Original Article

Investigation of the Neuroprotective Effect of Riluzole on Matrix Metalloproteinases in an Experimental Model of Glaucoma

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Abstract

Purpose: To investigate the neuroprotective effect of riluzole through matrix metalloproteinase-2 (MMP-2) and MMP-9 in rats, in an experimental glaucoma model.

Methods: A total of 28 *Wistar albino* rats, 3–4 months old, weighing 250–300 g, were divided into four groups; Group I (control), Group II (glaucoma), Group III (glaucoma + corn oil + dimethyl sulfoxide (DMSO), and Group IV (glaucoma + corn oil + DMSO + riluzole). A glaucoma model was created by episcleral vein cauterization in the left eyes of Group II, III, and IV subjects. After the formation of the model, daily intraperitoneal riluzole (5 mg/kg) administration was started. At the end of 7 weeks, retinal tissues were taken and some parts of them were stained with hematoxylin and eosin for histopathological examinations. In the other part, MMP-2 and MMP-9 expressions were determined using real-time quantitative PCR and immunohistochemical techniques.

Results: As a result, a statistically significant increase in intraocular pressures (IOP) was found in Groups II, III, and IV when compared with the control eyes after 7 weeks ($P < 0.001$). There was a decrease in IOP in the riluzole group compared to the glaucoma group. Expression levels in both genes decreased slightly with riluzole administration. In the histopathological evaluation of the groups, it was observed that there were no significant differences between the findings of degeneration in ganglion cells, hemorrhage, and differentiation in layers.

Conclusions: MMP-2 and MMP-9 exhibited decreased expression levels in both Group III (glaucoma + corn oil + DMSO) and Group IV (glaucoma + corn oil + DMSO + 5 mg/kg riluzole) compared to the glaucoma groups in Group II. Given that both Group III and Group IV received injections of corn oil + DMSO, this conclusion was drawn.

Keywords: Glaucoma, Intraocular pressure, Matrix metalloproteinase-2, Matrix metalloproteinase-9, Neuroprotection, Riluzole

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Submitted: 25-Dec-2023; **Revised:** 11-Apr-2024; **Accepted:** 13-Apr-2024; **Published:** 18-Jan-2025

Introduction

Glaucoma is one of the leading causes of vision loss and is a disease characterized by progressive degeneration of optic nerve fibers and retinal ganglion cells (RGCs).[1,2] It is estimated that there are approximately 80 million people diagnosed with glaucoma all over the world, and this number will increase to over 110 million by 2040.[3] In addition, it should be taken into account that there are many more undiagnosed glaucoma patients than those diagnosed and that visual impairments and losses will increase with the increase in the average age.[4] The most important risk factor for glaucoma is high intraocular pressure (IOP).[2] Since the nerve cells do not regenerate, it is not possible to reverse the optic nerve damage due to glaucoma

Access this article online

Quick Response Code:

Website: https://journals.lww.com/joco

DOI: 10.4103/joco.joco_290_23

How to cite this article: Yildiz F, Sahinoglu-Keskek N, Yilmaz IT, Candan İA, Korkmaz OT. Investigation of the neuroprotective effect of riluzole on matrix metalloproteinases in an experimental model of glaucoma. J Curr Ophthalmol 2024;36:136-44.

with current treatment methods. Treatment modalities such as standard therapy, medication, or surgery all focus on reducing IOP. Current treatments are aimed at preventing the disease from causing further damage and increasing the quality of life of individuals from the moment of diagnosis of glaucoma.[5,6] Although currently, available medical treatment options can lower IOP, the progression of glaucoma still cannot be completely prevented today. For these reasons, neuroprotective compounds recently approved for neurodegenerative diseases have been the focus of extensive reviews for glaucoma.[7]

Although increased IOP is still known as the most important risk factor in the development of optic nerve damage, it has been thought that different mechanisms play a role in the etiopathogenesis of glaucoma in recent years.[8] One of these mechanisms is the extracellular matrix (ECM) and its components. The ECM is a dynamic structure that undergoes constant remodeling to maintain cellular and tissue homeostasis.[9] It plays a crucial role in all parts of the eye, such as maintaining the clarity and hydration of the cornea and vitreous, IOP maintenance, regulation of angiogenesis, and vascular signaling.[10] Matrix metalloproteinases (MMPs) are a group of enzymes mostly responsible for the degradation of the ECM.[11] MMPs are involved in the protection and remodeling of the ocular structure by affecting a wide variety of processes such as basement membrane remodeling, neovascularization, and blood–retina barrier integrity and are found in almost all areas of the eye.[12,13] It is also believed that MMPs play a role in the pathophysiology of many ocular diseases, including age-related macular degeneration, diabetic retinopathy, and glaucoma. MMP-2 and MMP-9 from the MMP enzyme family play an important role in the pathogenesis of neurodegenerative diseases and the protection of the blood–brain barrier by destroying the endothelial basement membrane components. MMP activity affects IOP control, which is one of the pathological mechanisms of glaucoma.[13] In glaucoma, increased MMP activity in target intraocular tissues causes ECM remodeling, resulting in a decrease in IOP.

