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Melatonin enhances interleukin-10 expression and suppresses chemotaxis to inhibit inflammation in situ and reduce the severity of experimental autoimmune encephalomyelitis

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ABSTRACT

Melatonin is the major product secreted by the pineal gland at night and displays multifunctional properties, including immunomodulatory functions. In this study, we investigated the therapeutic effect of melatonin in experimental autoimmune encephalomyelitis (EAE). We demonstrated that melatonin exhibits a therapeutic role by ameliorating the clinical severity and restricting the infiltration of inflammatory Th17 cells into the CNS of mice with myelin oligodendrocyte glycoprotein (MOG)-induced EAE. Furthermore, melatonin enhances splenic interleukin (IL)-10 expression in regulatory T cells by inducing IL-27 expression in the splenic DC; it also suppresses the expression of IFN-γ, IL-17, IL-6, and CCL20 in the CNS and inhibits antigen-specific T cell proliferation. However, there were no significant differences in the percentage of splenic regulatory T cells. These data provide the first evidence that the therapeutic administration of melatonin is effective in mice with EAE and modulates adaptive immunity centrally and peripherally. Thus, we suggest that melatonin could play an adjunct therapeutic role in treating human CNS autoimmune diseases such as multiple sclerosis. Melatonin merits further studies in animals and humans.

1. Introduction

Multiple sclerosis (MS) is the most prevalent inflammatory demyelinating disease of the central nervous system (CNS) in adult humans, with an onset between 20 and 40 years of age [1]. Experimental autoimmune encephalomyelitis (EAE) is the most widely used animal model of MS, due to the high similarity between the clinical and histopathological features of EAE and human MS [2].

The immunopathogenic mechanisms of MS are complex and are attributed to T-cell-mediated inflammatory processes that eventually lead to demyelination and axonal injury [3]. T cells and macrophages infiltrate the CNS lesions of animals with EAE [4]. CD4 T cells play a critical role in the initiation of the autoimmune process in adaptive immunity, such as the recruitment of Th1 and Th17 cells, which are involved in the immunopathogenesis of both MS and EAE [5–7]. On the other hand, innate immune cells, such as monocytes and macrophages, engage in the inflammatory course byproducing cytokines, chemokines, nitrogen free radicals and, reactive oxygen species in response to stimulation by CD4 T cells [8–10].

Melatonin is a molecule that is produced by the pineal gland during the night and exhibits multifunctional properties [11,12]. It was initially considered to play a role in the regulation of circadian and seasonal rhythms [13,14]. In particular, melatonin dysregulation plays a role in sleep disturbances and fatigue of patients with MS [15]. Melatonin and its metabolites effectively scavenge antioxidants [16–18]. In addition, melatonin modulates adaptive and innate immunity [19–21]. Melatonin treatment increases the proliferation of T cells in mice [22] and the production of natural killer (NK) cells and monocytes in the bone marrow of mice [23]. It also increases cytokine production in human peripheral blood mononuclear cells [24]. A recent study also reported that melatonin induced the production of IL-4 in T cells, but decreased the

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Table 1
The list of primers (forward and reverse) tested against the related genes.

Primer		
IFN-γ	Forward	5′-AAG CGG CTG ACT GAA CTC-3′
	Reverse	5′-CTG TTA CTA CCT GAC ACA TTC G-3′
IL-4	Forward	5′-GCT AGT TGT CAT CCT GCT CTT C-3′
	Reverse	5′-TGG TGT TCT TCG TTG CTG TG-3′
IL-6	Forward	5′-ATG GAT GCT ACC AAA CTG GAT-3′
	Reverse	5′-TGA AGG ACT CTG GCT TTG TCT-3′
IL-10	Forward	5′-TGC TGC CTG CTC TTA CTG-3′
	Reverse	5′-GCA TTA AGG AGT CGG TTA GC-3′
CCL2	Forward	5′-TTC ACA GTT GCC GGC TGG-3′
	Reverse	5′-TGA ATG AGT AGC AGC AGG TGA GTG-3′
CCL5	Forward	5′-CAG CAG CAA GTG CTC CAA TCT T-3′
	Reverse	5′-TTC TTG AAC CCA CTT CTT CTC TGG-3′
CCL9	Forward	5′-TGC CCT CTC CTT CCT CAT TCT-3′
	Reverse	5′-GCT GTG CCT TCA GAC TGC TCT-3′
CCL20	Forward	5′-CTG CTG GCT CAC CTC TGC A-3′
	Reverse	5′-CAT CGG CCA TCT GTC TTG TG-3′
CXCL10	Forward	5′-GAA ATC ATC CCT GCG AGC CT-3′
	Reverse	5′-TTG ATG GTC TTA GAT TCC GGA TTC-3′
HPRT[a]	Forward	5′-ATC ATT ATG CCG AGG ATT TGG AA-3′
	Reverse	5′-TTG AGC ACA CAG AGG GCC A-3′

[a] Hypoxanthine-guanine phosphoribosyltransferase (HPRT).

levels of IL-2 and interferon (IFN)-γ in treated BALB/c mice [18]. These results suggest that melatonin plays a role in enhancing of Th2-mediated immunity.

The preventive effect of melatonin in EAE was previously studied by Kang and Shin et al. [25]. They revealed that a 5 mg/kg melatonin treatment for 15 days inhibited the onset of the disease and reduced the severity of the clinical signs. This protective effect resulted from the suppression of intracellular adhesion molecule 1 expression in the spinal cord [25]. However, the therapeutic role of melatonin in EAE and its immunomodulatory mechanisms have not yet been elucidated. To our knowledge, the role of IL-10 in melatonin treatment of MS is uncertain. In this study, we investigated the effectiveness of a high-dose melatonin treatment after the onset of EAE. We also analyzed the immunomodulatory effects of melatonin on EAE progression.

