Original Article

Neuroprotective Effects of Memantine in the Retina of Glaucomatous Rats: An Electron Microscopic Study

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Abstract

Purpose: In this experimental study, the effects of systemic memantine administration on the retinal ultrastructure of experimentally induced glaucomatous rats were investigated.

Methods: Twenty-four Wistar albino rats were included in this study. Glaucoma was induced by injecting sodium hyaluronate into the anterior chamber of the rats for a period of three weeks. As a control, 8 rats were sham treated (Group C). Glaucoma induced animals were divided into two groups; Group M ($n = 8$) received a single daily dose of 10 mg/kg memantine, and Group G received the same volume of saline ($n = 8$), via intraperitoneal route for a period of six weeks, starting with the induction of glaucoma. Then, all rats were sacrificed and the retinas were prepared for electron microscopic examination. Electron microscopic damage findings were graded between 0 and 4 and mean damage scores for each cell or layer was calculated for each group. Statistical comparison was made between group G and group M.

Results: Including the photoreceptor cells, marked ultrastructural changes were observed in the retinas of the animals in group G. The ultrastructural changes in group M were modest and there was no significant cell death. Statistical findings indicated these results.

Conclusion: Results of the present study suggest that memantine treatment, when started in the early phase of glaucomatous process, may help to preserve the retinal ultrastructure and thus prevent neuronal injury in experimentally induced glaucoma.

Keywords: Electron Microscopy; Glaucoma; Memantine; Neuroprotection; Rat

INTRODUCTION

Glaucoma is the second leading cause of blindness worldwide[1] and is characterized by an optic neuropathy accompanied by typical structural and functional defects (optic disc damage and visual field loss).[2] It is widely accepted that the death of a substantial number of retinal ganglion cells (RGCs) in the inner retina and loss of their axons in the optic nerve are pathophysiologic characteristics of glaucoma.[2] Glaucoma is a multifactorial condition with intraocular pressure (IOP) elevation as the major risk factor.[3] However, the pathophysiological relationship of elevated IOP with RGC death remains

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unclear. Understanding the underlying pathogenesis is necessary to determine the ideal treatment strategy.

Neuronal death occurs by several different mechanisms including growth factor deprivation, blockade of axonal transport in the optic nerve fibers due to IOP elevations, hypoxia-ischemia, oxidative stress, inflammatory cytokines, aberrant activity of the immune system, and glutamate excitotoxicity.[3-5] Glutamate receptor activational excitotoxicity has been associated with mitochondrial dysfunction in acute and chronic neurodegenerative disorders, and has been suggested to cause RGC death in glaucoma.[5-8] However, the relationship between IOP elevation and glutamate excitotoxicity and mitochondrial dysfunction remains unclear.[9] IOP elevations lead to mechanical stress or ischemic conditions, resulting in RGC death and glaucoma.[10,11]

Currently, the only management strategy for glaucoma is IOP reduction. However, despite successful IOP control, some patients continue to lose vision.[12,13] Therefore, it is clear that reduction of IOP may be inadequate for treatment of glaucoma patients, and the focus of research is now shifting toward other strategies, such as neuroprotection of RGCs and other neurons in the central visual pathway.[14-18]

Memantine is an uncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist and has been shown to be neuroprotective in mouse, rat, and monkey models of glaucoma.[19-22] Memantine has demonstrated some benefits in animal models, but there has been less convincing evidence in human studies to date.[23,24] Two phase 3 trials on the safety and efficacy of memantine failed to show statistical significance for the primary endpoint. Kersey et al reported that perhaps the benefits of memantine were underestimated due to study design.[25] According to Osborn, the result of a phase III clinical trial in the human open angle glaucoma was disappointing, which may be due to inappropriate endpoint and insufficient duration of treatment.[26] In spite of this study, the neuroprotective efficacy of memantine is still under investigation.[9,27,28]

The protective effects of memantine on the ultrastructural construction of glaucomatous retinas have not been fully elucidated. In the current study, we aimed to investigate the effects of systemic memantine administration on the retinal ultrastructure in an experimental model of glaucoma in rats.

METHODS

This study was conducted according to the principles regarding the care and use of animals adopted by the American Physiological Society and the Society for Neuroscience.

Animal Selection

A total of 24 young adult male Wistar albino rats (mean weight 220 ± 40 grams) were used for the purpose of the study. The animals were housed in a standard animal room with food and water *ad libitum* under controlled conditions of humidity and temperature (21 ± 2°C). The room was lit by fluorescent lights that were turned on and off automatically every 12 hours (on from 6 AM to 6 PM).

The animals were divided into three groups of 8 rats as follows: Group G, Group M, and Group C. In groups G and M, glaucoma was induced by injecting sodium hyaluronate into the anterior chamber of the rats for a period of three weeks. The rats in Group C were sham treated. Rats in Group M received a single daily dose of 10 mg/kg memantine, while rats in Group G received the same volume of saline via the intraperitoneal route for a period of six weeks, starting with induction of glaucoma.

Glaucoma Induction

All rats were anesthetized using intraperitoneal ketamine hydrochloride (15 mg/kg) and xylazine hydrochloride (0.3 mg/kg). Glaucoma was induced in the right eyes of the rats as described previously.[29,30] Briefly, 25 uL of sodium hyaluronate (23 mg/ml, Healon-5®) was injected into the anterior chamber of the rats, through the corneoscleral limbus via a Hamilton syringe (Hamilton, Reno, NV, USA) using a 30-gauge needle, after drawing an equal volume of the aqueous humor. Injections were performed under a surgical microscope (Topcon OMS 75, Topcon Corp., Tokyo, Japan). Glaucoma was induced by weekly injections for 3 weeks. In the sham-treated animals (Group C), an equal volume of saline solution was injected.

