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Melatonin suppresses markers of inflammation and oxidative damage in a human daytime endotoxemia model☆,☆☆

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ABSTRACT

Purpose: Melatonin used as an exogenous drug has been documented to have potent antioxidant and anti-inflammatory effects in animal model. We aimed to examine the effect of melatonin in an experimental human sepsis model.
Materials and Methods: Twelve healthy males were enrolled in a randomized, placebo-controlled, double-blinded cross-over trial. They received lipopolysaccharide endotoxin 0.3 ng/kg of body weight intravenously at 12:00. Before endotoxemia, an 8-hour infusion of melatonin (100 mg) or placebo (saline) was initiated. Blood samples were drawn before and at 2, 4, 6, and 8 hours after lipopolysaccharide administration. Proinflammatory (tumor necrosis factor α [TNF-α], interleukin [IL] 1β, IL-6, and YKL-40), anti-inflammatory markers (IL-1Ra, IL-10, soluble tumor necrosis factor receptor I, and soluble tumor necrosis factor receptor II), a marker for oxidative damage (malondialdehyde), and antioxidants (ascorbic acid and dehydroascorbic acid) were analyzed in plasma.
Results: Melatonin significantly reduced proinflammatory markers IL-1β ($P < .01$) and YKL-40 ($P < .05$) but not TNF-α and IL-6. None of the anti-inflammatory markers (IL-1Ra, IL-10, soluble tumor necrosis factor receptor I, and soluble tumor necrosis factor receptor II) were lowered by melatonin. Melatonin reduced the levels of ascorbic acid ($P < .05$) but not dehydroascorbic acid or malondialdehyde.
Conclusions: Melatonin administration before endotoxemia resulted in reduction of certain markers of inflammation and oxidative stress. Further studies are needed to clarify the role of melatonin in clinical setting.

1. Introduction

The proportion of patients with severe sepsis and septic shock has increased in the last 2 decades [1-3]. One third of patients with sepsis and half of all patients with severe sepsis require intensive care unit admission [4]. One in 8 patients with sepsis dies, and almost 1 in every 2 patients with septic shock dies [5]. The annual economic cost has been estimated to be 16.7 billion dollars in the United States [5]. Several drugs targeting blood pressure, coagulation system, inflammatory cytokines, and renal function have been investigated [5].

Melatonin is an endogenous molecule secreted by the pineal gland and has a modulatory effect on circadian rhythm [6,7]. Melatonin exhibits potent antioxidant and anti-inflammatory effects in endotoxemia [8-20]. Most of the scientific work has been done in animal models, and the effect of melatonin on sepsis in human models requires further evaluation [21,22]. Based on the low toxicity of melatonin and effects demonstrated in experimental and preliminary studies in human newborns, the effects of melatonin were investigated in a human sepsis model. Human endotoxemia as a model of systemic inflammation has been used widely in the scientific work of sepsis [23], and it imitates the acute phase response in sepsis. It is a reproducible systemic inflammatory response with a defined onset, and it is fully reversible. The acute phase response is induced by *Escherichia coli* endotoxin lipopolysaccharide (LPS) administered intravenously to healthy volunteers. Here, we examined the effect of daytime melatonin administration on endotoxin-induced acute phase response in young healthy male volunteers.

2. Methods

2.1. Ethics

Written informed consent was obtained from all participants, and the trial was conducted according to the Declaration of Helsinki. The

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trial was registered at www.clinicaltrials.gov (NCT01087359). This study was approved by the Danish Medicines Agency (EudraCT-nr 2009-017360-1), The Regional Committee on Biomedical Research Ethics (H-2-2010-010), and the Danish Data Protection Agency. Moreover, the study was monitored by the Good Clinical Practice unit at Copenhagen University Hospital.

2.2. Subjects

After written informed consent, healthy males aged 18 to 40 years were included. Exclusion criteria were smoking, alcohol abuse, medication, allergy for melatonin, known sleep difficulties, and infections in the past 14 days before the first measurement day. The subjects underwent a general clinical examination with electrocardiogram recording.

2.3. Materials

Melatonin (M5250, purity by thin layer chromatography 99%) from Sigma-Aldrich, St Louis, MO, was tested for sterility according to the European Pharmacopoeia requirements. Melatonin powder (100 mg) was dissolved in 2-mL ethanol (99%) and mixed with 1-L physiological saline. Placebo consisted of a mixture of 2-mL ethanol (99%) and 1-L physiological saline. *E coli* LPS endotoxin (Lot G3E069; US Pharmacopeia Convention, Rockville, MD) was dissolved in sterilized water in a concentration of 10 ng/mL.