2. Materials and methods

2.1. Animals

C57BL/6 mice (6–8 weeks old) were purchased from the National Laboratory Animal Center, Taiwan. They were maintained at the animal center of the National Defense Medical Center in Taipei, Taiwan under specific pathogen-free conditions. All animal protocols were approved by the Institutional Animal Care and Use Committee of Taiwan.

2.2. Melatonin treatment

The experimental mice were treated with melatonin on days 9, 11, and 13 after EAE induction. Each mouse was subcutaneously injected with melatonin at 200 mg/kg of body weight; the melatonin was dissolved in a solvent solution consisting of less than 25% ethanol in

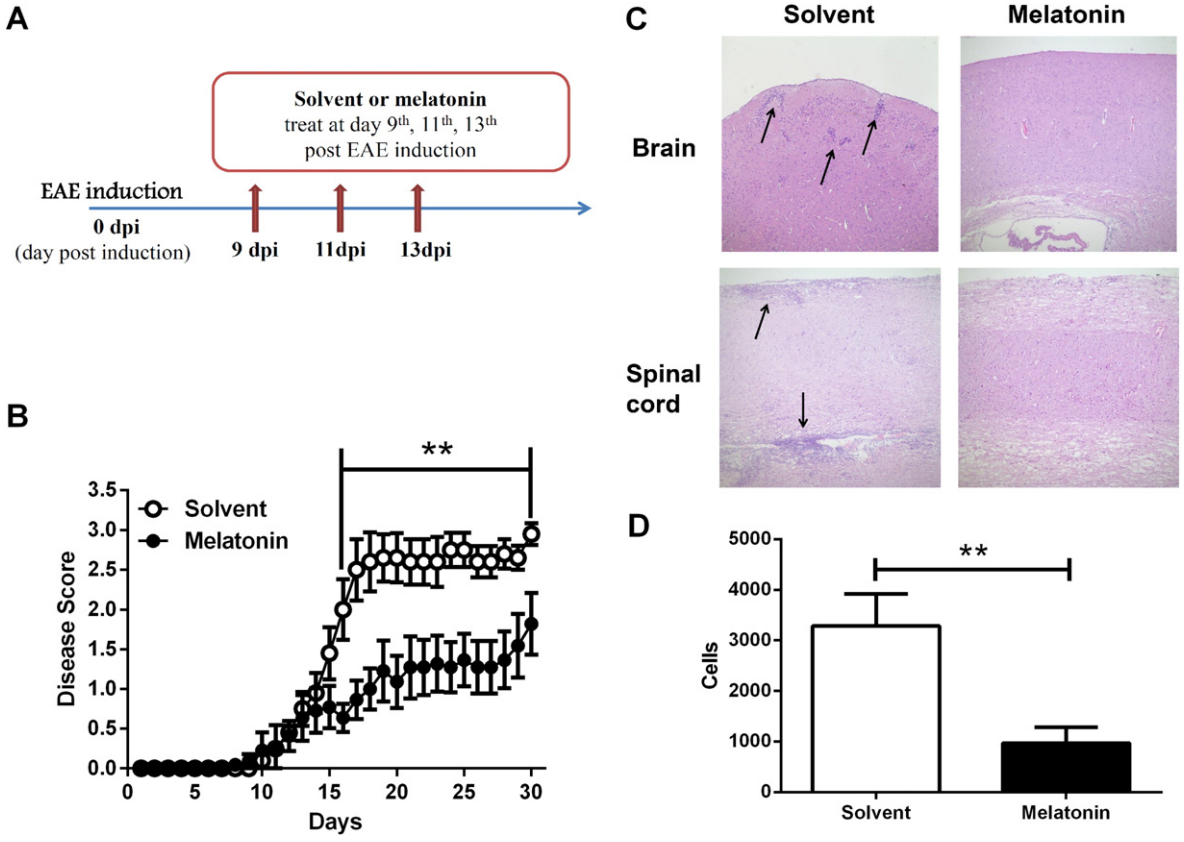

Fig. 1. Therapeutic effects of melatonin in mice with experimental autoimmune encephalitis (EAE). (**A**) Melatonin treatment procedure in EAE. The experimental mice were treated with melatonin on days 9, 11, and 13 after EAE induction. Each mouse was subcutaneously injected with 200 mg/kg/bw melatonin dissolved in a solvent solution (<25% ethanol in phosphate-buffered saline). The control mice were subcutaneously injected with the solvent solution. (B) The clinical signs in the solvent-treated (n = 10) and melatonin-treated mice (n = 11) were monitored daily. Melatonin treatment significantly reduced the severity of EAE compared with the solvent-treated control mice (**$P < 0.01$). The data are expressed as the means ± SEMs. (**C**) Leukocytes infiltration was evaluated at day 14 by hematoxylin and eosin staining. The histopathological analysis showed that fewer leukocytes had infiltrated into the brain and spinal cord of melatonin-treated mice compared with the solvent-treated control mice. The arrows indicate infiltrated leukocytes. (D) The number of leukocytes that had infiltrated the brain and spinal cord were counted on day 14. The number of infiltrated cells in the melatonin-treated mice was significantly reduced compared with the solvent-treated controls (**$P < 0.01$). The data are expressed as the means ± SEMs.