Intraocular Pressure Measurement and Animal Sacrifice

Intraocular pressure (IOP) measurements before and after the procedures were recorded during the 3 weeks of glaucoma induction, and weekly for another 3 weeks. IOP was measured with the Tono-Pen (Tono-Pen XL®, Medtronic Solan, Jacksonville, FL, USA) once mild anesthesia was achieved by 3 mg/kg of asepromazine and a single drop of topical 0.4% oxybuprocaine hydrochloride. The Tono-Pen was oriented perpendicular to the cornea, and using a swift and steady stroke, the tip was brought into contact with the cornea. For each eye, the mean of 15 valid readings per pressure level was calculated.[31] All injections and measurements were performed at the same time period each day (9:00-10:30 am), and by the same investigator (HC). IOP changes in the 3 groups are summarized in Table 1.

At the end of 6 weeks, all rats were sacrificed by and intracardiac perfusion of glutaraldehyde, and the globes were enucleated.

Electron Microscopic and Ultrastructural Evaluation

The posterior wall of the globes were cut into 1 mm pieces and fixed in 2.5% glutaraldehyde for one hour at +4°C and then washed in cold cacodylate buffer (0.05 M; pH 7.4) twice, for 10 minutes each. Samples were then post-fixed in 1% osmium tetroxide in 0.05 M Na cacodylate for one hour and were dehydrated in a series of graded alcohols (50%, 70%, 90%, 96%, and 100%, respectively). Propylene oxide-Epon 812 treated specimens were embedded in Epon–812 embedding media. Semi thin sections (1 μm thick) were obtained using a microtome (Reichert Optische Werke AG, Vienna, Austria) and stained with toluidine blue. Using retinal sections, uranyl acetate-lead citrate-stained ultrathin sections were prepared using LKB ultramicrotome (LKB, Sweden). Transmission electron microscopic examination was performed using a Jeol JEM - 1011B electron microscope (Peabody, MA, USA) with Megaview digital imaging system. Electron microscopic damage findings were graded between 0 and 4 (0 representing no damage and 4 representing the highest level of damage) and mean damage scores for each cell or layer was calculated for each group.

Statistical Analysis

Data for IOP measurements were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software for Windows (version 12.0; SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) test and *Post Hoc* Tukey's test were used for comparing the three groups in terms of IOP changes. Electron microscopic grades were compared between group G and group M employing the Mann-Whitney U test. P values less than 0.05 were considered as statistically significant.

RESULTS

Intraocular Pressure Measurements

Mean IOP values of the study groups at different time points are shown in Figure 1. Mean IOP in Group M and Group G were significantly higher than that of the control group after glaucoma induction ($P < 0.001$).

Electron Microscopic and Ultrastructural Evaluation

Fine structural changes in retinal layers, indicating reversible and irreversible cellular degeneration, were

Table 1. Intraocular pressure change at different time points

Week	IOP change (mean±SD)		
	Control group	Glaucoma group	Memantine group
0	14.02±0.69	16.0±0.74	14.07±0.86
1	14.01±0.56	31.0±0.75	32.0±0.82
2	14.02±0.70	32.0±0.83	32.0±0.96
3	14.02±0.54	37.0±0.74	37.0±0.76
4	14.01±0.58	35.0±0.56	38.0±0.56
5	14.03±0.82	31.9±0.92	37.0±0.89
6	14.04±0.83	33.0±0.96	35.0±0.52

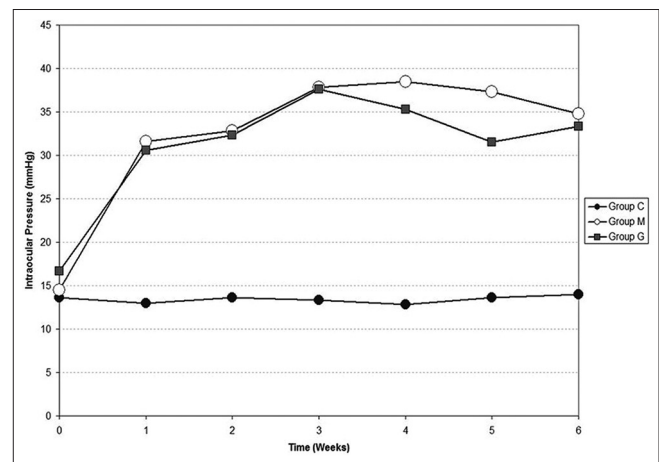IOP, intraocular pressure; SD, standard deviation

Figure 1. The mean intraocular pressure of the groups at different time points. Note that the mean intraocular pressure of Group M and Group G remained remarkably higher than that of Group C.

evaluated. Mitochondria and other membranous organelles of the retinal pigment epithelium (RPE) were intact in Group C [Figure 2a], whereas partial degeneration of mitochondrial cristae was noted in Group M [Figure 2b]. The inclusion bodies in the mitochondria and other organelles were more prominent in Group G as compared to Groups C and M [Figure 2c].

