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Melatonin attenuates amyloid beta$_{25–35}$-induced apoptosis in mouse microglial BV2 cells

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Abstract

Melatonin has been reported to possess strong antioxidant actions, and is able to directly scavenge a variety of reactive oxygen species (ROS). The present study investigated whether melatonin possesses protective effects against Aβ-induced cytotoxicity in microglial cells. Cells treated with Aβ exhibited several characteristic features of apoptosis, while cells pre-treated with melatonin prior to exposure to Aβ showed a decrease in the occurrence of such apoptotic features. Several previous studies have demonstrated the involvement of ROS in Aβ-induced neurotoxicity, and ROS generated by Aβ have been reported to lead to the activation of nuclear factor-kappa B (NF-κB), a transcription factor; pre-treatment with melatonin in the present study reduced the level of Aβ-induced intracellular ROS generation, inhibited NF-κB activation, and suppressed the Aβ-induced increase in caspase-3 enzyme activity. In addition, it was found that pre-treatment with melatonin inhibits Aβ-induced increase in the levels of bax mRNA and that it enhances the level of bcl-2 expression. Based on these findings, the authors speculate that melatonin may provide an effective means of treatment for Alzheimer's disease through attenuation of Aβ-induced apoptosis.

Keywords: Melatonin; Amyloid β; Apoptosis; Reactive oxygen species; Nuclear factor-kappa B; Caspase-3

Alzheimer's disease is a neurodegenerative disorder of the central nervous system (CNS) characterized clinically by progressive loss of memory and cognitive functions [19]. Molecular hallmarks of Alzheimer's disease include extracellular deposition of the amyloid β (Aβ) peptide in the form of senile plaques. Aβ is known to play a critical role in the pathogenesis of Alzheimer's disease [12]. It has been reported that Aβ accelerates neurodegeneration by activating microglia and that the activated microglia exert cytotoxic effects on neurons by releasing inflammatory mediators [8,19]. Several studies have demonstrated the involvement in Aβ-induced neurotoxicity of reactive oxygen species (ROS) [4,12,22,23], which have been shown to induce neuronal apoptosis [11,14]. Apoptosis, also known as programmed cell death, is a biological process that plays crucial roles in normal development and tissue homeostasis [20] and in a variety of human disorders [21].

Melatonin (*N*-acetyl-5-methoxytryptamine) is an endogenous neurohormone produced by the pineal gland in mammals [2]. Melatonin has been reported to possess strong antioxidant actions and to be able to directly scavenge a variety of ROS [1,16]. Recently, it has been suggested that melatonin offers protection against Aβ-induced apoptosis [13,17]. In the present study, the mechanism of protective effect of melatonin on BV2 microglial cells against Aβ$_{25–35}$-induced apoptosis was investigated.

The Aβ$_{25–35}$ used in this study was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cells of the BV2 cell line, derived from mouse microglia, were cultured

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in Dulbecco's Modified Eagle Medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) at 37 °C in 5% CO_2–95% air in a humidified cell incubator. Cell viability was determined using the MTT assay kit according to the manufacturer's instruction (Boehringer Mannheim GmBH, Mannheim, Germany). The percent viability was calculated as (OD of drug-treated sample/control OD) × 100. For flow cytometric analysis, BV2 cells were incubated with 100 μg/ml RNase and 20 μg/ml propidium iodide in PBS for 30 min at 37 °C and were analyzed using FACScan (Becton Dickinson, San Jose, CA, USA). 2′,7′-Dichlorofluorescein-diacetate (DCFH-DA), a dye which allows flow cytometric measurement of intracellular ROS production, was used. The cells were then observed under a fluorescence microscope (Zeiss GmBH, Oberköchen, Germany). Electrophoretic mobility shift assay (EMSA) was carried out according to a previously described procedure [7]. Total RNA was isolated from BV2 cells using easy-BLUE™ total RNA extraction kit according to the manufacturer's instruction (iNtRON Inc., Seoul, Korea). Expression of mRNAs was measured by reverse-transcriptional-polymerase chain reaction (PCR). The primer sequences for *bax* and *bcl-2* as used in the study were as reported by Jang et al. [6]. The expected sizes of the PCR products were 270 bp for *bax*, 333 bp for *bcl-2*, and 291 bp for *cyclophilin*. Caspase enzyme activity was measured using ApoAlert® caspase-3 assay kit according to the manufacturer's instruction (CLONTECH, Palo Alto, CA, USA). Data were expressed as mean ± standard error mean (S.E.M.). The data were analyzed by one-way ANOVA followed by Duncan's post hoc test using SPSS. Differences with *P*-values less than 0.05 were considered to be statistically significant.