Neuroprotective compounds approved for neurodegenerative diseases have been the focus of extensive reviews for glaucoma. One of these drugs is riluzole, and its neuroprotection mechanism has not yet been fully elucidated. Riluzole (2-amino-6-(trifluoromethoxy) benzothiazole) is a neuroprotective agent that inhibits glutamate release by blocking voltage-dependent Na^+ channels. Because of this feature, it is used in amyotrophic lateral sclerosis (ALS) disease and reduces the progression of motor neuron damage. It has been used in our country for about 15 years.[14] Previous studies have shown the protective effects of riluzole against ischemic damage in myorelaxant, psychotropic, anticonvulsant, sedative, hypnotic, anesthetic, hypothermic, global ischemia models, and neuroprotective effects against anoxia or excitotoxic damage.[15]

Riluzole may be useful in the treatment of glaucoma, as glaucoma displays the pathophysiological features of both chronic and neurodegenerative diseases. Within the literature information we could reach, no study was found that investigated the effect of riluzole on MMP-2 and MMP-9 gene expression in a glaucoma model. Therefore, the present study aimed to investigate the efficacy of riluzole in an experimental glaucoma model through MMP-2 and MMP-9 gene expression.

Methods

The study was conducted with the permission of Eskisehir Osmangazi University Animal Experiments Local Ethics Committee (HADYEK) with permission number 876/2021. All experimental animals were obtained from Anadolu University Experimental Animals Research and Application Unit production laboratory. In the study, 28 *Wistar albino* male rats, healthy, 3–4 months old, weighing 250–300 g, were used. The animals were kept alive at a temperature of 21°C ± 2°C with a 12-h light/dark cycle and 50% ± 5% humidity throughout the experiment. In addition, animals were given standard commercial rat pellets and tap water.

Rats were randomly divided into four groups, each of which had 7 rats. Group I (control): distilled water was injected intraperitoneally (i.p) for 40 days. Group II (glaucoma): after the glaucoma model was created in this group of experimental animals, no drugs were administered. The rats were sacrificed 7 weeks after glaucoma induction. Group III (glaucoma + corn oil + dimethyl sulfoxide [DMSO]): with the induction of glaucoma in experimental animals in this group, 2 ml corn oil + DMSO was given i.p simultaneously for 40 days and was sacrificed in the 7th week. Group IV (glaucoma + corn oil + DMSO + 5 mg/kg riluzole): with the induction of glaucoma in experimental animals in this group, 5 mg/kg/day riluzole was given i.p simultaneously for 40 days and was sacrificed in the 7th week.

Riluzole drug administration is started simultaneously with the onset of glaucoma induction, and at the same time every day (12:00–1:00 PM), a single dose of 5 mg/kg/day riluzole hydrochloride (MedChem Express, Cat no. HY-B0211A) is applied. It was dissolved in 90% DMSO + 90% corn oil and given by intraperitoneal injection. A total of 40 doses of riluzole were administered to this subject group until they were sacrificed at week 7.

Subjects were injected intramuscularly with a mixture of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). The glaucoma model was created by episcleral vein cauterization. Since it was planned to apply cauterization (Electromag M20-80 Cauter Device, Bipolar) to the left eyes of all subjects, the right-side position was given, and the subjects' necks were supported and carefully placed under the microscope. After the plate required for cauterization was placed under the heads of the subjects, 0.5% proparacaine hydrochloride (ALCAINE® ophthalmic solution, Alcon Laboratories, Inc., TX, USA) was dripped for topical anesthesia and three (two dorsal and one lateral) of the four episcleral veins in the left eyes were cauterized under sterile conditions. The right eye was used

as an internal control.[16] To protect the eye from infection, it was washed with povidone-iodine before the injection, and topical antibiotic (Moxifloxacin 0.5%) and 0.5% proparacaine hydrochloride were applied as a postoperative analgesic after the procedure. In addition, artificial tears in gel form were applied to the opposite eye during the procedure to prevent drying. By measuring IOP on the 1st day after the injection and then every 3 days, the model was considered successful if it was at least 5 mmHg above the contralateral eye (not exceeding 60 mmHg) and this elevation persisted for 7 days.[17]

Before experiments, all animals were kept in low light of 40–60 lux (measured with Sper Scientific) to avoid IOP changes. For IOP measurement, topical anesthesia with 0.4% proparacaine hydrochloride 0.5% (Alcaine 0.5%) was applied to both eyes of all animals, and a Tono-Pen® Avia (Reichert Inc., Depew, USA) tonometer device measurement was made. All tonometric measurements were performed at the same time (12:00–2:00 PM) weekly, and the average of 3 measurements was recorded as a single data.

Isolation was performed according to the manufacturer's instructions, using the appropriate commercial kit (GeneAll® Hybrid-RTM) for RNA isolation. After measuring the purity of the isolated RNAs with the ELISA Plate Reader (260/280 nm = 1.8–2.1), total RNA samples were stored at −80°C.