The structural organization of photoreceptor lamellae as well as mitochondrial cristae was preserved both in groups C and M [Figure 3a and b, respectively], whereas, the lamellar structure and mitochondria of the inner segment of the photoreceptors were damaged in Group G [Figure 3c].

Microvilli of Müller cells, and the mitochondria of cone and rod cells in the inner segment of the retina were preserved in Group C [Figure 4]. However, in Group M lamellar inclusions and focal loss of cristae were observed in some mitochondria, and obliteration was observed in the microvilli of Müller cells in the inner retina [Figure 5]. Loss of mitochondrial cristae and swelling of inner segments were more prominent in Group G [Figure 6].
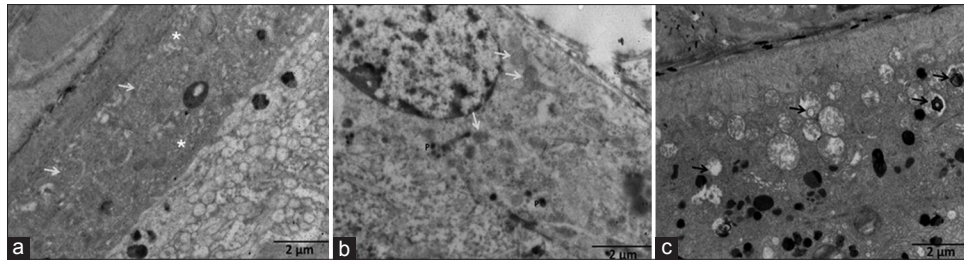
Figure 2. Electron micrographs showing ultrastructural changes in mitochondria of the retinal pigment epithelium. (a) Electron micrograph of mitochondria (white arrows) and other membranous structures (asterisk) in the retinal pigment epithelium of Group C. (b) Electron micrograph of mitochondria (white arrows) and pigment granules (P) in the retinal pigment epithelium of Group M. (c) Electron micrograph of swollen and degenerated mitochondrial cristae containing several inclusions (arrows) in the retinal pigment epithelium of Group G.
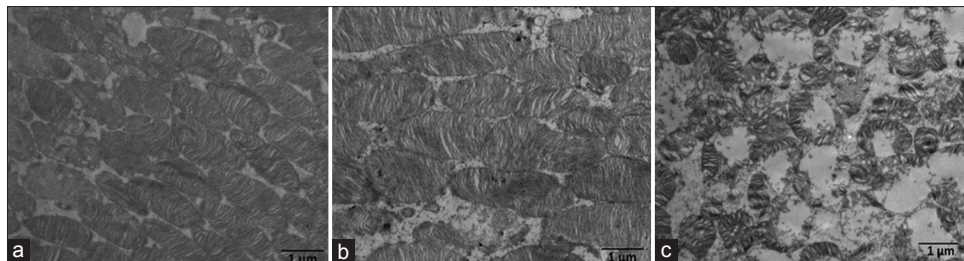

Figure 3. Electron micrographs showing photoreceptor structure. (a) Electron micrograph of photoreceptors in Group C. (b) Electron micrograph of photoreceptors in Group M. (c) Electron micrograph of photoreceptors in Group G.
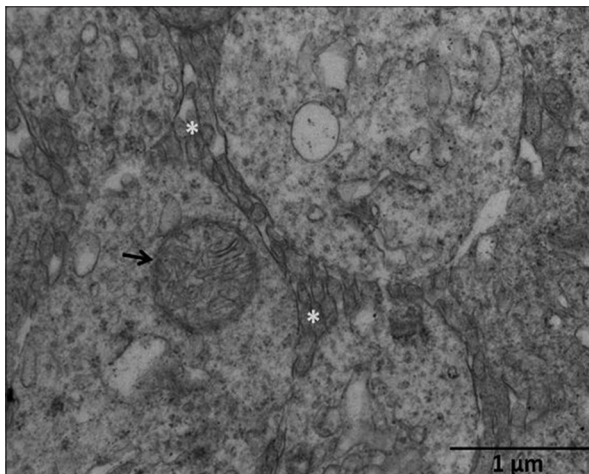

Figure 4. Electron micrograph of the inner retina in Group C is viewed. It shows well preserved microvilli of Müller cells (asterisk), and mitochondria of cone and rod cells (arrow).

Attachments amongst outer nuclear layer cells were preserved in Group C [Figure 7a]. Cellular attachments forming the external limiting membrane were intact and there were minimal detachments between the cells in Group M [Figure 7b]. Severe cytoplasmic and nuclear vacuolization was detected in the cells of the outer nuclear layer in Group G [Figure 7c].

Cell attachments, membranous compartments, synaptic contacts, and synaptic vesicles in the outer plexiform layer were preserved in Group C [Figure 8a and b]. Synaptic contacts were preserved, but synaptic vesicles were diminished and mitochondrial cristae were partially destructed in Group M [Figure 8c]. In contrast, severe mitochondrial degeneration and separation of the intercellular junction were evident in the cells of the outer plexiform layer in Group G [Figure 8d].

In Group M, vacuolization and edema was observed in the cells of the inner plexiform layer [Figure 9a] but cellular organelles, such as the endoplasmic reticulum, were intact in both interneurons and ganglion cells [Figure 9b]. Nuclear membrane invaginations, dilatation in endoplasmic reticulum cisternae, and autophagosomes were common in bipolar and ganglion cells of Group G [Figure 10a-c, respectively]; however such findings were detected only in two animals in Group M.

