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Investigation of Apoptotic Effects of Melatonin in H_2O_2 Induced Glioma Cell Lines

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Aging, cancer and neurodegenerative diseases are relationship between of free oxygen radicals, which is still one of the research topics. In terms of the etiology of neurodegenerative diseases, genetic and environmental factors as well as the biochemical processes in the body related to aging and the mechanisms put forward to be effective, but could not reach a definitive conclusion. Most emphasis on reasons are oxidative stress and cellular apoptosis. Also, due to the increase of oxygen free radicals are also known as increased apoptosis. Especially, brain tissue is susceptible to oxidative stress [1].

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous neurohormone derived from tryptophan, that is, mainly released from the pineal gland which is participates in a number of physiological processes like the reproduction regulation and circadian rhythms, at the same time is a well-known potent antioxidant and well tolerant upon its administration [2,3].Moreover melatonin has both neurogenic and neuroprotective effects in mammalian cell lines such as neuroblastoma cells. The mechanisms of action include receptor-coupled processes, direct binding and modulation of calmodulin and protein kinase C, and direct scavenging of free radicals. [4].

We designed this study to investigate the effects of melatonin treatment on oxidative stress induced by H_2O_2 C6 Glioma Cell line using immunocytochemistry methods related to apoptosis and oxidative stress.

This study was performed on C6 glioma cell line at Istanbul Science University's Multidisciplinary Research Laboratory. The cell line was cultured in a medium which contained Dulbecco's Modification of Eagle's Medium (DMEM) and Ham's F12 Medium (3:1). DMEM/Ham's F12 medium was supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin + L-glutamine (100 U/mL / 100 µg/mL). Cells were incubated in a humidified 5% CO2 atmosphere at 37 °C. After cells were incubated with H_2O_2 for 1 hour, they were treatment with melatonin for 72 hours. Melatonin (Sigma) was taken, and dissolved in ethanol (%95) to form 1 ml of stock solution (10^{-1}M). Thereafter, final concentration ($5x10^{-4}$ M) were obtained by dilution of stock solution with growth medium. At the end of incubation, For immunocytochemical staining, the cells were fixed with methanol. Then cells were incubated for 1 h at room temperature with primary antibodies: a monoclonal Apaf-1, Caspase-3 and CAMK. After the serial washing and secondary antibody labelling procedure, cells were incubated DAB (Dako) for 5min to stain immunolabelling. Detection of the apoptotic cell death in situ using as TUNEL method was used for programme cell death mechanism. It can be detected in cells using a terminal deoxynucleotidyltransferase-biotin nick end-labeling method (TUNEL) performed with a commercial kit (ApopTag® Peroxidase In Situ Apoptosis Detection Kit, Millipore) according to the manufacturer's instructions.

According to our results, there was a significant increase in TUNEL positive cells in H_2O_2-induced group compared with the control and melatonin treated groups (Table-1). Also Apaf-1, caspase-3 and CAMK positive cells in H2O2-induced group compared with the control and melatonin treated groups as expected (Fig-1-2). In Melatonin/H_2O_2 group, we found apoptotic markers decreased compared with H_2O_2-induced group (Fig-3-4). With our findings, melatonin has been shown to prevent apoptosis.

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4. We kindly acknowledge the help of the organizers of MC 2013 with the online submission procedure.

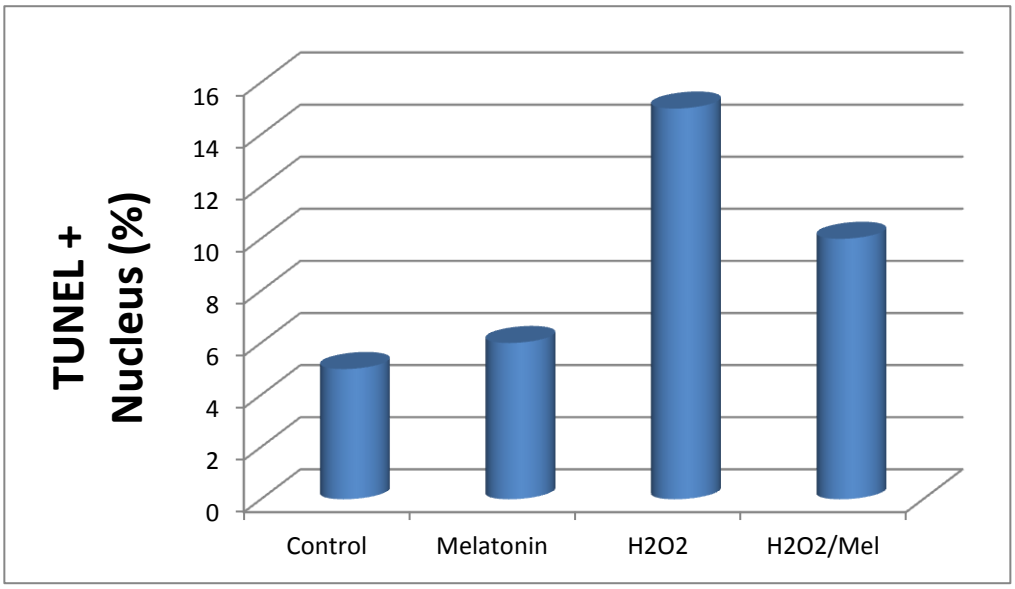

Figure 1. results of the TUNEL + nucleus numbers on all groups. H_2O_2-induced group was shown to be a significant increase in apoptosis compared to control and H_2O_2/Mel group.

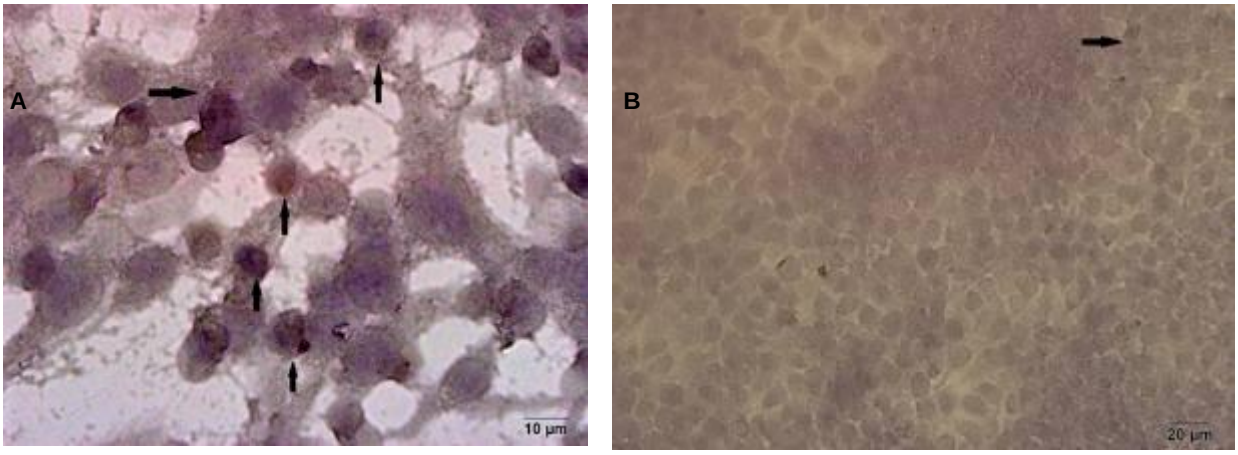

Figure 2. A-H_2O_2 (x100), and B-H_2O_2/Melatonin (x40). TUNEL + cells indicated by an arrow. 500 mM H2O2 H2O2 treated group than the control group in the number of TUNEL + cells led to a significant increase.