Neuroprotective Effects of Memantine on Retinas of Experimental Glaucomatous Rats: Histopathological and Immunohistochemical Analysis*

Deneysel Sıçan Glokomunda Memantinin Retina Üzerindeki Nöron Koruyucu Etkileri: Histopatolojik ve İmmünohistokimyasal İnceleme

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ABSTRACT

Purpose: The present work aimed to investigate the effects of early and late systemic memantine treatment on retinal ganglion cell (RGC) count, retinal layer thickness and retinal apoptotic cell density in a rat model of experimental glaucoma.

Material and Methods: Glaucoma was induced by injecting sodium hyaluronate into the anterior chamber of rats for a period of three weeks. Animals received a single daily dose of 10mg/kg memantine intraperitoneal either starting with the glaucoma induction for a period of six weeks (early phase treatment) or following the glaucoma induction for a period of three weeks (late phase treatment). Glaucoma and control groups were no treated. After sacrification, RGCs and immunohistochemically stained apoptotic cells were counted and retinal thickness were measured.

Results: We observed that retinal thickness was preserved significantly in memantine treated animals. Similar results were seen when RGC counts compared. When started with glaucoma induction, memantine treatment significantly reduced RGC loss. Even started in the late phase of glaucoma, memantine treatment seemed to preserve the number of RGCs significantly. In nontreated animals widespread apoptotic cells were observed both in RGC layer and inner nuclear layer, whereas the intensity of apoptotic cells were found to be significantly less in memantine treated groups.

Conclusions: The results of the present study suggest that memantine may act as an effective neuroprotective agent in retinal injury associated with experimental glaucoma. Also it seems possible that the neuroprotective effect of memantine is much significant when administrated in the early phase of glaucoma.

Key Words: Glaucoma, memantine, neuroprotection, rat, retina.

ÖZ

Amaç: Bu çalışmanın amacı deneysel glokom oluşturulan sıçanlarda erken ve geç dönem sistemik memantin uygulamasının etkilerinin retinal ganglion hücre sayımı (RGH), retina kalınlık ölçümü ve apoptotik hücre sayımı metodları ile araştırılmasıdır.

Gereç ve Yöntem: Üç hafta süresince sıçanların ön kamaralarına sodyum hyaluronat enjeksiyonu uygulanarak glokom oluşturuldu. Erken memantin grubuna glokom indüksiyonu ile birlikte 10mg/kg memantin intraperitoneal başlandı, 6 hafta süresince verildi. Geç memantin grubunda ise glokom indüksiyonunun 3. haftasında tedavi başlandı, 3 hafta süresince memantin intraperitoneal yolla verildi. Glokom ve kontrol gruplarına tedavi uygulanmadı. Sakrifikasyon sonrası RGH ve TUNEL boyalı apoptotik hücreler sayıldı, retina kalınlığı ölçüldü.

Bulgular: Memantin gruplarında retina kalınlığının anlamlı düzeyde korunduğu gözlendi. Benzer sonuçlar RGH sayımlarında da görüldü. Glokom indüksiyonu ile birlikte başlandığında memantin tedavisinin RGH kaybını anlamlı düzeyde azalttığı izlendi. Geç memantin grubunda ise RGH sayısının anlamlı düzeyde korunduğu gözlendi. Tedavisiz deneklerde yaygın apoptotik hücreler hem RGH katında hem de iç nükleer tabakalarda izlenirken apoptotik hücrelerin yoğunluğu memantin tedavi gruplarında anlamlı düzeyde az bulundu.

Sonuç: Memantin deneysel glokom ilişkili retinal hasarda nöroprotektif etkinlik gösterebilmektedir. Ayrıca glokomatöz sürecin erken döneminde başlandığında memantinin nöroprotektif özelliğinin daha belirgin olduğu gözlenmiştir.

Anahtar Kelimeler: Glokom, memantin, nöroproteksiyon, retina, sıçan.