In Group G, apoptotic cells characterized by fragmented nuclei and dark cytoplasm were mostly observed in the ganglion cell layer and in inner plexiform layer [Figure 11]. The number of apoptotic cells was smaller in the outer plexifom layer and they were rare in the photoreceptor cell layer.

One of the major findings noted in Group M was the presence of widely scattered Müller cells, exhibiting increased number of phagosomes, among the degenerated neurons. In addition, structural integrity of the mitochondria was preserved in Group M.

In contrast to the normal vascular structure [Figure 12c] in Group M, vacuolar degeneration in the nerve fiber layer [Figure 12a] and endothelial swelling

and vacuolization in some retinal vessels [Figure 12b] were observed in Group G. Ultrastructural changes related with glaucomatous damage are summarized in Table 2.
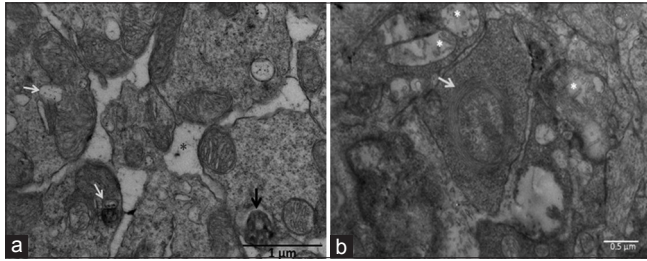

Figure 5. Electron micrographs show lamellar inclusions and focal loss of cristae in some mitochondria, and obliteration of the microvilli of Müller cells in the inner retina in Group M. (a) Electron micrograph shows sparse lamellar inclusions and focal loss of mitochondrial cristae (arrows) and obliteration of microvilli of Müller cells (asterisk) in the inner retina in Group M. Note a cilium (black arrow). (b) Electron micrograph reveals loss of mitochondrial cristae (asterisk) and a part of lamellar body in the apical portion of inner segment (arrow) in Group M.
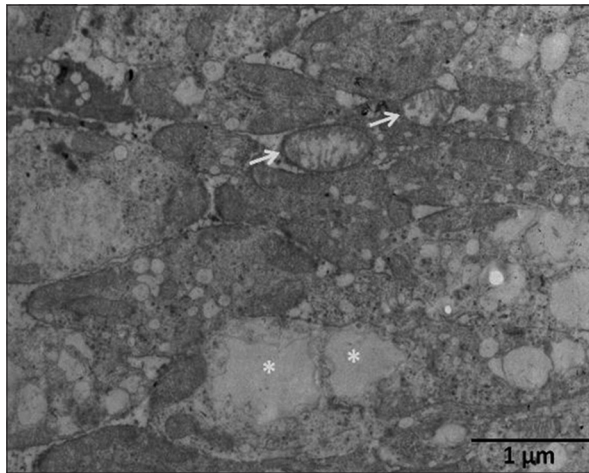

Figure 6. Electron micrograph showing loss of mitochondrial cristae (arrows) and swelling of inner segments (asterisk) in Group G.

DISCUSSION

In this study, a rat model of experimental glaucoma was used to investigate the effects of systemic memantine treatment on retinal ultrastructure. Although ultrastructural changes in experimentally induced glaucoma have been previously studied, there is no published data on ultrastructural changes in the retina during pharmacological treatment to prevent excitotoxic neuronal death in glaucoma.[32] The present study constitutes the first demonstration of ultrastructural changes in glaucomatous retinas treated with systemic memantine, an uncompetitive NMDA glutamate receptor antagonist for neuroprotection.

An increased number of phagosomes in the RPE, and nuclear membrane invaginations in ganglion cells without evident neuronal loss were observed in memantine treated animals, and these alterations were not as severe as those of non-treated animals.

A noteworthy finding in the present study was the presence of evident degeneration and apoptosis in photoreceptor cells. There are contradictions about photoreceptor damage in glaucoma in the literature.[33] In most studies, electroretinogram findings were reported to be normal in patients with glaucoma suggesting that photoreceptors remain intact in glaucoma.[33-35] Quigley and Green[36] demonstrated damage only in the RGC and their axons in a light and electron microscopic study on postmortem glaucoma patients. In contrast to those studies, there are studies proposing that photoreceptors are affected as glaucoma progresses.[37] Data of the present study seem to support the idea that outer retinal layers including photoreceptors and RPE were all affected following sub-acute glaucoma induction. These data support the opinion that glaucoma may cause damage not only in RGCs and in their axonal extensions but also in all retinal layers, including the photoreceptors.

Müller cells are some of the most important glial cells of the vertebrate retina, with important functions including homeostasis, regulation of retinal angiogenesis and retinal blood flow, and development of reactive
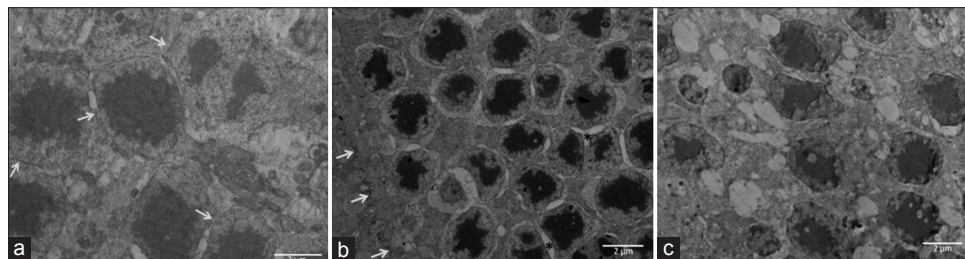