As shown in Fig. 1, the viabilities of cells incubated with $A\beta_{25–35}$ at concentrations of 0.1 μg/ml, 0.5 μg/ml, 1 μg/ml, 5 μg/ml, and 10 μg/ml for 24 h were 91.41 ± 2.14%, 82.15 ± 3.34%, 74.19 ± 4.58%, 58.47 ± 2.59%, and 49.52 ± 4.41% of the control value, respectively. A trend of decreasing viability with increasing $A\beta_{25–35}$ concentration was observed. The viabilities of cells treated with melatonin only at concentrations of 0.5 μg/ml, 1 μg/ml, 5 μg/ml, and 10 μg/ml for 24 h were 102.54 ± 5.68%, 99.82 ± 7.14%, 101.24 ± 4.52%, and 98.17 ± 3.89% of the control value, respectively, indicating that melatonin in itself does not possess overtly toxic effects on BV2 cells. The viability of cells treated with 10 μg/ml $A\beta_{25–35}$ for 24 h was 49.52 ± 4.41%. This figure was increased to 48.58 ± 3.54%, 53.47 ± 4.17%, 67.48 ± 5.21%, and 71.41 ± 4.93% in cells treated prior to exposure to $A\beta_{25–35}$ with melatonin for 1 h at concentrations of 0.5 μg/ml, 1 μg/ml, 5 μg/ml, and 10 μg/ml, respectively. These results show that at a concentration of 10 μg/ml $A\beta_{25–35}$ has cytotoxic effects and that melatonin protects BV2 cells from this $A\beta_{25–35}$-induced cytotoxicity (Fig. 1).

Flow cytometric analysis of the protective effect of melatonin on cells against $A\beta_{25–35}$-induced cell death was also carried out. Following treatment with 10 μg/ml $A\beta_{25–35}$ for

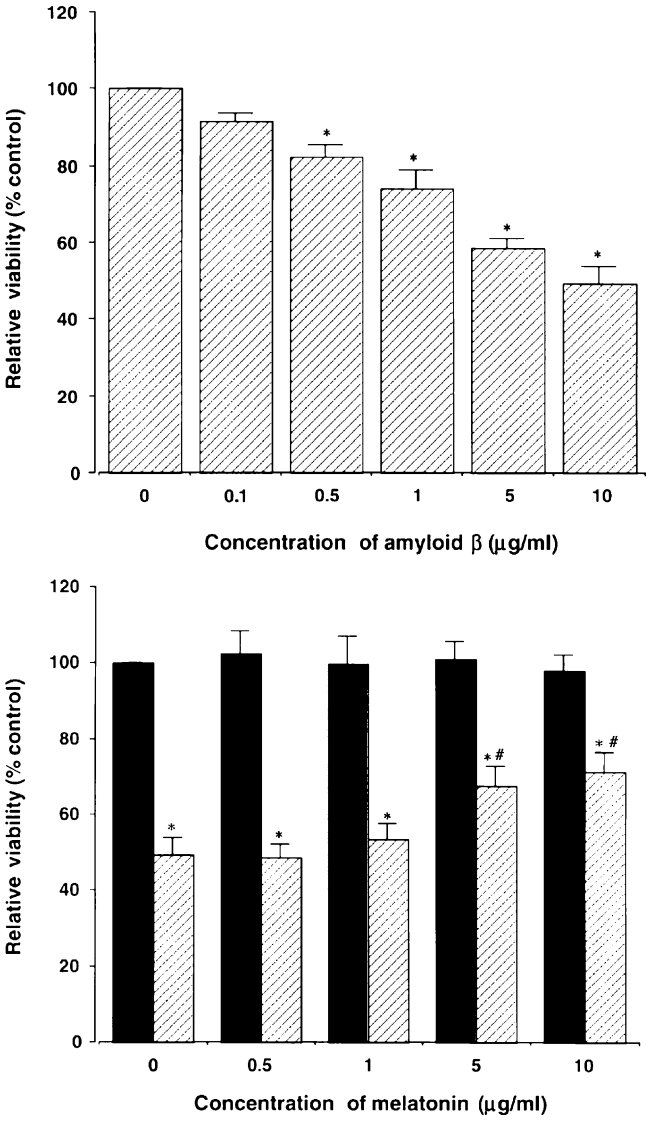

Fig. 1. $A\beta_{25–35}$-induced cytotoxicity and protective effect of melatonin. Top: Graph demonstrating the cytotoxic effect of $A\beta_{25–35}$. Bottom: Graph demonstrating the protective effect of melatonin. (■) Melatonin treatment without $A\beta_{25–35}$ treatment and (▨) melatonin treatment with 10 μg/ml $A\beta_{25–35}$ treatment. Data are presented as mean ± standard error mean (S.E.M.). $^{*}P < 0.05$ compared to the control. $^{\#}P < 0.05$ compared to cells treated with $A\beta_{25–35}$ only.

24 h, the fraction of cells in the sub-G1 phase was increased from 5.92% (control) to 63.39%. On the other hand, when treated with 10 μg/ml melatonin for 1 h prior to exposure to 10 μg/ml $A\beta_{25–35}$ for 24 h, the fraction of cells in the sub-G1 phase was decreased to 45.18% (Fig. 2).

To verify whether $A\beta_{25–35}$ induces cell death through the generation of ROS, the effect of the antioxidant *N*-acetylcysteine (NAC) on $A\beta_{25–35}$-induced cell death was assessed. As shown in Fig. 3 (top), the viability of cells exposed to 10 μg/ml $A\beta_{25–35}$ for 24 h was 50.84 ± 3.97% of the control value, while the viability of cells pre-treated with NAC for 1 h at concentrations of 10 μg/ml and 50 μg/ml before exposure to 10 μg/ml $A\beta_{25–35}$ was increased in a statistically