Gene expression levels of selected genes were determined by real-time quantitative PCR (RT-qPCR) amplification of cDNAs synthesized complementary to mRNAs resulting from transcription. Obtained RNAs were measured with an ELISA Plate Reader at a wavelength of 260 nm and microgram values per microliter were determined. Using random hexamers as primers, cDNA synthesis was performed from total RNA with cDNA synthesis kit (A.B.T.™ cDNA Synthesis Kit Cat no. C03-01-20).

Relative gene expression RT-qPCR experiments with primers suitable for MMP-2, MMP-9, and housekeeping gene determined beta-actin and RT-qPCR master mix kit (A.B.T.™ 2X miRqGreen Master Mix, Cat no. Q04-02-05) carried out. The results were calculated using the Ct (Cycle threshold) method according to the $2^{-\Delta\Delta Ct}$ formula and their graphics were prepared.

Retinal tissues taken from the eye for histological studies were placed in 10% buffered formaldehyde and kept for 24 h for fixation. After routine tissue follow-up procedures, 4–5 µm thick sections were taken with a microtome (Leica RM 2025) from the blocks that were embedded in paraffin. Then, hematoxylin and eosin staining was done, and the preparations were made permanent by sealing with entellan. The sections were examined, evaluated, and photographed using the ZEISS AX10 (Axicocam ERc5s) computer-assisted binocular microscope imaging system.

Tissue sections taken from the experimental groups were deparaffinized in xylene, passed through a decreasing alcohol series, and kept in distilled water for 5 min. The tissue was treated with ethylenediamine tetraacetic acid buffer solution in a microwave oven to expose the receptor sites closed by formaldehyde. After the sections were cooled at room temperature, the tissues on the section were scratched with a pappen. After washing the tissues with phosphate buffered saline (PBS) for 3 × 5 min, they were blocked in Ultra V block solution for 10 min. Then, without washing the tissues, their primary antibodies (individually) were left active. Primary antibodies MMP-2 (Cat. No. AP05482, BTLAB) and MMP-9 (Cat. No. 393859, Santa Cruz Biotechnology) were used for immunostaining. After the relevant time, the tissues washed with PBS were exposed to the appropriate FITC-conjugated secondary antibody for 1 h in the dark. After that, the slides were washed with PBS and then covered by counterstaining with DAB (3,3'diaminobenzidine) chromogen and hematoxylin. Preparations ZEISS A × 10 Lab. A1 (Axicocam ERc5s) was evaluated in a computer-assisted imaging system and photographed.

Statistical analysis

Statistical analyses were performed using SPSS Version 21.0 (IBM Corporation, Armonk, USA) and GraphPad 7 Prism software (GraphPad Software, Inc., San Diego, USA). For normally distributed data, the difference between the experimental groups was demonstrated using a one-way analysis of variance test. The conformity of the data to the normal distribution was evaluated with histogram, q-q graphs, and Shapiro–Wilk test. Tukey and Dunn tests were used for multiple comparisons and $P < 0.05$ was considered significant. IOP measurements were evaluated with the Wilcoxon signed-rank test, which is a nonparametric test.

Results

Unilateral IOP elevation with episcleral vein cauterization was performed in the left eye (OS) to induce glaucoma-like damage and the right eye (OD) was used as an internal control. All subjects who underwent glaucoma induction showed elevated IOP. Adequate IOP values were achieved in all groups during 7 weeks. The weekly IOP trends of the groups during the experiment are shown in Figure 1. After stable IOP elevation, simultaneous drug administration was started in Group III and Group IV. Six weeks later, IOP increased to 35.14 ± 1.93 mmHg in the riluzole group (Group IV), 36.24 ± 2.05 mmHg in the corn Oil + DMSO group (Group III), and 37.43 ± 2.27 mmHg in the glaucoma group (Group II) ($P < 0.01$). In addition, IOP in the control group (Group I) was 24.19 ± 0.17 for 7 weeks [Figure 1]. When IOP results were compared between groups, there was no significant difference between Group III and Group IV ($P > 0.05$), and there was a statistically significant difference between Group II and Group I ($P < 0.01$). A decrease in IOP was observed in the riluzole group compared to Group II, but this decrease was not statistically significant ($P > 0.05$). In addition, the highest IOP increase was seen in Group II. There was a statistically significant difference

between Group II, Group III, Group IV, and Group I in terms of IO*P* values ($P < 0.01$) [Figure 1]. In addition, the IOP in the OD of the respective groups did not change significantly over 7 weeks [Figure 2]. Mean IOP was significantly higher in glaucomatous eyes than in the control group ($P < 0.05$). When the OD values of the experimental groups were compared; Group I (OD; 23.43 ± 0.9 mmHg), Group II (OD; 26.74 ± 0.7 mmHg), Group III (OD; 26.17 ± 1.8 mmHg), and Group IV (OD; 25.21 ± 0.44 mmHg) were similar [Figure 2].

