

MECHANISM OF NEUROPROTECTION BY MAGNESIUM ACETYLAURATE ON  
EXPERIMENTAL GLUTAMATE-INDUCED EXCITOTOXICITY IN RAT RETINAL GANGLION CELLS



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Prof. Dr,

**SUBMISSION OF FINAL REPORT FOR PROJECT RMI 600-RMI/RAGS 5/3 (103/2013).**

I wish to submit the final report entitled "*Mechanism of Neuroprotection by Magnesium Acetyltaurate on Experimental Glutamate-Induced Excitotoxicity in Rat Retinal Ganglion Cells*" for project RMI 600-RMI/RAGS 5/3 (103/2013).

Glutamate excitotoxicity plays a major role in the loss of retinal ganglion cells (RGCs) in glaucoma. The toxic effects of glutamate on RGCs are mediated by the over stimulation of N-methyl-d-aspartate (NMDA) receptors. Accordingly, NMDA receptor antagonists have been suggested to inhibit excitotoxicity in RGCs and delay the progression and visual loss in glaucoma patients. The purpose of the present study was to examine the potential of neuroprotective effect of Mg acetyltaurate (MgAT) on RGC death induced by NMDA. MgAT was proposed mainly due to combination of magnesium (Mg) and taurine which may provide neuroprotection by dual mechanisms of action, i.e. inhibition of NMDA receptors and antioxidant effects.

In our study, the morphometric measurement on H&E staining was observed with reduction in number of ganglion cells in NMDA group compared to the MgAT pre- and co-treated groups. Similar results were observed in Caspase-3 staining. The results further corroborated by the estimation of markers of oxidative stress, caspase-3 activity in retina.

For the first time, we showed that pre-treatment with intravitreal MgAT prevents RGC apoptosis induced by NMDA. Overall, our data demonstrated that the pretreatment with MgAT was more effective than co- and post-treatment. This protective effect of MgAT against NMDA-induced retinal cell apoptosis could be attributed to reduction of retinal oxidative stress and activation of neuroprotective mechanisms.

Thanking you.



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## 5.2 Enhanced Executive Summary

**Background:** Glutamate excitotoxicity plays a major role in the loss of retinal ganglion cells (RGCs) in glaucoma. The toxic effects of glutamate on RGCs are mediated by the overstimulation of N-methyl-D-aspartate (NMDA) receptors. Accordingly, NMDA receptor antagonists have been suggested to inhibit excitotoxicity in RGCs and delay the progression and visual loss in glaucoma patients. The purpose of the present study was to examine the potential neuroprotective effect of Mg acetyltaurate (MgAT) on RGC death induced by NMDA. MgAT was proposed mainly due to the combination of magnesium (Mg) and taurine which may provide neuroprotection by dual mechanisms of action, i.e., inhibition of NMDA receptors and antioxidant effects.

**Methods:** Rats were divided into 5 groups and were given intravitreal injections. Group 1 (PBS group) was injected with vehicle; group 2 (NMDA group) was injected with NMDA while groups 3 (pre-), 4 (co-), and 5 (post-) treatments were injected with MgAT, 24 h before, in combination or 24 h after NMDA injection respectively. NMDA and MgAT were injected in PBS at doses 160 and 320 nmol, respectively. Seven days after intravitreal injection, the histological changes in the retina were evaluated using hematoxylin & eosin (H&E) staining. Optic nerves were dissected and stained in Toluidine blue for grading on morphological neurodegenerative changes. The extent of apoptosis in retinal tissue was assessed by caspase-3 immunofluorescence staining. The estimation of oxidative stress in retina was done using enzymelinked immunosorbent assay (ELISA) technique

**Results:** The retinal morphometry showed reduced thickness of ganglion cell layer (GCL) and reduction in the number of retinal cells in GCL in NMDA group compared to the MgAT-treated groups. Caspase-3 staining showed increased number of apoptotic cells in inner retina. The results were further corroborated by the estimation of oxidative stress in retina.

**Conclusion:** In conclusion, current study revealed that intravitreal MgAT prevents retinal and optic nerve damage induced by NMDA. Overall, our data demonstrated that the pretreatment with MgAT was more effective than co- and posttreatment. This protective effect of MgAT against NMDA-induced retinal cell apoptosis could be attributed to the reduction of retinal oxidative stress.

### 5.3 Introduction

Glaucoma is characterized by damage to the optic nerve, which leads to progressive and irreversible vision loss (Casson et al. 2012). It is the second most common cause of blindness worldwide after cataract. It is estimated that 4.5 million people are suffering from glaucoma-related blindness and this number is expected to rise to 11.2 million by 2020 (Quigley and Broman 2006). Most therapies for glaucoma are directed at the reduction of the intraocular pressure (IOP). Conventional wisdom holds that excessive pressure within the eye leads to retinal ganglion cell (RGC) loss and optic nerve damage seen in this disease. However, the elevated IOP is only one of the risk factors that lead to RGC apoptosis. Several other risk factors also contribute to RGC loss in glaucoma. Studies have shown that many of these risk factors culminate into glutamate-mediated toxicity to RGCs and glutamate selectively damages RGCs in the mammalian eye (Dreyer and Lipton 1999). Glutamate is the principal excitatory neurotransmitter in the central nervous system (CNS). However, excessive activation of glutamate-gated membrane channels may lead to irreversible injury to neurons. Glutamatergic excitotoxicity, results primarily from intracellular accumulation of calcium ions and in retina it was first described by Lucas and Newhouse (1957). Because of the relatively high permeability of N-methyl-D-aspartate (NMDA)-type glutamate-gated channels to calcium ions, neurons are particularly sensitive to injury associated with excessive activity of this channel subtype (Choi 1988). RGCs are known to express NMDA-type channels, and glutamatergic excitotoxicity, mediated by NMDA channels, has been demonstrated to contribute significantly to RGC injury in both the in vitro and animal models (Levy and Lipton 1990; Sucher et al. 1991; Kitano et al. 1996; Pang et al. 1999; Luo et al. 2001). From a therapeutic standpoint, NMDA receptors are potential targets of intervention to prevent RGC death (Lipton 1993; Chidlow et al. 2007; Russo et al. 2008). Glutamate, however, mediates synaptic transmission essential for normal function of the nervous system. Hence, complete blockade of NMDA receptor activity causes unacceptable side effects (Seki and Lipton 2008). From this point of view, magnesium (Mg) as physiological antagonist of NMDA receptors might be good therapeutic instrument in the treatment of NMDA-mediated ocular pathological conditions (Agarwal et al., 2014). Mg ions block NMDA receptor-mediated component of synaptic transmission and systemically administered Mg antagonizes the neurotoxic effects of NMDA in in vivo and in vitro experiments. For example, systemic treatment with Mg can prevent the death of motoneurons that are rendered susceptible to the excitotoxic effects of NMDA by nerve injury (Gougoulas et al. 2004). In an autoradiographic study, Mg sulfate treatment decreased NMDA receptor binding in the rat brain (Hallak et al. 1994). Previous studies