MELATONIN VIA SIRT1ACTIVATION ATTENUATES MICROGLIAL NEUROTOXIC EFFECTS DURING HYPOXIA

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Introduction: Melatonin is a pineal gland-produced hormone endowed with neuroprotective properties and known to activate protective deacetylase SIRT1. Melatonin exerts neuroprotection against hypoxia-generated damage, but its selective role on microglial cells in this condition is not yet fully characterized. Microglia is activated by hypoxia and triggers neuroinflammatory responses, eventually taking part in exacerbation of neuronal damage. We here aimed to evaluate melatonin's effects on microglia, with a focus on the role played by SIRT1and on the indirect fallouts on neuronal susceptibility to hypoxic injury.

Materials and methods: Cobalt chloride ($CoCl_2$, 250 μM) was used to obtain chemical hypoxia in SH-SY5Y neuronal-like cells and in primary rat microglia or murine BV2cell line. Melatonin was used at 1μM concentration and selective SIRT1inhibitor EX527(5μM) was always added 15minutes prior to other treatments. Co-culture experiments were carried out by plating primary microglia on top of transwell inserts placed in multiwell plates containing SH-SY5Y cells. Conditioned medium (CM) was obtained by pulsing (3h) BV2cells with $CoCl_2$, alone or in combination with other drugs, followed by recovery (18h) in fresh medium devoid of any drug. Cell viability was assessed by the MTT assay. Protein expression was analyzed by Western blot, immunocytochemical or cytofluorometric analyses.

Results: $CoCl_2$significantly reduced cell viability of microglia (both primary and BV2cell line) and neuronal-like SH-SY5Y cells in a time and concentration dependent manner. At 250 μM, $CoCl_2$impaired SIRT1nuclear translocation in BV2microglia. In addition, it induced the expression of hypoxia inducible factor-1alpha subunit and pro-inflammatory markers NF-kB and pFAK. Melatonin was able to prevent NF-kB and pFAK upregulation and to restore SIRT1localization in the nucleus, all effects sensitive to SIRT1inhibitor EX527. Melatonin also significantly attenuated toxicity by $CoCl_2$on microglial cells. $CoCl_2$toxicity was slightly but significantly increased in neuronal cells when co-cultured with primary microglia, or treated in CM from BV2cells exposed to a pulse/recovery with $CoCl_2$. This effect was prevented by CM from microglia pulsed with $CoCl_2$in combination with melatonin alone, but not with the additional presence of EX527.

Conclusions: Altogether, these data add information on the mechanisms involved in the neuroprotective effects of melatonin during hypoxia, identifying microglia as a significant target and evidencing the role played by SIRT1as a mediator. Given its positive safety profile and high permeability through the blood-brain barrier, melatonin is confirmed to be a valuable drug against hypoxia-induced CNS damage with microglia as an additional target.