MMP-2 and MMP-9 gene expression results are shown in Figure 3. To investigate the effect of increased IOP on MMP-2 and MMP-9 gene expression at the end of the 7 weeks, left eyes were taken in each group and the expressions of these components were compared between the groups. MMP-2 gene expression was higher in Group II than in all groups. When MMP-2 gene expression results were compared, there was a statistically significant difference between Group II and all groups (3, 16-fold, $P < 0.0001$). Administration of 5 mg/kg riluzole was associated with significantly lower expression of MMP-2 mRNA compared to that in Group II (0.95-fold, $P < 0.0001$). Riluzole and corn oil + DMSO group was associated with significantly lower expression of MMP-2 mRNA than that in the control group (0.95 and 0.94-fold, $P < 0.0001$). MMP-9 gene expression level was quite low in the control and corn oil + DMSO groups compared to the other groups (0.12 and 0.27-fold, respectively, $P < 0.0001$). Compared to that in the control group, glaucoma and riluzole group had significantly higher expression of MMP-9 mRNA (1.26-fold and 0.96-fold, respectively; $P < 0.0001$ for each pairwise comparison). The glaucoma group was associated with 1.26 times higher MMP-9 mRNA expression compared to all groups ($P < 0.0001$) [Figure 3].

Histopathological analyses on the retinal tissue sections of all experimental groups are shown in Figures 4 and 5. A significant difference was found between Group II and the other groups (control, riluzole, DMSO + oil) in staining retinal tissue sections ($P < 0.001$). When the control group was examined, the pigment epithelial cells and the limiting membrane structure were regular. In addition, the outer and inner membrane structures were homogeneous, and the ganglion cells had polygonal regular in the histological section of the retina [Figure 4A and 4A1]. When Group II was

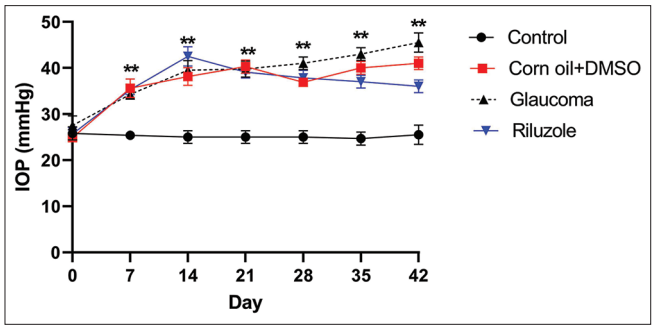

Figure 1: Comparison of intraocular pressure (IOP) measurements of the experimental groups ($n = 7$). IOP was measured once a week with a tonometer device. After episcleral vein cauterization, IOP increased to a stable level within 2 weeks. Then, riluzole (5 mg/kg) administration was started simultaneously with the IOP rise. After episcleral vein cauterization, IOP increased in all groups except the control. $^{**}P < 0.001$ as compared to the control groups. The data are given as mean ± standard deviation ($n = 7$)

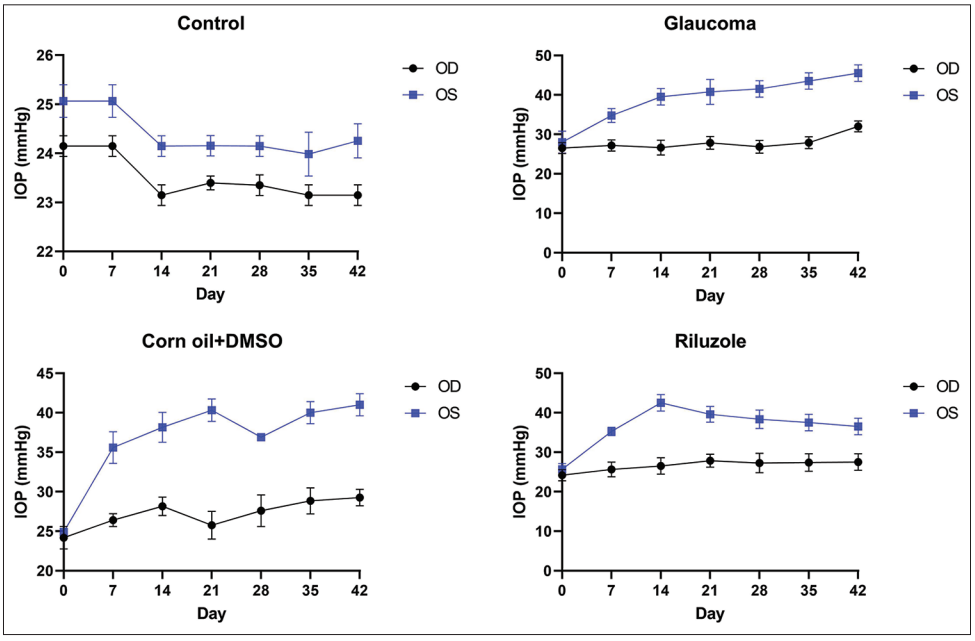

Figure 2: Mean intraocular pressures (IOPs) ($n = 7$) of the hypertensive left (OS) eyes and contralateral right (OD) eyes of the experimental groups. After episcleral vein cauterization, the IOP values in the OS eyes of the subjects increased significantly compared to the OD eyes in all experimental groups. The data are given as mean ± standard deviation ($n = 7$)