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INTRODUCTION

Glaucoma is a kind of progressive optic neuropathy with characteristic optic nerve head appearance and related visual field loss changes and occurs more frequently than other optic neuropathies.[1,2] It has been reported that characteristic pathologic changes in glaucomatose retina are decrease in retinal thickness, and significant retinal ganglion cell death.[3-5]

Progression in glaucoma usually results in vision loss because of the insufficiency of the current treatments. The vision loss in glaucoma has been accepted as the result of retinal ganglion cell (RGC) death.[6] The best known and curable risk factor in glaucoma is intraocular pressure increase hence the glaucoma treatment is mainly focused on intraocular pressure decrease today. Because glaucoma is not only a situation of intraocular pressure increase, but also a neurodegenerative disease, glaucoma may show progression despite achieving decreasement in intraocular pressure.[1,6-7]

In several studies, high glutamate levels has been reported in glaucoma similar to high glutamate levels shown in retinal ischemia-reperfusion states. Glutamate-dependent excitotoxicity resulting from N-methyl D- Aspartate (NMDA) receptor hyperactivation and apoptosis-mediated cell death are thought to be the two main mechanisms which may play role in RGC loss in glaucoma.[8-12]

Recently, excitotoxic mechanism became an attractive target for neuroprotective efforts in glaucoma related neuronal damage. After various ischemic and traumatic retinal injury models supported the exicotoxic theory about RGC death in glaucoma, neuroprotective effectivity of various NMDA receptor antagonists in experimental glaucoma models has been demonstrated.[13,14]

In the present study memantine (1-adamantanamine hydrochloride), a noncompetitive NMDA receptor antagonist, is used systemically in an experimental glaucoma model in the rat. The effect of early and late systemic memantine treatment on retinal ganglion cell count, retinal layer thickness and retinal apoptotic cell density was investigated histopathologically and immunohistochemically.

MATERIAL AND METHODS

Animals

Young adult male Wistar albino rats (mean weight 220±40 gram) were used in the study. The rats were allowed free access to water and standart laboratory chow, maintained on a 12 hours light (06:00-18:00); 12 hours dark cycle and housed under controlled conditions of temparature (21±2 °C) and humidity.

Ten rats served as control (C) group and no surgical intervention was made in these animals. In 55 rats glaucoma induction was performed and the rats were grouped as follows:

1) Early period glaucoma (EPG) group: 10 rats which received no treatment and were sacrificed at the end of the third week following glaucoma induction.

2) Late period glaucoma (LPG) group: 15 rats which received no treatment and were sacrificed at the end of the sixth week following glaucoma induction.

3) Early period memantine (EPM) group: 15 rats which received 10 mg/kg/day intraperitoneal memantine treatment starting with glaucoma induction and were sacrificed at the end of the sixth week following glaucoma induction.

4) Late period memantine (LPM) group: 15 rats which received 10 mg/kg/day intraperitoneal memantine treatment starting three weeks after glaucoma induction and were sacrificed at the end of the sixth week following glaucoma induction.

In the experiment design, the reason of forming EPG group was to reveal the mean remaining RGC count to search the potential protective effect of memantine on those cells at the end of the 3rd week in LPM group. No drugs were applied to glaucoma induced groups (EPG, LPG). Intraocular pressures were recorded before and after the procedures during the 3 weeks of glaucoma induction and weekly in the later 3 weeks. EPG group was sacrificed at the end of the 3rd week while other groups were sacrificed at the end of the 6th week.

Glaucoma Model

Rats were anesthesized with intraperitoneal ketamine hydrochloride (15 mg/kg) xylazin hydrochloride (0.3 mg/kg) and asepromazin (1.5 mg/kg). 0.03-0.05 ml of sodium hyaluronate (23 mg/ml) were injected into anterior chamber of the right eyes of the rats via a 30 Gauge needle. Topcon OMS 75 (Topcon Europe BV) was used as surgical microscope. Injections were made trough corneoscleral limbus into anterior chamber.[15] Glaucoma induction was achieved by repeating weekly injections for 3 weeks. Intraocular pressures were recorded before and after the procedures during the 3 weeks of glaucoma induction and weekly in the later 3 weeks. Intraocular pressure was measured using tonometer (Tono-Pen; Medtronic Solan XL). Rats were sedated with 3.0-mg/kg IM acepromazine during IOP measurements. Proparacaine 0.5% was applied topically on the eyes to anesthetize the cornea. As tip of Tono-Pen probe contacted cornea, the mean value of 5 consecutive automatic measurements, done by the microprocessor inside the device, was recorded as the intraocular pressure value.