Figure 7. Electron micrographs showing the structure of the outer nuclear layer in the study groups. (a) Electron micrograph of the outer nuclear layer shows attachments (arrows) between cells in Group C. (b) Electron micrograph from the outer nuclear layer shows minimal detachment between cells (asterisk) and intact cellular attachments forming the external limiting membrane (arrows) in Group M. (c) Electron micrograph shows severe cytoplasmic and nuclear vacuolization in the cells of the outer nuclear layer in Group G.
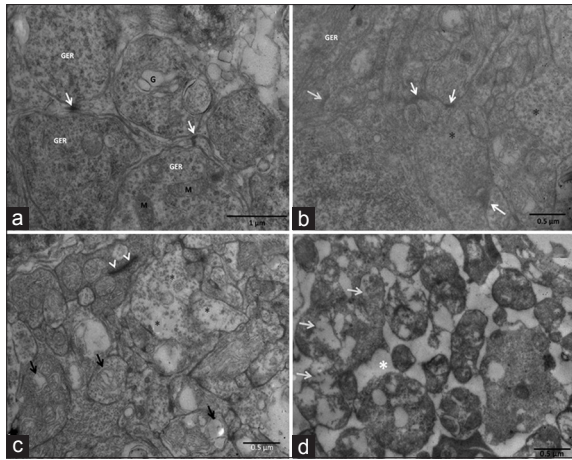
Figure 8. Electron micrographs of the study groups showing ultrastructural changes in the outer plexiform layer: (a) Well preserved cellular attachments (arrow) and compartments in Group C. G: golgi; M: mitochondria; GER, granular endoplasmic reticulum. (b) Group C demonstrated synaptic contacts (arrows) and synaptic vesicles (asterisk). GER: granular endoplasmic reticulum. (c) Electron micrograph from the outer plexiform layer shows limited destruction in mitochondrial cristae (arrows), diminished synaptic vesicles (asterisk) and a ribbon synapse (arrow head) in Group M. (d) Electron micrograph from the outer plexiform layer shows severe degeneration in mitochondrial cristae (arrows) and detachment of cells (asterisk) in Group G.
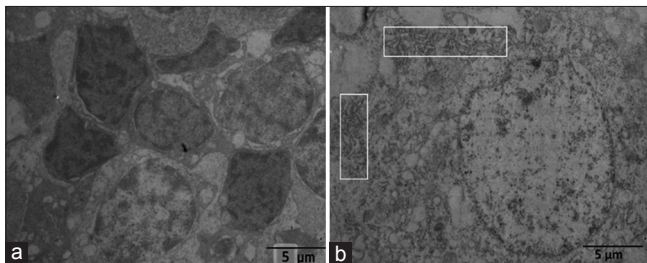

Figure 9. Electron micrograph examples for Group M showing the ultrastructural changes in the inner plexiform layer and ganglion cells. (a) Electron micrograph from inner plexiform layer of Group M, shows edema in interneurons. (b) Electron micrograph of ganglion cells with preserved granular endoplasmic reticulum (frames) in Group M.
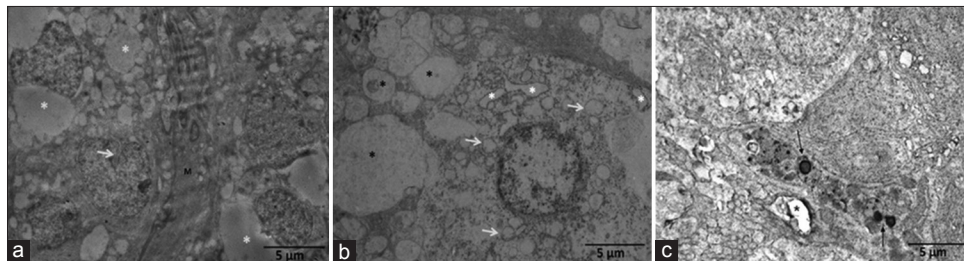
responses in pathologic conditions.[38] It has also been reported that Müller cells are effective in preventing excitotoxicity, uptake of glutamate from the extracellular environment and the release of glutathione.[38] We observed that in non-treated glaucomatous rats, Müller cells were scattered around the degenerated neurons, with their dense and long microfilamentous processes. However, Müller cells in memantine-treated animals did not show wide dispersion around the neurons and they contained dense phagosomes. Regarding the configuration of Müller cells, the difference between memantine treated and non-treated groups may be explained as a "workload relieving" effect by memantine on Müller cells.

Another finding, which was not observed in memantine-treated eyes but frequently noted in the non-treated glaucoma group, was villous transformation and vacuolization of endothelial cells in inner retinal layers. This finding indicates possible degeneration in retinal vessels that is ultrastructurally visible.