compared with the other experimental groups, spaces between the pigment epithelium, irregularity in the limiting membrane, degenerative changes in some cone and bacillus cell nuclei, thinning of the inner limiting membrane, degeneration of ganglion cells, and thickening of the small capillary walls were observed ($P < 0.001$) [Figure 4B and 4B1]. A slight decrease was observed in histopathological findings such as degeneration of ganglion cells and congestion in vascular structures with riluzole administration ($P < 0.001$) [Figure 4C and 4C1]. As a result of the histopathological evaluation and analysis of the groups, it was concluded that there was no statistically significant difference between the control, corn oil + DMSO [Figure 4D and 4D1], and riluzole groups in terms of degeneration in ganglion cells, congestion in vascular structures, bleeding, and differentiation in layers ($P > 0.05$) [Figure 5].

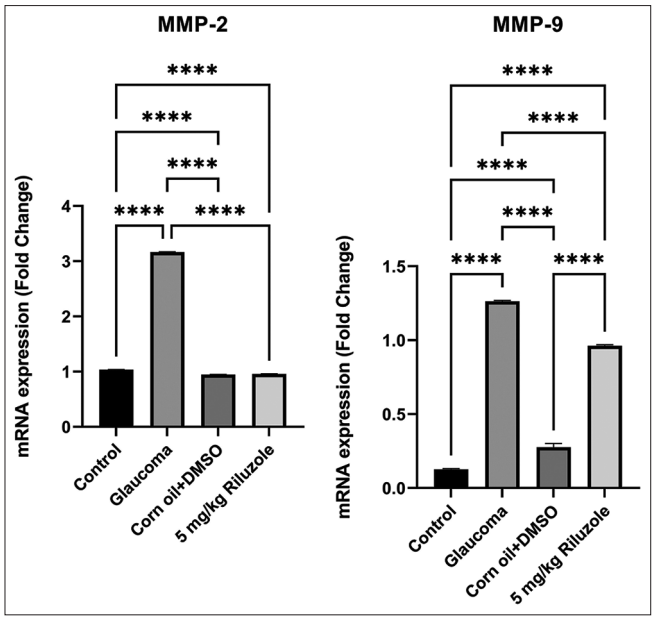

Figure 3: Matrix metalloproteinase-2 (MMP-2) and MMP-9 mRNA expression levels of experimental groups. Mean value ± standard deviation ($n = 7$). It was determined that MMP-2 and MMP-9 gene expression decreased after riluzole administration compared to the glaucoma group. This decrease was found to be 0.95-fold at the MMP-2 gene level compared to the glaucoma group, and 0.96-fold at the MMP-9 gene level compared to the glaucoma group. ****$P < 0.0001$ (when compared between control and groups). The data are given as mean ± standard deviation ($n = 7$)

MMP-2 and MMP-9 expression analysis results of all experimental groups are shown in Figures 6 and 7. MMP-2 expressions were significantly different in all groups compared to the control group [Figure 6A]. In the immunohistochemical application of MMP-9, negative MMP-9 expression was observed in the pigment epithelium and outer limiting membranes in the sections of the control group and in the inner limiting membrane and ganglion cells in cone and bacillus cell nuclei [Figure 6A1]. MMP-2 expressions were higher in Group II compared to the control group. In addition, MMP-2 expression was found positive in the inner and outer limiting membranes of the pigment epithelium in the glaucoma group [Figure 6B] ($P < 0.01$). In normal eye sections, MMP-9 is weakly expressed in a single layer of retinal pigment epithelium. In the riluzole and Group II, MMP-9 expression was weak in pigment epithelial and cone bacillus cell nuclei and the ganglion cell layer on the limiting membrane surfaces [Figure 6B1 and C1]. MMP-2 expressions were decreased in riluzole and corn oil + DMSO group compared to Group II [Figure 6C and D] ($P < 0.05$).

Discussion

Glaucoma is the leading cause of irreversible blindness worldwide and is a neurodegenerative disease that is affected