Retinal Ganglion Cell Count and Retinal Thickness Measurement

4 μm thick sections cut from paraffine blocks of retina were placed on to poly-L-lisine coated lammel and kept in incubator at 45 °C overnight. After dehidration with graded alcohol series and clearing with toluol, sections were placed into water and stained with routine hematoxyline-eosine and 1% cresyl violet for gang-

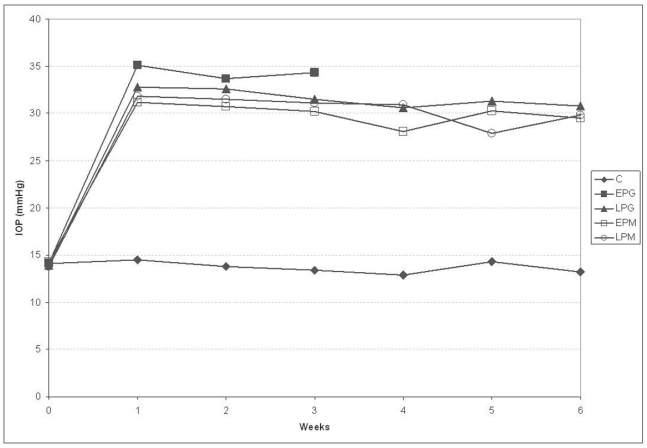
Table: Mean values (± standard error of mean) of retinal ganglion cell (RGC) counts, retinal layer thickness (RT) and apoptotic cell counts of all groups are shown. RGC counts and RT measurements in EPG, LPG, EPM and LPM groups were also calculated as percent loss compared with the control group and are shown in parenthesis.

Groups	Mean Retinal Ganglion Cell Count (number of cells/mm2)	Mean Retinal Layer Thickness (micrometers)	Mean Apoptotic Cell Count (number of cells/mm2)
C *(n=10)*	**1787,70 ± 8,94**	**91,40 ± 3,56**	**0,42 ± 0,34**
EPG *(n=10)*	**1566,90 ± 26,77** *(12,36% ± 1,49)*	**70,30 ± 2,26** *(23,09% ± 2,47)*	**2,59 ± 0,44**
LPG *(n=15)*	**1320,40 ± 18,05** *(26,14% ± 1,00)*	**63,46 ± 3,50** *(30,57% ± 3,82)*	**4,14 ± 0,42**
EPM *(n=15)*	**1765,46 ± 14,35** *(1,25% ± 0,80)*	**87,86 ± 3,06** *(3,88% ± 3,34)*	**2,18 ± 0,42**
LPM *(n=15)*	**1527,53 ± 68,57** *(14,56% ± 4,65)*	**68,38 ± 3,17** *(25,19% ± 3,46)*	**2,87 ± 0,33**

C: Control Group, EPG: Early Period Glaucoma Group, LPG: Late Period Glaucoma Group, EPM: Early Period Memantine Group, LPM: Late Period Memantine Group.

lion cell counting. Morphologically identifable glial cells according to their shape and size and vascular endothelial cells were not counted. RGCs containing Nissl bodies were screened under microscope (Olympus BX 51, Japan). Microphotographic images obtained by SPOT Insight Firewire camera were analyzed using SPOT Advanced Software Windows: Version 4.1. RGCs per area (mm^2) were counted by two double-blinded researchers. Retinal thickness were measured using the same digital analyze system.

Apoptosis Anaylze with TUNEL (deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling) Method



Graphic 1a: Time distribution of mean intraocular pressure (IOP) values for normal rat eyes (C), non-treated glaucomatous eyes (EPG and LPG) and memantine treated glaucomatous eyes (EPM and LPM) is shown. The IOP values of both treated and non-treated groups are obviously higher than those of the control group at each time point after glaucoma induction ($P<0.05$). 10 mg/kg memantine treatment, whether started in the early phase or late phase of glaucoma caused no effect on IOP.