In late 2008, some of the results of the second phase III trial on the clinical use of memantine in primary open-angle glaucoma were reported, and it was stated that while higher doses of memantine reduced progression of glaucoma, lower doses did not cause a significant effect.[23,24] The most detailed comment on these results was made by Osborne.[26] This author emphasized the fact that the results of this phase III trial on memantine should not be seen as a disappointment, but should be used as a basis for further studies and stated that memantine, might be effective in slowing down apoptosis during the glaucomatous process only in cells containing a defined density of NMDA receptors and also stressed the necessity of using agents with different mechanisms of actions for neuronal protection.[26]

In the current study, by demonstrating the efficacy of memantine at the organelle level, we introduce data that may provide a basis for the combined use of memantine with different agents in order to enhance its efficacy. The employed dose of memantine showed no toxic effects at the level of the organelle. We assert that demonstration

Figure 10. Examples of electron micrographs for Group G, which demonstrate several ultrastructural changes in the inner nuclear layer, in ganglion cells and in the inner plexiform layer. (a) Electron micrograph of the inner nuclear layer shows nuclear membrane invaginations (arrow) and prominent edema (asterisk) in bipolar cells M: Müller cell. (b) Electron micrograph of ganglion cells with dilated cisternae of granular endoplasmic reticulum (asterisk) and destroyed mitochondria (arrows). Also note the swollen axonal processes (black asterisk). (c) Electron micrograph of autophagosomes in the inner plexiform layer of Group G.

Cell/layer	Ultrastructural findings	Group C	Mean score±SD		P*
			Group G	Group M	
RCE	Vacuolization and loss of villi	0	3.75±0.46	0.25±0.46	<0.001
RNFL	Cellular edema	0	3.00±0.75	1.13±0.64	0.001
RNFL	Inclusion bodies	0	3.62±0.51	0.50±0.53	0.001
RNFL	Lipid vacuolar degeneration	0	3.63±0.51	0.50±0.53	0.001
GCL	Mitochondrial damage	0	2.88±0.83	0.50±0.53	0.001
GCL	Mitochondrial inclusion bodies	0	3.38±0.74	0.50±0.53	0.001
GCL	Apoptotic cells	0	3.63±0.51	0.88±0.35	<0.001
INL	Condensation in cytoplasm and chromatin	0	3.38±0.74	0.63±0.74	0.001
INL	Vacuole formation and edema	0	3.00±0.53	0.75±0.46	<0.001
INL	Apoptotic cells	0	3.00±0.75	0.50±0.53	0.001
BCL	Mitochondrial damage	0	2.75±0.71	0.75±0.71	0.001
BCL	Mitochondrial inclusion bodies	0	3.38±0.74	0.75±0.71	0.001
BCL	Dilatation in ER cisternae and axons	0	3.50±0.53	0.50±0.53	0.001
MC	Phagosome content	0	0.38±0.51	3.00±0.75	0.001
MC	Cellular degeneration	0	3.00±0.75	0.50±0.53	0.001
MC	Mitochondrial damage	0	3.25±0.88	0.38±0.52	0.001
MC	Mitochondrial inclusion bodies	0	3.25±0.71	0.63±0.74	<0.001
OPL	Detachment in layers	0	2.87±0.64	0.50±0.53	0.001
ONL	Apoptotic cells	0	2.00±0.75	0.38±0.51	0.002
PRC	Degeneration in lamellar bodies	0	3.75±0.46	0.50±0.53	<0.001
PRC	Mitochondrial damage	0	3.38±0.74	0.75±0.71	0.001
PRC	Mitochondrial inclusion bodies	0	3.38±0.91	0.38±0.51	0.001
PRC	Apoptotic cells	0	2.00±0.75	0.38±0.51	0.002
RPE	Loss of pigment granules	0	3.63±0.74	0.88±0.35	<0.001
RPE	Formation of inclusion bodies	0	3.50±0.75	2.00±0.53	0.003
RPE	Vacuolization	0	3.00±0.75	0.38±0.51	0.001
RPE	Detachment at intercellular junctions	0	3.25±0.88	1.00±0.53	0.001
RPE	Loss of villous processes	0	3.75±0.46	0.50±0.53	<0.001
RPE	Mitochondrial damage	0	3.75±0.46	0.38±0.52	<0.001
RPE	Mitochondrial inclusion bodies	0	3.38±52	0.50±0.53	0.001

*Statistical comparisons were made between groups G and M. Electron microscopic findings of damage were graded between 0 and 4 (with 0 representing no damage and 4 representing the highest level of damage); mean damage scores for each cell or layer was calculated for each group. SD, standard deviation; RCE, retinal capillary endothelium; RNFL, retinal nerve fiber layer; INL, inner nuclear layer; BCL, bipolar cell layer; MC, Müller cells; OPL, outer plexiform layer; ONL, outer nuclear layer; PRC, photoreceptor cells; RPE, retinal pigment epithelium; GCL, ganglion cell layer; ER, endoplasmic reticulum
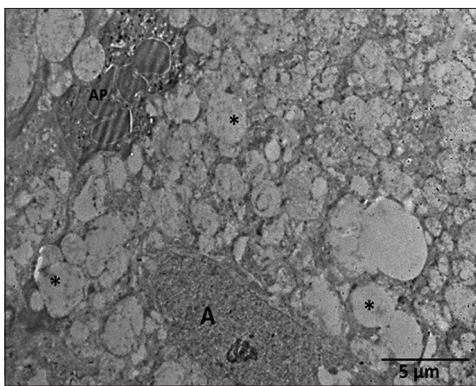

Figure 11. Electron micrograph shows an amacrine cell (A), diffuse vacuolization (asterisk) and an apoptotic cell (AP) in Group G.