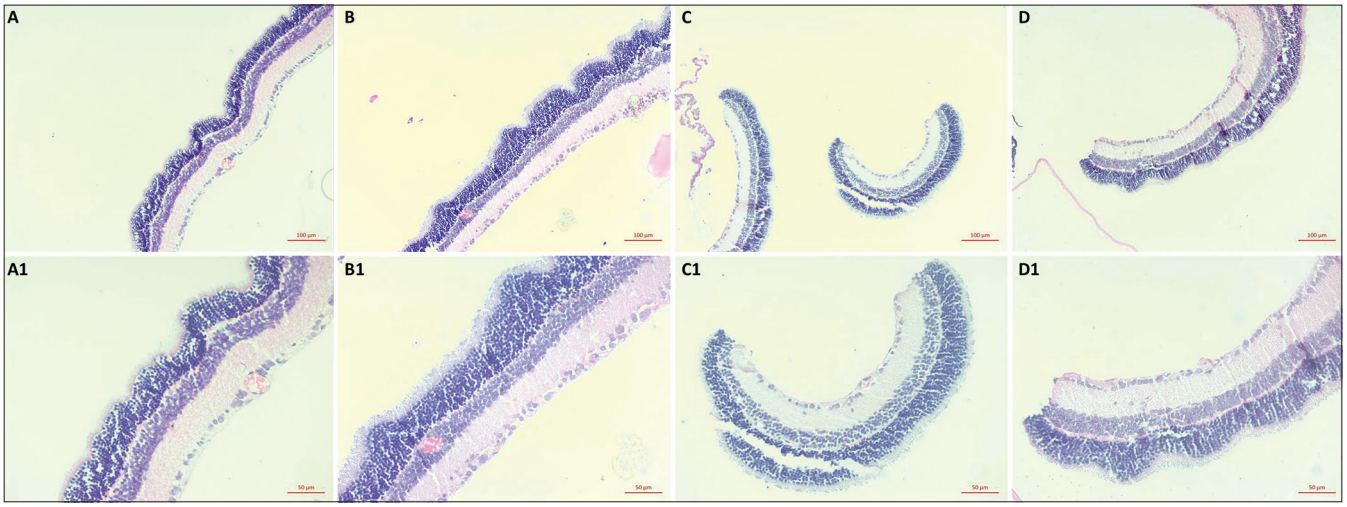

Figure 4: Hematoxylin-eosin staining of control (A and A1), glaucoma (B and B1), riluzole (C and C1), and DMSO + corn oil (D and D1) groups. No histopathological findings (degeneration in ganglion cells, congestion in vascular structures, hemorrhage, and differentiation in layers) were found in the images (A, C, and D ×10) (A1, C1, and D1 ×20). The glaucoma group differed significantly from the other experimental groups in terms of degeneration in ganglion cells, occlusion in vascular structures, bleeding, and differentiation in layers (B, ×10) (B1, ×20)

by many factors, including genetic and environmental, during the damage process.[13,18] Since IOP elevation is the most important risk factor for the development and progression of the disease, the most important clinical treatment today is lowering IOP. [19] Studies have shown that IOP-lowering treatments play a protective role in the progressive damage process of glaucoma.[14,20] However, despite the use of existing IOP-lowering treatments, many patients still continue to lose their sight.[19] Therefore, many researchers have recently focused on developing new treatment strategies in addition to conventional glaucoma treatment to lower IOP. Treatment options in newly initiated studies include drugs that increase blood flow to the eye and have neuroprotective effects.[21]

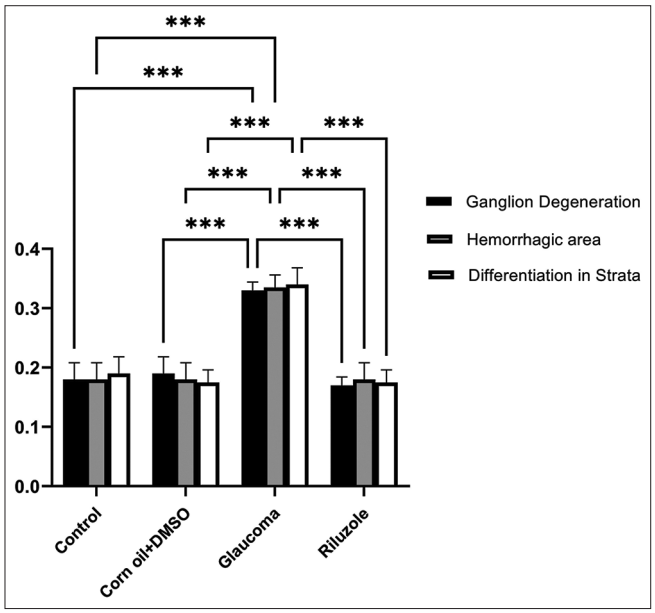

Figure 5: Statistical evaluation results of histopathological parameters of degeneration in ganglion cells, congestion, and hemorrhage in vascular structures and differentiation in layers according to groups in retinal tissue ***$P < 0.001$ (when compared between control and groups)

When studies are examined, the exact mechanism by which high IOP and many other factors affect glaucoma at molecular or cellular levels and cause progression has not been fully understood.[22] Many studies report changes in the activity and expression of various MMPs in the aqueous humor, retina, optic nerve, and trabecular network in experimental glaucoma models and glaucoma patients.[23] However, reports on this subject are conflicting, and research explaining the underlying mechanisms is lacking. Therefore, in our study, we investigated the neuroprotective effect of riluzole on MMP-2 and MMP-9 in rats with an experimental glaucoma model and examined the relationship between MMPs and glaucoma.