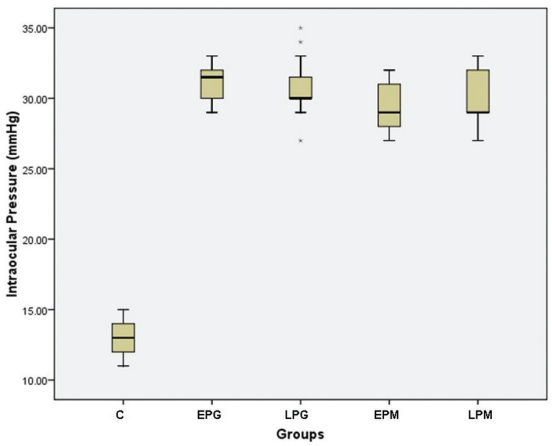
4 μm thick sections were obtained from paraffine embedded retina blocks starting from the optic nerve head region. In situ cell death detecting kit (Roche Applied Science, Germany) was used for immunohistochemical staining. After washing with phosphate-buffered salin solution (PBS), endogenous peroxidase was blocked by 3% hydrogen peroxide containing methanole for 15 minutes. Sections were then kept in room temparature for 30 minutes in Proteinase K solution (in 10 mM tris/HCL), washed in PBS, and incubated for 1 hour at 37 °C in a mixture of solution containing an enzyme (deoxinucleotidyl transferase) and a marker. Following incubation, sections were washed in PBS and photographed with fluorescence microscope under 540 nm green filter. Later, sections were processed with converter-POD, marked using diaminobenzidin or AEC choromogens, counter-stained with Mayer's hematoxyline, and examined with light microscope after closure. TUNEL positive apoptotic cells were counted under microscope by two double-blinded researchers. Microphotographic images obtained by SPOT Insight Firewire camera and were analyzed using SPOT Advanced Software for Windows (Version 4.1).

Statistical Analysis

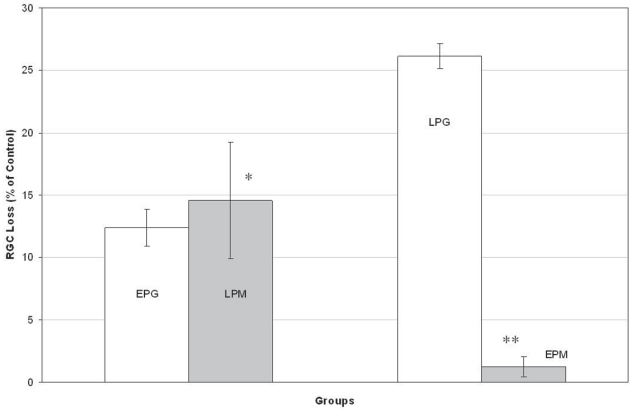
The data are expressed as mean±SEM. Kruskal-Wallis test was used for analysing the variances and Mann-Whitney U test was used for comparing two groups. P values less than 0.05 were considered to be statistically significant.

RESULTS

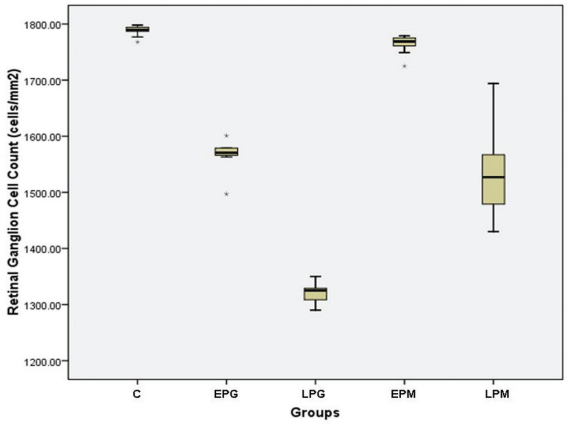
Mean values of retinal ganglion cell (RGC) counts, retinal thickness (RT) measurements and apoptotic cell (AC) counts are summarized in Table. RGC counts and RT measurements in memantine treated or non-treated glaucoma induced groups were calculated as percent loss compared with the control group and are also shown in Table.



Graphic 1b: Intraocular pressure (IOP) values for normal group (C), non-treated groups (EPG and LPG) and memantine treated groups (EPM and LPM) are shown in box-plot graphics.



Graphic 2a: Neuroprotective effect of memantine on retinal ganglion cells in glaucoma induced rats. When started with glaucoma induction (EPM Group), memantine treatment significantly reduced retinal ganglion cell (RGC) loss compared to non-treated rats (LPG) at the end of the sixth week (**p<0.05). The ratio of RGC loss after the first 3 weeks without treatment (EPG group) was compared to the ratio of RGC loss in LPM group which received treatment with memantine for three weeks thereafter. The difference was found to be insignificant (*p>0.05) which meant that memantine treatment significantly prevented further cell loss when started 3 weeks after glaucoma induction. Values are expressed as mean±SEM.