of glaucomatous damage and the efficacy of agents, such as the NMDA receptor antagonist memantine, at the organelle level may be considered as an approach to elucidate the etiopathogenesis of glaucoma, although the reflection of its efficacy to clinical practice is controversial. It might be a rational method to reduce RGC loss due to glaucoma damage by the combined use of agents with different mechanisms of action and to effectively protect remaining neuronal populations by the combined use of multi-dimensional neuroprotective aegnts.[26,39] Gabelt reported evidence for a neuroprotective effect by memantine on ganglion cell function in their experimental study on monkeys.[28] Therefore, memantine is still considered to be valuable, and its neuroprotective efficacy is still under investigation.[9,27]
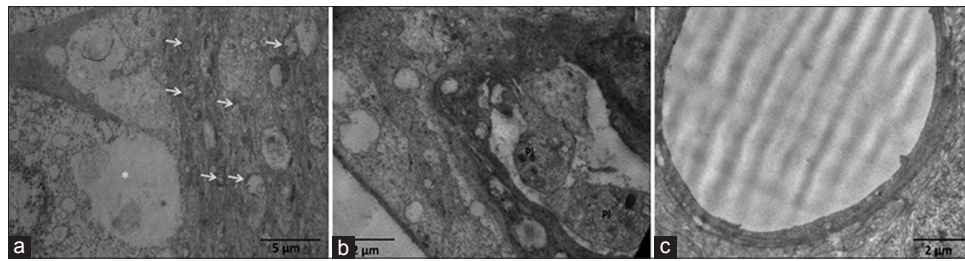
Figure 12. Electron micrographs show vacuolar degeneration in the nerve fiber layer and endothelial swelling in Group G and preserved vascular structure in Group M. (a) Electron micrograph of the nerve fiber layer shows destruction in mitochondria (arrows) and swelling (asterisk) in Group G. (b) Electron micrograph of a capillary in Group G shows endothelial swelling and platelets (Pl) in its lumen. (c) Electron micrograph of a capillary in Group M shows normal endothelium.

Data of the present study demonstrate that in an experimentally induced glaucoma model in rats, memantine demonstrated a neuroprotective effect without any significant impact on IOP. Our results seem to support the idea that glutamate-induced excitotoxicity may play a significant role in glaucoma induced neuronal injury. Currently, in addition to conventional glaucoma treatment, which directly aims to decrease IOP, anti-excitotoxic medications are being explored as new treatment strategies to prevent neuronal injury. Memantine treatment may preserve retinal ultrastructure and thus prevent neuronal injury in experimental glaucoma. Based on our knowledge, this is the first published report on qualitative ultrastructural changes in glaucomatous retinas treated with systemic memantine for neuroprotection. We hope that by demonstrating organelle protection in the neuronal cells, these observations contribute to further neuroprotective investigations and lead to a better understanding of the pathogenesis of glaucoma at the ultrastructural level.

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Conflicts of Interest

There are no conflicts of interest.

REFERENCES

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol* 2006;90:262-267.
2. Quigley HA. Glaucoma. *Lancet* 2011;377:1367-1377.
3. Lipton SA. Possible role for memantine in protecting retinal ganglion cells from glaucomatous damage. *Surv Ophthalmol* 2003;48 Suppl 1:S38-S46.
4. Osborne NN, Chidlow G, Wood J, Casson R. Some current ideas on the pathogenesis and the role of neuroprotection in glaucomatous optic neuropathy. *Eur J Ophthalmol* 2003;13 Suppl 3:S19-S26.
5. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet* 2004;363:1711-1720.
6. Beal MF. Aging, energy, and oxidative stress in neurodegenerative diseases. *Ann Neurol* 1995;38:357-366.
7. Mattson MP, Pedersen WA, Duan W, Culmsee C, Camandola S. Cellular and molecular mechanisms underlying perturbed energy metabolism and neuronal degeneration in Alzheimer's and Parkinson's diseases. *Ann N Y Acad Sci* 1999;893:154-175.
8. Fan MM, Raymond LA. N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog Neurobiol* 2007;81:272-293.
9. Ju WK, Kim KY, Angert M, Duong-Polk KX, Lindsey JD, Ellisman MH, et al. Memantine blocks mitochondrial OPA1 and cytochrome c release and subsequent apoptotic cell death in glaucomatous retina. *Invest Ophthalmol Vis Sci* 2009;50:707-716.
10. Anderson DR, Drance SM, Schulzer M; Collaborative Normal-Tension Glaucoma Study Group. Factors that predict the benefit of lowering intraocular pressure in normal tension glaucoma. *Am J Ophthalmol* 2003;136:820-829.
11. Leske MC, Heijl A, Hussein M, Bengtsson B, Hyman L, Komaroff E; Early Manifest Glaucoma Trial Group. Factors for glaucoma progression and the effect of treatment: The early manifest glaucoma trial. *Arch Ophthalmol* 2003;121:48-56.
12. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. Collaborative Normal-Tension Glaucoma Study Group. *Am J Ophthalmol* 1998;126:487-497.
13. The advanced glaucoma intervention study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. The AGIS Investigators. *Am J Ophthalmol* 2000;130:429-440.
14. Cheung W, Guo L, Cordeiro MF. Neuroprotection in glaucoma: Drug-based approaches. *Optom Vis Sci* 2008;85:406-416.
15. Vorwerk CK, Lipton SA, Zurakowski D, Hyman BT, Sabel BA, Dreyer EB. Chronic low-dose glutamate is toxic to retinal ganglion cells. Toxicity blocked by memantine. *Invest Ophthalmol Vis Sci* 1996;37:1618-1624.
16. Levin LA. Retinal ganglion cells and neuroprotection for glaucoma. *Surv Ophthalmol* 2003;48 Suppl 1:S21-S24.
17. Kaushik S, Pandav SS, Ram J. Neuroprotection in glaucoma. *J Postgrad Med* 2003;49:90-95.
18. Osborne NN, Chidlow G, Nash MS, Wood JP. The potential of neuroprotection in glaucoma treatment. *Curr Opin Ophthalmol* 1999;10:82-92.
19. Schuettauf F, Quinto K, Naskar R, Zurakowski D. Effects of anti-glaucoma medications on ganglion cell survival: The DBA/2J mouse model. *Vision Res* 2002;42:2333-2337.
20. Hare WA, WoldeMussie E, Lai RK, Ton H, Ruiz G, Chun T, et al. Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, I: Functional measures. *Invest Ophthalmol Vis Sci* 2004;45:2625-2639.
21. Yücel YH, Gupta N, Zhang Q, Mizisin AP, Kalichman MW, Weinreb RN. Memantine protects neurons from shrinkage in

