B-NU-4

Melatonin Protects Neuronal Cells Against Cell Death Induced by Oxygen-Glucose Deprivation *in vitro*

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Background: Melatonin is a neurohormone secreted from the pineal gland. Our previous results have showed that treatment with melatonin protects against focal cerebral ischemia in rats *in vivo*. The present *in vitro* study was designed to study whether melatonin also protects against cell death due to oxygen-glucose deprivation (OGD) using cultured SHSY5Y neuronal cells and whether melatonin membrane receptors are involved.

Methods: SHSY5Y neuronal cells were seeded in 96-well microtiter plates and subjected to 1 hour of OGD four days later. OGD was achieved via placing the cells in glucose-free culture medium and exposing them to an atmosphere with less than 0.8 % oxygen. Different doses of melatonin were added at 5 minutes before OGD and maintained for 23 hours. Cell death was quantitatively assessed using the measurement of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in the culture medium at 24 hours after OGD, and data of all OGD groups were expressed as percentages of that from the sham OGD group. Results from melatonin-treated groups were compared with that of vehicle-treated control group using 2-tailed student's *t* test. Presence of melatonin membrane receptors (MT_1 and MT_2) in the neuronal cells was detected using reverse transcription-polymerase chain reaction (RT-PCR).

Results: The relative MTT values were, in mean ± SEM, 56.7± 2.3% (32 wells) in the group treated with vehicle and 78.4± 3.2% (24 wells; $p<0.05$), 82.3± 2.8% (32 wells; $p<0.05$), 73.7± 2.2% (32 wells; $p<0.05$), 63.9± 2.3% (32 wells; $p>0.05$), 62.7± 3.2% (24 wells; $p>0.05$), and 59.4± 4.1% (16 wells; $p>0.05$) in the groups treated with melatonin at 10^{-3}M, 10^{-4}M, 10^{-5}M, 10^{-6}M, 10^{-7}M, and 10^{-8}M, respectively. In other words, cell death was significantly reduced following treatment with melatonin at 10^{-3}M, 10^{-4}M, or 10^{-5}M. The results of RT-PCR showed that neither MT_1 nor MT_2 was expressed in this cell line.

Conclusion: Melatonin protects neuronal cells against OGD-induced *in vitro* cell death in a dose-dependent manner. The neuroprotective action of melatonin is independent of its known membrane receptors.

B-RC-1

Effects of Specific Cytokines on Antioxidant Expressions in A549 Human Lung Adenocarcinoma Cell Line

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Introduction: Preliminary study on antioxidant expressions in human non-small cell lung carcinoma tissues showed up-regulation of manganese superoxide dismutase (Mn SOD) and down-regulation of catalase in tumours compared to tumour-free lung tissues. However the exact cause of such alterations of antioxidant expressions is unknown.

Hypothesis: Specific cytokines (tumour necrosis factor alpha [TNF], interleukin-1 beta [IL-1], interferon gamma 1b [IFN]), which are elevated in lung cancer patients, may be responsible for increased Mn SOD and decreased catalase expressions in A549 human lung adenocarcinoma cell line.

Methods: A549 cells were either unstimulated or stimulated at 70% confluence in 100-mm diameter culture dishes with TNF, IL-1, and IFN at 10ng/ml, 0.5ng/ml, and 10^4U/ml respectively. Cells were harvested at baseline, 8, 24, 48, and 72 hours after stimulation for RNA and protein extractions. Activity of major antioxidants (catalase, and superoxide dismutase [SOD]) in protein extracts was measured by chemical kinetic reactions spectrophotometrically. Western blot and Northern blot analyses were performed for specific antioxidant protein and mRNA expressions. All cell culture experiments were performed in triplicate. Comparisons were made between cytokine-stimulated and unstimulated groups.

Results: Higher SOD activity in cells stimulated with specific cytokines compared with unstimulated cells was detected starting at 8 hours post-stimulation ($p=0.01$). The catalase activity failed to increase in the cytokine-stimulated cells compared with unstimulated cells starting at 24 hours post-stimulation ($p=0.04$). Northern blot analysis showed a marked increase in Mn SOD ($p=0.004$) and a decrease in catalase ($p=0.007$) mRNA expression in stimulated cells compared with unstimulated cells starting at 8 hours after stimulation. Western blot analysis showed a parallel significant increase in Mn SOD and decrease in catalase protein expression in stimulated compared with unstimulated cells.

Conclusion: Specific cytokines can be responsible for the up-regulation of Mn SOD and down-regulation of catalase at transcriptional level in lung carcinoma cells.