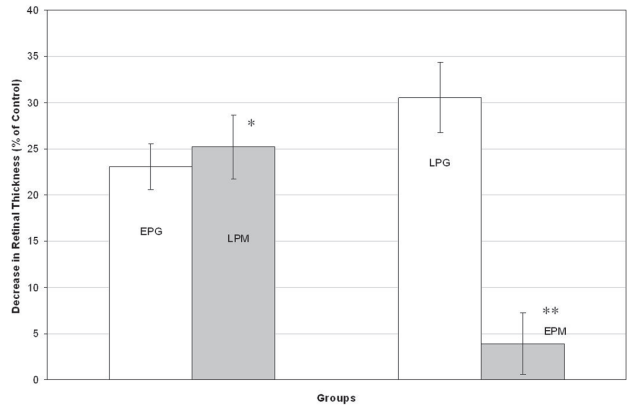
Graphic 2b: Retinal ganglion cell counts for normal group (C), non-treated groups (EPG and LPG) and memantine treated groups (EPM and LPM) are shown in box-plot graphics.

Intraocular Pressure Measurements

Time distribution of mean intraocular pressure (IOP) values for each group is shown in graphic 1. The mean IOP values of both memantine treated (EPM and LPM) and non-treated (EPG and LPG) groups remained obviously higher than those of the control group after glaucoma induction process has started (P<0.001). Memantine treatment, whether started in the early phase or late phase of the experimental glaucoma caused no effect on IOP.

Retinal Ganglion Cell Count

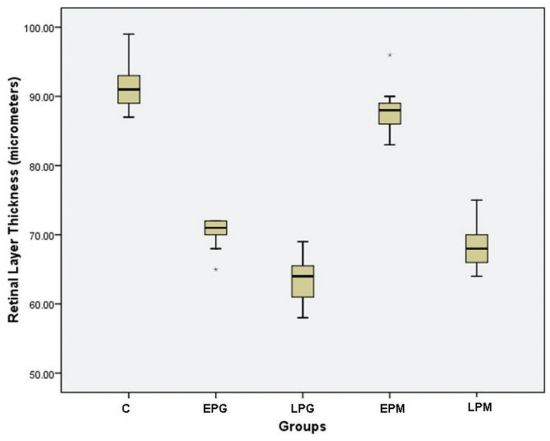
When started with glaucoma induction (EPM Group), memantine treatment significantly reduced retinal ganglion cell (RGC) loss as compared to non-treated rats (LPG) at the end of the sixth week. EPM group showed 1.25%±0.8% RGC loss in proportion to control group. This rate was 26.14%±1.0% in LPG group and the difference was found to be statistically significant. The rate of RGC loss after the first 3 weeks was calculated as 12.36%±1.49% (EPG group). Treatment with memantine for 3 weeks thereafter (LPM group) significantly prevented further cell loss. The rate of RGC loss in LPM group was calculated as 14.56%±4.65% of control animals and when compared to EPG group the difference was not statistically significant. The results concernning RGC counts are shown in graphic 2.



Graphic 3a: Protective effect of memantine on retinal thickness (RT) in glaucoma induced rats. When started with glaucoma induction (EPM Group), memantine treatment significantly preserved RT compared to non-treated rats (LPG) at the end of the sixth week (** p<0.05). The ratio of RT loss after the first 3 weeks without treatment (EPG group) was compared to the ratio of RT loss in LPM group which received treatment with memantine for three weeks thereafter. The difference was not significant (* p>0.05) which indicated that memantine treatment significantly preserved RT when started 3 weeks after glaucoma induction. Values are expressed as mean±SEM.

Retinal Thickness Measurements

In EPM group, in which memantine treatment was started with glaucoma induction, retinal thickness (RT) was found to be significantly preserved as compared to non-treated rats (LPG) at the end of the sixth week. EPM group showed 3.88%±3.34% of decrease in RT. This rate was 30.57%±3.82% in LPG group and the difference was found to be statistically significant. The rate of RT decrease after the first 3 weeks was calculated as 23.09%±2.47% (EPG group). Treatment with memantine for 3 weeks thereafter (LPM group) significantly prevented further decrease in RT. The rate of RT decrease in



Graphic 3b: Retinal layer thickness values for normal group (C), non-treated groups (EPG and LPG) and memantine treated groups (EPM and LPM) are shown in box-plot graphics.

