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Role of melatonin in HT22 cells challenged with serum deprivation

Gianna Di Sario - Erica Cesarini - Barbara Canonico - Sara Gabrielli - Loris Zamai - Stefano Papa - Francesca Luchetti

University of Urbino Carlo Bo, Department of Biomolecular Sciences, Urbino, Italia

In vitro serum deprivation (SD) is one model for investigating the molecular mechanisms underlying apoptosis as well as autophagy, which generally function as defense strategies upon cell injury by eliminating damaged organelles [1]. Furthermore, SD injury in vitro is widely used to mimic the ischemic environment [2]. In serum deprived conditions, cells show different parameters of apoptosis and autophagy. Melatonin (MLT), a lipophilic indole secreted by pineal and non-pineal cells, is a well-known potent free radical scavenger acting as neuroprotective molecule that prevents apoptotic cell death in several models of neurodegenerative diseases. In the present study we investigated the neuroprotective effects of MLT during SD condition on mouse hippocampal HT22 cells, considering that intracellular ROS are usually linked to autophagy and apoptosis. To explore potential effects of combining SD with melatonin we studied clonogenic survival of HT22 cells. Clonogenic assay demonstrated a significative ($p< 0.01$) reduction of HT22 total cell numbers challenged for 24h with SD, whereas the pre-treatment with 200nM of MLT for 24hr noticeably reduced this effect of about 30%. In HT22 starved cells the percentage of MitoTracker Red (MTR) positive cells doubled ($P< 0.05$) if compared to the control condition, suggesting that SD induced a remodelling of mitochondrial network. It is noteworthy that MLT pre-treatment produced a MTR positivity similar to that of controls. We next investigated whether melatonin was able to influence the autophagic pathway. Autophagy was detected by measuring the aggregation of LC3B protein coupled to green fluorescence protein (GFP). Confocal images show that SD induced an increase in the GFP-LC3 puncta, whereas the melatonin treatment reduces these aggregations. Taken together, our results suggest that MLT treatment may play protective roles against cellular modifications induced by SD treatment in HT22 cells.

References

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Keywords

Melatonin; HT22; serum deprivation.