UNIVERSITI TEKNOLOGI MARA

NEUROPROTECTIVE AND ANTIAPOPTOTIC EFFECTS OF MAGNESIUM ACETYLTAURATE AGAINST NMDA-INDUCED RETINAL AND OPTIC NERVE DAMAGE IN SPRAGUE-DAWLEY RATS

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PhD

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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.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Glaucoma is a primary cause of irreversible blindness worldwide and is characterized by retinal ganglion cells (RGCs) loss. Glutamate excitotoxicity underlies the RGCs death in glaucoma. Overstimulation of N-methyl-*d*-aspartate NMDA receptors causes intracellular Ca^{2+} influx and triggers the apoptosis signalling cascade by increasing the Bax/Bcl-2 ratio and caspase-3 activation via up-regulating the activity of a number of transcription factors such as NF-κB, p53 and AP-1. None of the current therapies protects RGCs from excitotoxicity but only target the elevated intraocular pressure (IOP), which is often associated with progressive visual loss despite treatment. In view of the neuroprotective effects of magnesium and taurine, we investigated the effect of magnesium acetyltaurate (MgAT), a combined salt of magnesium and taurine, on retinal injury induced by NMDA in *Spraque-Dawley* rats. The objective of this study was to the elucidate effect and antiapoptotic mechanism of neuroprotection by MgAT against NMDA-induced retinal damage in rats. In this study, rats were separated into 5 groups: group 1, group 2 received intravitreal injection of NMDA, while groups 3, 4, and 5 received MgAT as pre-, co- and post-treatment to NMDA, respectively. Seven days after injections, rats were euthanized and eyes were enucleated, fixed and processed for histopathological examination of retinal and optic nerve morphology using H&E and toluidine blue staining, respectively, while retinal cells apoptosis was detected by TUNEL assay and caspase-3 immunostaining. Estimation of pro/antiapoptotic proteins (Bax/Bcl-2), caspase-3 activity and BDNF level in retina were done using ELISA. This study revealed that intravitreal MgAT prevents retinal and optic nerve damage induced by NMDA through suppression of apoptosis in retinal cells. Overall, our data demonstrated that pretreatment with MgAT was more effective than co- and post-treatment. This neuroprotective effect of MgAT against NMDA-induced retinal cell apoptosis could be attributed to activation of BDNF-related neuroprotective mechanisms. In the next part of the study, a similar experiment was set to compare the neuroprotective effects of MgAT and taurine (TAU) alone. This part of the study demonstrated that MgAT surpassed neuroprotective effect of TAU in all studied parameters. In the subsequent study, antiapoptotic mechanism of neuroprotection by MgAT against NMDA-induced retinal damage in rats was elucidated. It was observed that pretreatment with MgAT abolished NMDA-induced increase transcriptional activity of NF-κB, p53 and AP-1 family members (c-Jun/c-Fos), as shown by both real time PCR and western blot analysis. Antiapoptotic effect of MgAT correlated with the number of survived RGCs after NMDA exposure. Both retrograde labelling with fluorogold and BRN3A immunostaining showed significant increase in a number of RGCs in the group which received MgAT when compared with the NMDA group. The final study was done to evaluate visual function of rats using object recognition tests conducted in an “open field arena” and “Morris water maze”. Both studies showed that MgAT improved difficulties for rats to recognise the visual cues after NMDA exposure thus showing that visual function of rats was relatively preserved by pretreatment with MgAT. In conclusion, pretreatment with MgAT prevents NMDA induced retinal and optic nerve injury by inhibiting NMDA-induced neuronal apoptosis via downregulation of transcriptional activity of NF-κB, p53 and AP-1-mediated c-Jun/c-Fos. The results were further corroborated by evaluation of visual function of rats using ORT.

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TABLE OF CONTENT