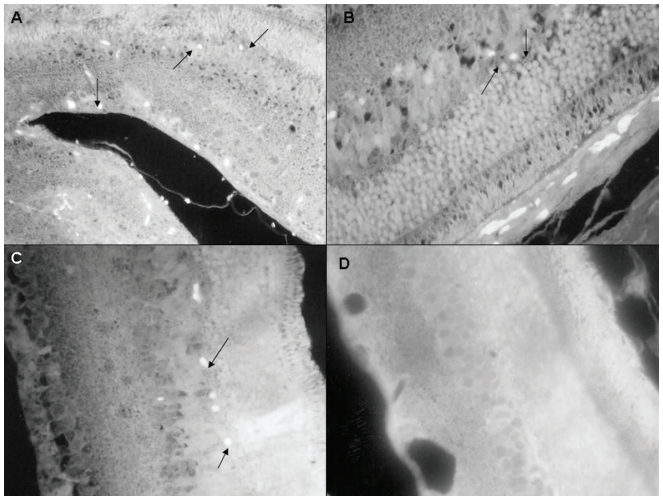


Figure 2: TUNEL (deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling) marked fluorescence microscopic images of the groups are shown in 6A through 6D. 2A: An example for "late period glaucoma group" is viewed under X400 magnification. Numerous apoptotic cells (arrows) are scattered throughout the retinal layers. 2B: An example for "late period memantine group" is viewed under X400 magnification. Apoptotic cells (arrows) are seen internal nuclear layer. 2C: An example for "early period memantine group" is viewed under X400 magnification. Apoptotic cells (arrows) are seen both in internal and outer nuclear layers. Retinal ganglion cell layer seems to be preserved. 2D: An example for "control group" is viewed under X400 magnification. No apoptotic cell is observed.

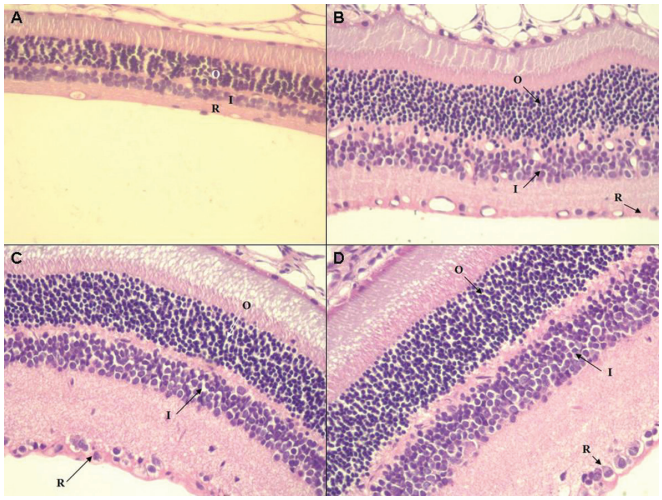
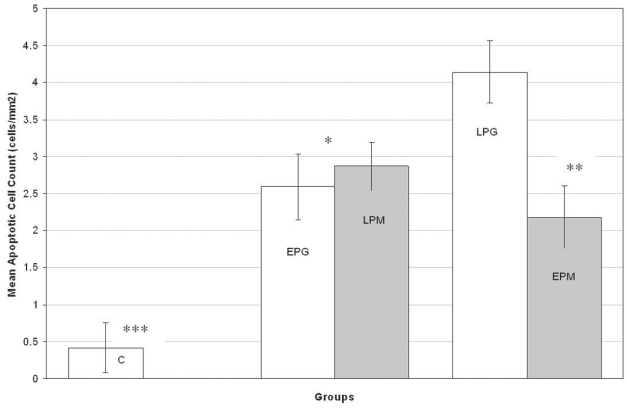


Figure 1: Histological photographs demonstrating retinal layers of the groups are shown in 1A through 1D. All examples were stained with hematoxyline-eosine and viewed under X400 magnification. I: Internal nuclear layer, O: Outer nuclear layer, R: Retinal ganglion cell layer. 1A: An example for "late period glaucoma group". Atrophic changes in retinal layers and excessive decrease in number of retinal ganglion cells are seen. Also separations of the cells are observed both in internal nuclear layer and outer nuclear layer. 1B: An example for "late period memantine group". Moderate thinning in retinal layers and a moderate decrease in number of retinal ganglion cells are observed. There is no significant separation of the cells in internal and outer nuclear layers. 1C: An example for "early period memantine group". Thicknesses of the internal and outer nuclear layers seem to be preserved. A slight disorganization is observed in retinal ganglion cell layer but there is no significant decrease in number of retinal ganglion cells. 1D: An example for "control group". Normal appearance of the internal and outer nuclear layers and the normal organization of the retinal ganglion cell layer are seen.