2.4. Study design

This study was conducted as a randomized, double-blinded, placebo-controlled, cross-over trial. Both the subjects and the investigator were blinded with respect to whether the drug was placebo or melatonin. The trial consisted of 2 intervention days with a wash-out period of at least 3 weeks in between to eliminate endotoxin tolerance. One week before the intervention day, the subjects were instructed to adapt to a standardized sleep schedule (8 hours of sleep between 23:00 and 08:00), no caffeine intake, no alcohol intake, and wearing sleep mask during sleep. Time of onset of endotoxemia was standardized for all subjects and in both intervention days because the acute phase response exhibits a circadian variation throughout the day (unpublished data). At each intervention day, the volunteers had intravenous catheters inserted in cubital veins bilaterally and were monitored with hourly measurement of blood pressure, temperature, and heart rate. Baseline blood samples were obtained, and melatonin infusion was started at 11:00 and continued for 8 hours. An intravenous bolus injection of *E coli* LPS of 0.3 ng/kg was administered at 12:00. Additional blood samples were collected 2, 4, 6, and 8 hours after injection of LPS.

2.5. Blood samples and analysis

The blood samples were drawn for analysis of oxidative stress markers malondialdehyde (MDA), ascorbic acid (AA), and dehydroascorbic acid (DHA) and the inflammatory markers tumor necrosis factor α (TNF-α), interleukin (IL) 1β, IL-6, IL-10, IL-1Ra, soluble tumor necrosis factor receptor I (sTNF-RI), and soluble tumor necrosis factor receptor II (sTNF-RII), and YKL-40. Blood samples for the determination of the inflammatory markers were drawn into tubes containing EDTA and trasylol and centrifuged at 3100 rpm for 3 minutes. The plasma was then stored at −80°C until analyses. The concentration of YKL-40 in the plasma was determined by a commercial an enzyme-linked immunosorbent assay (Quidel, Santa Clara, CA). Blood samples and plasma analyses for the determination of the oxidative markers, MDA, AA, and DHA, have been described previously [24,25], using high-pressure liquid chromatography.

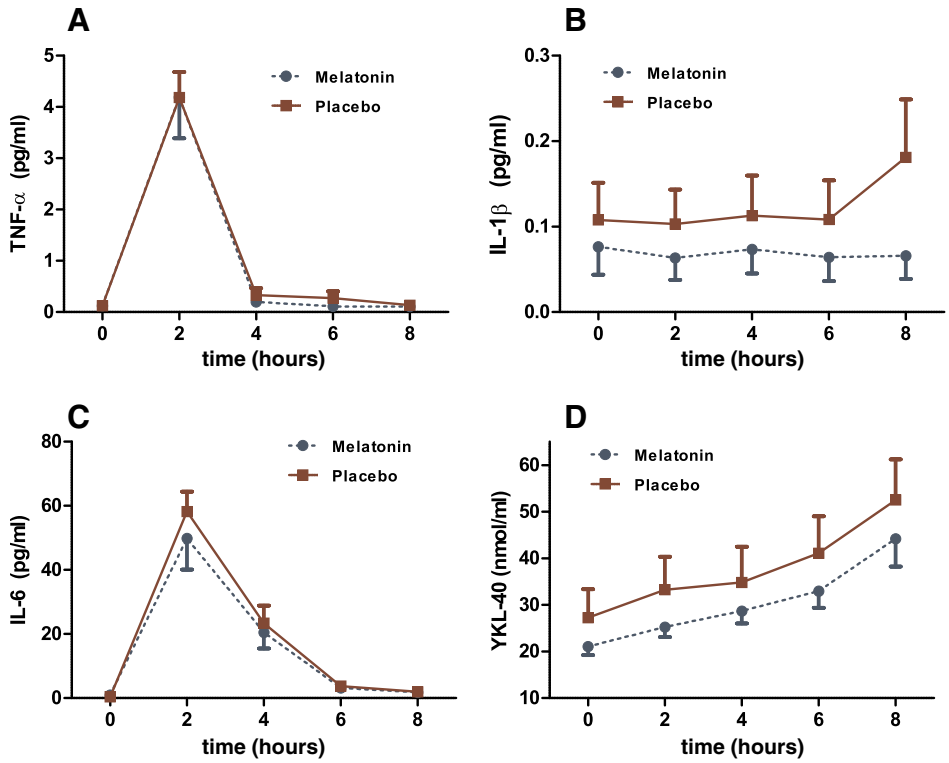

Fig. 1. Plasma levels of 3 proinflammatory markers and YKL-40. The time point 0 indicates the administration of *E coli* endotoxin. The endotoxemia was induced at 12:00, and melatonin (blue curve) or placebo (red curve) was initiated before the onset of the endotoxemia. Results from the 2-way ANOVA: (1) interaction term (time * intervention) was not significant for any of the markers; (2) between-groups difference was significant for IL-1B ($P < .01$) and YKL-40 ($P < .05$).