the lateral geniculate nucleus in experimental glaucoma. *Arch Ophthalmol* 2006;124:217-225.

22. Zhong L, Bradley J, Schubert W, Ahmed E, Adamis AP, Shima DT, et al. Erythropoietin promotes survival of retinal ganglion cells in DBA/2J glaucoma mice. *Invest Ophthalmol Vis Sci* 2007;48:1212-1218.

23. Allergan Trials, Memantine in Patients with Chronic Glaucoma; 2008. [Unpublished data only]. Available from: http://www.clinicaltrials.gov/ct2/show/NCT00141882. [Last accessed on 2015 May 20].

24. Allergan Trials, Memantine in Patients with Chronic Glaucoma; 2008. [Unpublished data only]. Available from: http://www.clinicaltrials.gov/ct2/show/NCT00168350. [Last accessed on 2015 May 20].

25. Kersey T, Clement CI, Bloom P, Cordeiro MF. New trends in glaucoma risk, diagnosis and management. *Indian J Med Res* 2013;137:659-668.

26. Osborne NN. Recent clinical findings with memantine should not mean that the idea of neuroprotection in glaucoma is abandoned. *Acta Ophthalmol* 2009;87:450-454.

27. Hare WA, Wheeler L. Experimental glutamatergic excitotoxicity in rabbit retinal ganglion cells: Block by memantine. *Invest Ophthalmol Vis Sci* 2009;50:2940-2948.

28. Gabelt BT, Rasmussen CA, Tektas OY, Kim CB, Peterson JC, Nork TM, et al. Structure/function studies and the effects of memantine in monkeys with experimental glaucoma. *Invest Ophthalmol Vis Sci* 2012;53:2368-2376.

29. Törngren L, Lundgren B, Madsen K. Intraocular pressure development in the rabbit eye after aqueous exchange with ophthalmic viscosurgical devices. *J Cataract Refract Surg* 2000;26:1247-1252.

30. Benozzi J, Nahum LP, Campanelli JL, Rosenstein RE. Effect of hyaluronic acid on intraocular pressure in rats. *Invest Ophthalmol Vis Sci* 2002;43:2196-2200.

31. Moore CG, Milne ST, Morrison JC. Noninvasive measurement of rat intraocular pressure with the Tono-Pen. *Invest Ophthalmol Vis Sci* 1993;34:363-369.

32. Wang X, Tay SS, Ng YK. An electron microscopic study of neuronal degeneration and glial cell reaction in the retina of glaucomatous rats. *Histol Histopathol* 2002;17:1043-1052.

33. Trick GL. Pattern reversal retinal potentials in ocular hypertensives at high and low risk of developing glaucoma. *Doc Ophthalmol* 1987;65:79-85.

34. Korth M, Horn F, Storck B, Jonas J. The pattern-evoked electroretinogram (PERG): Age-related alterations and changes in glaucoma. *Graefes Arch Clin Exp Ophthalmol* 1989;227:123-130.

35. Hare WA, Ton H, Ruiz G, Feldmann B, Wijono M, WoldeMussie E. Characterization of retinal injury using ERG measures obtained with both conventional and multifocal methods in chronic ocular hypertensive primates. *Invest Ophthalmol Vis Sci* 2001;42:127-136.

36. Quigley HA, Green WR. The histology of human glaucoma cupping and optic nerve damage: Clinicopathologic correlation in 21 eyes. *Ophthalmology* 1979;86:1803-1830.

37. Panda S, Jonas JB. Decreased photoreceptor count in human eyes with secondary angle-closure glaucoma. *Invest Ophthalmol Vis Sci* 1992;33:2532-2536.

38. Bringmann A, Pannicke T, Grosche J, Francke M, Wiedemann P, Skatchkov SN, et al. Müller cells in the healthy and diseased retina. *Prog Retin Eye Res* 2006;25:397-424.

39. Mandel SA, Avramovich-Tirosh Y, Reznichenko L, Zheng H, Weinreb O, Amit T, et al. Multifunctional activities of green tea catechins in neuroprotection. Modulation of cell survival genes, iron-dependent oxidative stress and PKC signaling pathway. *Neurosignals* 2005;14:46-60.