Neuroprotective effect of melatonin in neonatal hypoxia-ischemia: role of Sirt1

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Silent information regulator 1 (Sirt1) is a nicotinamide adenine dinucleotide (NAD^+)- dependent deacetylase, which has been shown to play an important role in the regulation of several biological processes, including metabolism, cell differentiation, apoptosis, oxidative stress and senescence. Sirt1 regulates these functions by targeting histone and non-histone proteins in the nucleus to modulate gene expression or downstream of transcription, by targeting specific proteins and other transcription factors in the mitochondria and cytosol. Accumulating evidence suggests that Sirt1 is also involved in several brain functions, modulating neuronal differentiationand participating in neuronal protection against ischemia/hypoxia.

Melatonin is a neurohormone secreted by pineal gland and it has diverse pharmacological activities, such as antioxidant, anti-inflammatory and anti-apoptotic properties, due to its pleiotropic effects, including the ability to scavenge free radicals. Studies have also revealed that melatonin attenuates ischemia injury under different experimental conditions in various organs, including brain. The aim of the present study was to investigate the role of Sirt1 in neuroprotection effects of melatonin in neonatal rats subjected to hypoxia-ischemia (HI).

Recently, we have observed that Sirt1-expression was significantly reduced 1h after HI, in both cytosolic and nuclear cortical fractions. Melatonin, administered immediately after the ischemic insult, increased Sirt1 expression in cytosol and nucleus. The increased Sirt1 expression in the lesioned hemisphere of melatonin-treated ischemic animals was confirmed by immunohistochemical studies. In addition, we also investigated the modulation of miR-34a, a well known miRNA that directly targets Sirt1. We found that the expression of miR-34a was significantly reduced after melatonin administration compared to ischemic group. To further assess the effects of melatonin on Sirt1 pathway, we studied the expression of transcription factor FoxO3a, a well known Sirt1-target, and the acetylation levels of p53, a tumor suppressor that is inhibited by Sirt1 during stress conditions. We found that neonatal HI decreased the cytosolic expression of FoxO3a, increasing the nuclear localization of the protein. After melatonin administration, the expression of FoxO3a significantly increased both in the cytosol and in the nucleus. The western blot analysis of p53 revealed that the protein expression increased during HI in nuclear cortical fraction, as well as its acetylation. Melatonin completely inhibited these HI-induced effects and these results were confirmed by the ability of melatonin to also reduced apoptosis, which is strictly connect to p53 activity. Further, we analyzed the cytochrome C in the mitochondrial fraction both in the ischemic cortex and in melatonin-treated ischemic cortex. We found that cytochrome C expression was significantly decreased in the mitochondrial fraction and increased in the cytosol fraction of the ischemic cortex. Melatonin reduced the expression of the protein in the cytosol and increased it in the mitochondria, indicating a reduced outer mitochondrial membrane permeabilization in the melatonin-treated ischemic animals.

The results clearly indicated that melatonin represents an important neuroprotective strategies for neonatal HI. Melatonin protection is strictly correlated to the regulation of Sirt1 signaling.