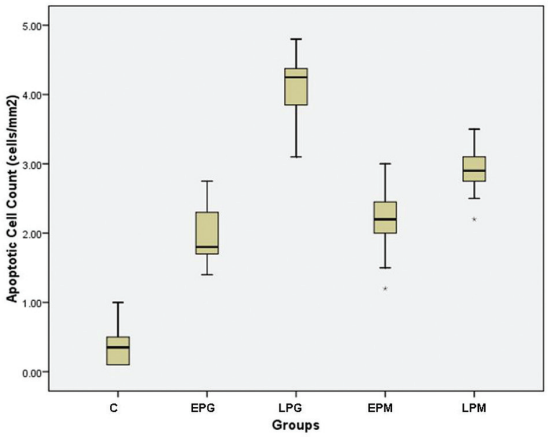
LPM group was calculated as 25.19%±3.46% of control group and when compared to EPG group the difference was not significant. The results are summarized in graphic 3. Histological photographs demonstrating retinal layers of the groups are shown in Figure 1.

Apoptosis Evalution

All glaucoma induced groups were found to have significantly higher AC counts compared to control group (0.42±0.34). At the end of the sixth week, mean AC count value of the non-treated LPG group (4.14±0.42) was found to be significantly higher than the mean AC count value of EPM group (2.18±0.42) in which memantine treatment has been started with glaucoma induction (p<0.001). As the mean AC counts of EPG group (2.59±0.44) and LPM group (2.87±0.33) were compared, the difference was not significant which indicated that memantine treatment significantly prevented further increase in AC population when started 3 weeks after glaucoma induction. The results concerning AC counts are shown in graphic 4. TUNEL marked fluorescence microscopic images of the groups are shown in Figure 2.



Graphic 4a: The results concerning apoptotic cell (AC) counts are shown. All glaucoma induced groups were found to have significantly higher AC counts compared to control group. At the end of the sixth week, mean AC count value of the non-treated LPG group was found to be significantly higher than the mean AC count value of EPM group in which memantine treatment has been started with glaucoma induction (** p<0,05). As the mean AC counts of EPG group and LPM group were compared, the difference was not significant (* p>0, 05) which indicated that memantine treatment significantly prevented further increase in AC population when started 3 weeks after glaucoma induction. Values are expressed as mean±SEM.



Graphic 4b: Apoptotic cell counts for normal group (C), non-treated groups (EPG and LPG) and memantine treated groups (EPM and LPM) are shown in box-plot graphics.

DISCUSSION

Glaucoma is described as an optic neuropathy leading to vision loss by damaging inner layers of retina and causing retinal ganglion cell (RGC) death with characteristic optic nerve head changes.[16] Revealing the underlying pathology is necessary to find the ideal treatment strategy. Randomized controlled clinical trials showed that disease progression occurs in some patients despite successful intraocular pressure control. As well as normotensive glaucoma patients, there are cases suffering vision loss despite decreased intraocular pressure.[17-19] These data direct most researchers to develop new treatment strategies in addition to the classic glaucoma treatment focusing on decreasing intraocular pressure. Today most of the popular studies about glaucoma are devoted to prevent neuronal loss.[20,21] The basic opinion about the pathophysiology of neuronal loss in glaucoma is necrosis and/or apoptosis-mediated cell death. There are several possible mechanisms of neurodegeneration in glaucoma, including a reduction in the transport of neurotrophic factors, induction of neuronal nitric oxide synthesis, and excitotoxicity due to extracellular glutamate.[22-26]

As concentration of vitreal glutamate has been shown to be elevated in humans[10] and dogs[12] with primary glaucoma, as well as in monkeys[10] with experimentally induced glaucoma, the excitotoxicity process have become a target for neuroprotective studies in glaucoma. Glutamate-induced neuronal death has been shown to begin with uptake of excess calcium into the cell by activated N-methyl D-Aspartat (NMDA) type glutamate receptors.[27,28] The presence of NMDA receptors in the retinal ganglion cells makes them especially vulnerable to glutamate toxicity.[14] In a recent study Kim et al.: demonstrated up-regulation of NMDA receptor 1 expression in some RGCs in a rat model of chronic ocular hypertension and they concluded that the excessive stimulation of NMDA receptor 1 by glutamate is involved in causing the death of RGCs in glaucoma.[26]

