UNIVERSITI TEKNOLOGI MARA

NEUROPROTECTIVE EFFECT AND MOLECULAR MECHANISMS OF ACTION OF MAGNESIUM ACETYLTAURATE AGAINST NMDA-INDUCED RETINAL INJURY IN RATS: FOCUS ON CALCIUM REGULATED PROTEINS

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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ABSTRACT

The excitotoxicity refers to excessive calcium influx intracellularly due to stimulation of glutamate receptors particularly the NMDA subtypes. This triggers apoptosis via calpain, calcineurin, CaMKII activation and cabin 1 inhibition. Altered expression of these Ca^{2+} regulated proteins cause increased retinal oxidative stress and nitric oxide synthase (NOS) expression. Altered NOS expression leads to production of nitrogen free radicals that further perpetuate the oxidative stress, activate the caspases and this culminates to apoptosis. Retinal ganglion cell (RGC) apoptosis is the hallmark feature of glaucoma and excitotoxic injury is the prominent pathophysiological mechanism. None of the current antiglaucoma medications counteract excitotoxicity but act only by reducing elevated intraocular pressure (IOP). Elevated IOP is the major risk factor for glaucoma, however, its lowering does not always prevent RGC apoptosis. Since, magnesium (Mg) is a natural calcium antagonist and taurine also possesses Ca^{2+} antagonistic and antioxidant properties, current study investigated the neuroprotective effect of Magnesium Acetyltaurate (MgAT), a combined salt of Mg and taurine, against NMDA-induced RGC apoptotic in *Sprague Dawley* rats. Animals weighing 200-250 g were divided into 5 groups. Groups 1 and 2 received PBS and NMDA, respectively, intravitreally. Groups 3, 4 and 5 received MgAT intravitreally as pre-, co- and post-treatment with NMDA. Rats were sacrificed 7 days post-injection and eyes were enucleated for histopathological examination of retina, detection of TUNEL positive retinal cells, expressions of 3 NOS isoforms and retinal oxidative stress. MgAT pre-treatment most prominently protected against NMDA-induced retinal injury by restoring the expression of NOS isoforms, lowering the nitrosative and oxidative stress and preserving the retinal morphology. In the next part of study, the neuroprotective effect of taurine alone was studied using the same parameters. Although pre-treatment with taurine provided neuroprotection against NMDA-induced injury, the same effects of pre-treatment with MgAT was significantly greater than taurine alone. Therefore, in the subsequent study, mechanisms of neuroprotective effect of MgAT pre-treatment against NMDA-induced retinal injury involving expression of calcium regulated proteins were investigated. It was observed that pre-treatment with MgAT abolishes the NMDA-induced increase in the retinal expression of calpain 1 and CaMKII and decreases the expression of cabin 1. To determine if MgAT pre-treatment could prolong the RGC survival, retrograde labelling of RGC was done using fluorogold as a neuronal tracer. Additionally, retinal sections were subjected to Brn3a immunostaining. Both the retrograde labelling and Brn3a staining specifically detected live RGCs, hence indicating the RGC survival. Both experiments showed significantly enhanced RGC survival in the MgAT pre-treatment group compared to NMDA treated group. Lastly, rats were subjected to visual function test to determine the functional outcome of the effects of MgAT. The visual recognition test using open field and Morris water maze test showed that pre-treatment with MgAT protects against NMDA-induced impairment of the visual functions. In conclusion, the current study showed that the pre-treatment with MgAT prevents NMDA-induced loss of retinal cells and preserves retinal morphology by reducing retinal nitrosative and oxidative stress more effectively than taurine alone. Mechanisms underlying these effects of MgAT are likely to involve restoration of NMDA-induced changes in the expression of calpain 1, CaMKII and cabin 1. These effects of MgAT was associated with greater RGC survival and improved visual functions. Further studies are needed to fully explore the potential of MgAT as an antiglaucoma agent.

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