While creating the glaucoma model, the episcleral vein cauterization method was preferred. With this method, the IOP level was increased by applying cautery to 3 of the 4 episcleral veins.[24] We prefer this method because, unlike laser-induced glaucoma, in the episcleral vein cauterization method, the ciliary nerve is not damaged, trabecular tissue integrity is not impaired, and IOP elevation can be sustained for approximately 6 months (a rat's lifetime).[25] In our study, when IOP measurements that were taken before episcleral vein cauterization and IOP measurements obtained on the 7th day following the episcleral vein cauterization were compared, the increase in IOP measurements in the left eyes of the subjects in Group II, Group III, and Group IV showed that the model formation was successful. In our study, after the glaucoma model was created with episcleral vein cauterization in rats, riluzole injection, which was started simultaneously and administered once a week, showed a decrease in IOP measurements at the end of the experiment compared to the glaucoma group, but this decrease was not statistically significant. In a study by Hernández *et al.* on rats, i.p., treatment was started 3 days after the glaucoma model was created with episcleral vein cauterization and at weekly intervals for 12 weeks reported that injection of brimonidine tartrate administered through the main route did not cause a

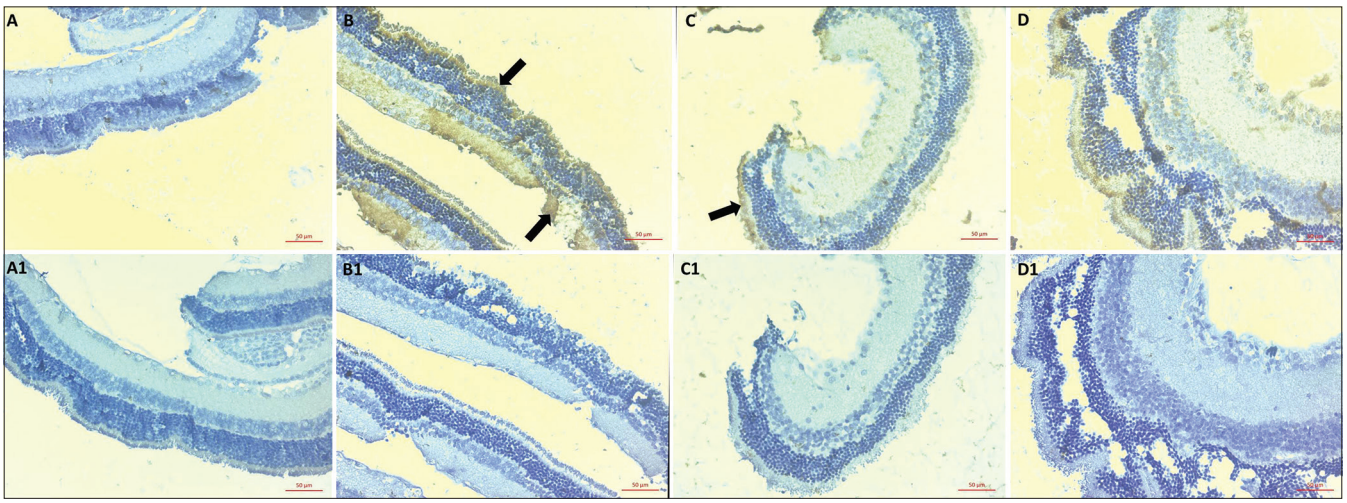

Figure 6: Matrix metalloproteinase-2 (MMP-2) and MMP-9 immunostaining of control (A and A1), glaucoma (B and B1), riluzole (C and C1), and oil + DMSO (D and D1) groups. MMP-2 staining was positive in the glaucoma group (×20)

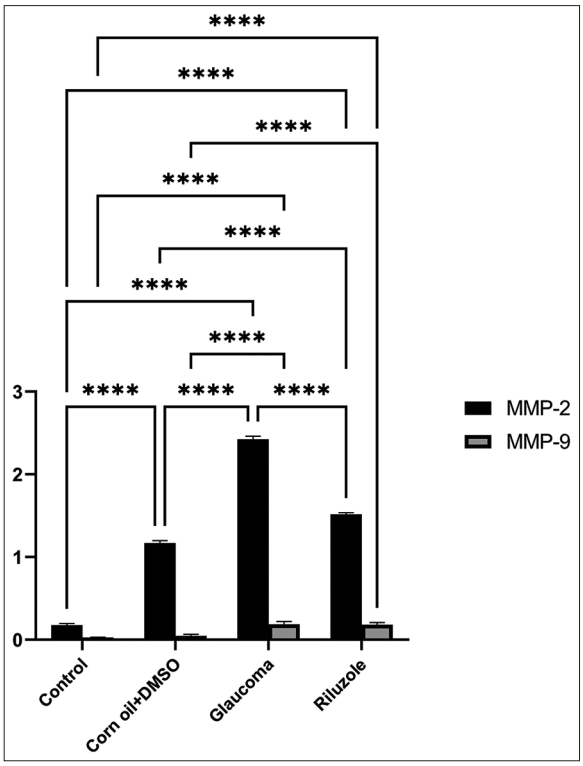

Figure 7: Statistical evaluation results of immunostaining degrees of matrix metalloproteinase-2 (MMP-2) and MMP-9 in retinal tissue according to groups. ****$P < 0.0001$ (when compared between control and groups)

significant decrease in IOP levels.[26] This result is similar to our study.

