UNIVERSITI TEKNOLOGI MARA

PROTECTIVE EFFECT AND MOLECULAR MECHANISMS OF MAGNESIUM ACETYLTAURATE AGAINST ENDOTHELIN-1-INDUCED RETINAL AND OPTIC NERVE DAMAGE IN RATS: FOCUS ON NEUROINFLAMMATION

NATASHA NAJWA BINTI NOR ARFUZIR

Thesis submitted in fulfillment
of the requirements for the degree of
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(Pharmacology)

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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.

Name of Student	:	Natasha Najwa binti Nor Arfuzir
Student I.D. No.	:	2014991763
Programme	:	Doctor of Philosophy in Medical Science (Pharmacology) – MD958
Faculty	:	Medicine
Thesis Title	:	Protective Effect and Molecular Mechanisms of Magnesium Acetyltaurate Against Endothelin-1-Induced Retinal and Optic Nerve Damage in Rats: Focus on Neuroinflammation
Signature of Student	:	.....
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ABSTRACT

Ischaemic retinal and optic nerve injury causes several ocular conditions including glaucoma, the leading cause of irreversible blindness. Glaucoma is characterized by loss of retinal ganglion cells (RGCs). All current antiglaucoma medications primarily act by lowering elevated intraocular pressure, the major risk factor for glaucoma, without providing direct neuroprotection to RGCs. The ischaemia in glaucomatous retina causes excessive glutamate accumulation and excitotoxicity. As a result, Ca^{2+} overload in glial cells causes release of reactive oxygen and nitrogen species (RONS) and inflammatory cytokines including interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α via activation of c-Jun and NFKβ. In addition to glial derived RONS and inflammatory cytokines, Ca^{2+} overload causes c-Jun and NFKβ activation in RGCs as well. This further escalates RONS generation and activates caspases and pro-apoptotic factors. Since both the Mg^{2+} and taurine are known to inhibit ischaemia, Ca^{2+} overload and apoptotic pathways, magnesium acetyltaurate (MgAT) was synthesised and its neuroprotective effects and mechanisms of action against endothelin (ET1) induced retinal and optic nerve injury were investigated in Sprague dawley rats. MgAT was administered intravitreally as pre-, co- and post-treatment with ET1. Seven days later rats were euthanised and eyes were enucleated. Eyeballs were processed for examination of retinal morphology using hematoxylin and eosin staining, optic nerve morphology using toluidine blue staining, retinal expression of 3 nitric oxide synthase (NOS) isoforms using immunohistochemistry, retinal nitrosative and oxidative stress markers using ELISA, extent of retinal cell apoptosis using caspase-3 and TUNEL immunostaining. Among the 3 MgAT treatment groups, pre-treatment was found to most effectively preserve retinal and optic nerve morphology by restoring the expression of NOS isoforms and reducing nitrosative and oxidative stress. In this group there were significantly lower number of caspase-3 and TUNEL positive cells compared to vehicle treated rats indicating lower number of retinal cells undergoing apoptosis. In the next part of study, the neuroprotective effects of MgAT were compared with that of taurine alone using the same parameters. It was observed that MgAT provides greater neuroprotection compared to taurine alone against ET1 induced ischaemic retinal injury. Since the pre-treatment with MgAT was found to be most effective, in subsequent studies, the mechanisms of neuroprotective effect of MgAT were investigated by administering it as pre-treatment with ET1. MgAT pre-treatment prevents ET1 induced increase in retinal expression of cytokines, c-Jun, phospho c-Jun, NFKβ and phospho NFKβ. Further, it was investigated if MgAT pre-treatment could prolong the RGC survival and retrograde labelling of RGC was done using fluorogold as a neuronal tracer. It was observed that there was significantly greater number of fluorogold positive cells in MgAT pre-treated group, indicating greater RGC survival. Additionally, Brn3a immunostaining was also done on retinal sections to assess RGC survival. It was observed that treatment with ET1 results in significantly lower number of Brn3a+ RGCs, however, pre-treatment with MgAT caused appearance of several Brn3a+ RGCs indicating greater RGC survival. At last, visual function test was performed to determine the functional outcome of the neuroprotective effect of MgAT. Object recognition test using Morris water maze showed that MgAT pre-treatment abolishes the ET1 induced impairment of visual functions. In conclusion, MgAT protects RGCs by inhibiting expression of inflammatory cytokines, restoring the expression of NOS isoforms and preventing retinal oxidative and nitrosative stress.

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TABLE OF CONTENTS