As glutamate-mediated excitotoxic neuronal damage in glaucoma has become a popular topic, the neuroprotective effect of glutamate antagonists, especially memantine (1-amino-3.5-dimethyladamantane), became one of the most frequently discussed subjects in glaucoma treatment.[29,30] The results of several studies suggest that memantine, an NMDA receptor antagonist, may provide a safe and effective treatment for reduction of any NMDA-type glutamatergic contribution to glaucomatous injury of retinal neurons.[14,21,26,31]

The present work aimed to investigate the effects of early and late systemic memantine treatment on retinal ganglion cell count, retinal layer thickness and retinal apoptotic cell density in a rat model of experimental glaucoma. The results of this study indicated that memantine treatment in the early and late phases of glaucoma yielded no significant effect on intraocular pressure. The data of the present study showed that retinal thickness was significantly preserved in memantine treated animals and memantine treatment provided an almost normal structure when started in the early phase of glaucoma. Similar results were seen when RGC counts compared. When started with glaucoma induction, memantine treatment significantly reduced RGC loss. Even started in the late phase of glaucoma, memantine treatment seemed to preserve the number of RGCs significantly. WoldeMussie et al.: have reported that daily 10 mg/kg memantine reduced RGC loss to 12% when applied immediately after first glaucoma induction, and prevented any further loss when applied ten days after first glaucoma induction.[14] The data of the present study seems to support these results.

The protective effect of memantine on RGC cell and retinal thickness in glaucomatous retina has been explained with the presence of NMDA receptors especially in RGCs[33] It is thought that NMDA receptors in RGCs made these cells defenseless and vulnerable to glutamate toxicity.[33-37] The findings of the present study, which seemed to verify the protective effect of memantine on RGC counts and on retinal layer thickness, can be explained in the same manner.

It is well known that exitotoxic neuron loss is not limited with RGCs. Response of cell groups comprising retinal layers to exitotoxic damage may be understood better with excitotoxicity-induced apoptosis investigation. In the present study TUNEL method was used for this purpose. TUNEL positive (i.e. apoptotic) cells were seen in all but most significantly in EPG and LPG groups. The presence of significant apoptotic cell density both in RGC and inner nuclear layers were noted. These results are in consistence with Sisk and Kuwabara's study in which significant damage has been reported both in RGC and inner nuclear layers following intravitreal injection of monosodium L-glutamate in rats.[38]

In a recent study, it was reported that memantine was effective in reversing acute experimental excitotoxicity at concentrations that have little effect on retinal light signaling. This study was designed to characterize the retinal response to experimental manipulations that mimic features of glutamatergic excitotoxic insult and also to determine whether memantine, an NMDA-type glutamatergic channel blocker, is effective in reversing experimental excitotoxicity. These results support a conclusion that memantine may provide a safe and effective treatment for retinal disorders associated with excessive activity of NMDA-type glutamatergic channels. The data of the our study (especially EM group) seems to support these results.[39]

The significant decrease in TUNEL positive cells in memantine treated groups can be interpreted as memantine has a possible role in protecting ganglion cell layer and inner nuclear layer from exitotoxic apoptosis. According to our data we suggest that early memantine treatment may have a significant role in preventing apoptosis in glaucoma-induced neuron damage. Our results also showed that memantine treatment, when started in the late phase of glaucoma, protected the remaining cells

from further apoptosis. The results of the present study suggest that NMDA receptor antagonist memantine may provide an effective neuroprotection in retinal injury associated with experimental glaucoma without any significant impact on intraocular pressure. Our data indicate that the beneficial effects of memantine would be more significant when given earlier in glaucoma. All these results seem to support the idea that glutamate-induced excitotoxicity may play a significant role in glaucoma induced neuronal injury. Also, the presence of considerable apoptotic cells, which seems to be significantly reduced by memantine treatment, suggests that cell death in glaucoma mainly occurs via apoptosis under NMDA-type glutamatergic contribution.

In summary, the present work indicates that memantine may provide a safe and effective treatment for reduction of neuronal injury in glaucomatose retina. However, it seems possible that the neuroprotective effect of memantine is much significant when administrated in the early phase of glaucoma process.

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