MMPs are a family of enzymes that play a role in ECM degradation and remodeling processes.[23,27] Evidence suggests that MMPs are associated with disease development in various neurodegenerative disorders.[14] MMPs are major matrix-degrading enzymes and have been identified as important modulators of IOP in the healthy human eye. MMPs function in a feedback mechanism that continually reshapes the ECM composition to maintain IOP homeostasis and a stable aqueous humor outflow resistance. It is known that MMPs are expressed by various glial and also neuronal cell types in both the retina and optic nerve.[23,28] The role of MMP-2 and MMP-9 *in vivo* remodeling of the hypertensive sclera has not yet been clarified.[29] One of the most studied MMPs in the retina is MMP-9. Studies have reported that IOP, which is an important risk factor for glaucoma, induces MMP-9 activation and eventually leads to retinal dysfunction.[2,16] In addition, increased MMP-1,-2,-3,-7, and -9 activity in neurodegenerative disease disrupts the blood–brain barrier and neuronal damage.[13,30,31] However, the role of MMP-2 in RGC death is still controversial. While a few studies in the literature reported that MMP-2 activity did not change after RGC death, some studies reported that MMP-2 activity increased after injury.[32,33] The results of RT-qPCR and immunohistochemistry analysis showed that MMP-2 was significantly increased in the glaucoma group. MMP-2 expression was significantly lower in the riluzole group than in the other groups, and the mean IOP level remained lower for 4 weeks than in the other groups. Riluzole administration showed that MMP-2 expression was significantly decreased compared to Group II. MMP-2 mRNA levels of both DMSO and riluzole groups were lower than the control group. The mechanisms underlying these findings and their physiological significance remain to be clarified.

MMP-9 expression was very low in the control and corn oil + DMSO groups. In a study, similar to our study, no MMP-3 or -9 expression could be detected in the healthy retina.[34] However, Agapova *et al*. reported that MMP-2 is expressed in RGC somata and their axons.[35] Similarly, Liu *et al.* reported that only MMP-1, -2, and -14 expressions were detected, but MMP-3 and -9 expression was not detected in a study they conducted by creating a glaucoma model on rabbits.[36] In our study, we could not detect MMP-9 expression levels in our control and corn oil + DMSO groups because they were very low. In some studies, MMP-2 gene expression levels increased significantly in the glaucoma model group compared to the control group,[37] while in others, they decreased significantly.[38] The emergence of different results may be due to the difference in the method of constructing the glaucoma model and the number of samples. In addition, decreased MMP-2 and -9 activity may be associated with high IOP. In addition, other studies have previously shown to upregulate the expression of MMP-2, MMP-3, and MMP-14 after IOP elevation, all of which are involved in the remodeling of the ECM.[39]

In vitro and animal model studies have shown that riluzole exerts a neuroprotective activity on injured axons.[18] Since DMSO + corn oil was used as the solvent of riluzole in the study, the DMSO + corn oil group was formed to evaluate the effect of the solvent. Riluzole is an American Food and Drug Administration-approved neuroprotective agent used for the treatment of ALS. However, the mechanism of the neuroprotective effect of riluzole has not been fully elucidated. Studies have shown that riluzole has a neuroprotective effect in many acute and chronic neurodegenerative diseases, including Parkinson's disease, Huntington's disease, and brain ischemia.[14,40] There are also studies indicating the neuroprotective activity of riluzole in retinal neuron damage.[41] Lagrèze *et al.* investigated whether riluzole, a glutamate release inhibitor, has neuroprotective activity in a retinal ischemia model. As a result of the study, 43% of the neurons were preserved in the riluzole group, and the results were statistically significant.[42] In addition, in another study by Pirhan *et al.,* the neuroprotective effect of the systemic use of riluzole and resveratrol on RGSs was investigated in an experimental glaucoma model. As a result of the study, it was shown that the number of RGCs was significantly preserved in the groups given riluzole and resveratrol compared to the glaucoma group. Although there is only one study in the literature that riluzole is neuroprotective on glaucoma, it has been reported to have neuroprotective effects in many publications.[14]

In conclusion, the findings of the study show that MMP-2 and MMP-9 exhibited decreased expression levels in both Group III (glaucoma + corn oil + DMSO) and Group IV (glaucoma + corn oil + DMSO + 5 mg/kg riluzole) compared to the glaucoma groups in Group II. Given that both Group III and Group IV received injections of corn oil + DMSO, this conclusion was drawn. In future, we believe that after the molecular mechanisms of this drug are elucidated, it will be valuable in the development of new candidate neuroprotective agents. However, further studies are needed on various issues, including the etiology and treatment of glaucoma and MMPs. Future studies will likely reveal the function of MMPs in glaucoma and may play a role in the prevention and successful treatment of glaucoma.

Financial support and sponsorship

This study was supported by Alanya Alaaddin Keykubat University Scientific Research Projects (BAP) [project grants 2022-04-03-MAP04].

Conflicts of interest

There are no conflicts of interest.

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