonin treatment enhanced the efficiency of mice immunization against VEE virus [86].

In conclusion, melatonin seems to have an immune effect in the VEE viral infection, probably mediated by IL-1β. This interleukin has been considered one of the earliest host mediators during infectious diseases of the CNS [88]. In these studies, the immunomodulatory properties of melatonin are evident and suggest this indoleamine as a therapeutic alternative to resist VEE and other viral diseases, including SARS as no antimicrobial agent has been proven to be effective for its treatment. The use of melatonin to treat SARS has been suggested by Shiu et al. [89].

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