Effect of the ACE inhibitor Zofenopril on the Oxidative Status of the Eye in Animals with Experimental Glaucoma

Mikheytseva Irina, Kolomiichuk Serhii, Siroshtanenko Tatiana, Storozhuk Nataliia, Kuznetsov Maxim, Alobaisi Mayyar

Department of Biochemistry, State Institution "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine", Odesa, Ukraine

Submitted to the editorial board: September 29, 2025
Accepted for publication: November 5, 2025
Available on-line: January 6, 2026

We confirm that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. This research was conducted using equipment and resources provided by State Institution "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine", Odesa, Ukraine. No additional external funding was received. The authors declare that there are no conflicts of interest related to this work.

Prof. Dr. Irina Mikheytseva

Correspondence address:
Head of Biochemistry department, State Institution «The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine»
49/51 Frantsuzkyi Boulevard
65061 Odesa
Ukraine
E-mail: iradocira@gmail.com

SUMMARY

Aims: To evaluate the effect of zofenopril on oxidative stress markers and antioxidant enzyme activity in ocular tissues of rabbits with experimentally induced glaucoma.

Materials and Methods: An experimental model of adrenaline-induced glaucoma (AIG) was developed in 2–2.5-year-old rabbits. Zofenopril, an angiotensin-converting enzyme (ACE) inhibitor, was administered orally as an aqueous suspension (1 mg/kg body weight) daily for three months. Oxidative stress was assessed by measuring malondialdehyde (MDA) levels as a marker of lipid peroxidation (LPO), hydroxyl and superoxide radical generation, and the activity of antioxidant enzymes (glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT)).
Intraocular pressure (IOP) in rabbits was measured using an applanation tonometer under local anesthesia with 0.5% Alcaine. Spectrophotometric analysis was performed on the retina, optic nerve, and drainage zone tissues.

Results: In the AIG modeling group, a dynamic increase in IOP was observed: by 28.3% after 30 days, by 34.2% and 46.7% after 60 and 90 days, respectively, compared to baseline data. Oral administration of zofenopril suspension during AIG modeling resulted in a milder elevation of IOP – by 17.4% on day 30 ($p < 0.05$) – followed by a gradual decline to 110.1% and 105.4% of baseline values on days 60 and 90, respectively. On day 90 of the study, rabbits with AIG exhibited significant activation of LPO and elevated MDA levels in ocular tissues: by 54.1% in the retina, 39.9% in the optic nerve, and 70.1% in the drainage zone, compared to controls (intact animals) ($p < 0.01$). Enhanced free radical generation was observed: hydroxyl radical levels increased by 71.3% in the retina, 58.9% in the optic nerve, and 81.8% in the drainage zone; superoxide radical levels increased by 78.4%, 64.4%, and 94.2%, respectively. Antioxidant enzyme activity declined in the retina, with GPx decreasing by 35.9%, SOD by 42.4%, and CAT by 30.7%, compared to the controls.
Zofenopril administration during glaucoma modeling resulted in reduced MDA levels: by 19.3% in the retina, 16.1% in the optic nerve, and 28.1% in the drainage zone, compared to the untreated AIG group. Hydroxyl radical generation also decreased by 23.0% in the retina, 21.9% in the optic nerve, and 23.9% in the drainage zone. Superoxide radical levels were reduced by 20.3% in the retina and 24.9% in the drainage zone. In contrast, antioxidant enzyme activity increased in the retina: GPx rose by 33.6%, SOD by 26.2%, and CAT by 21.6%, compared to the untreated AIG group.

Conclusion: Prolonged systemic administration of zofenopril in a rabbit model of glaucoma effectively attenuated oxidative stress and stabilized intraocular pressure. Zofenopril prevented a progressive rise in IOP observed in untreated animals, maintaining IOP values close to physiological levels throughout the experiment. The treatment resulted in a marked decrease in LPO and MDA accumulation within the retina, optic nerve, and drainage zone, along with reduced generation of hydroxyl and superoxide radicals and a significant restoration of antioxidant enzyme activity.

Key words: glaucoma, experimental model, rabbits, retina, optic nerve, drainage zone tissues, ACE inhibitor zofenopril, free radical processes, oxidative stress, antioxidant enzymes

Čes. a slov. Oftal., 81, 2025, No. x, p.

INTRODUCTION

Glaucoma is one of the leading causes of blindness worldwide, due to the progressive damage to the optic nerve and the loss of retinal ganglion cells. Oxidative stress and vascular dysfunctions play a key role in the pathogenesis and progression of this disease. Modern ophthalmology continues to explore pharmacological treatments that target the pathogenic mechanisms underlying glaucoma.

Despite advances in understanding the molecular and physiological basis of glaucoma and the development of

novel therapies, the treatment and prevention of complications associated with the disease remain highly relevant challenges [1].

The renin-angiotensin system (RAS) plays a pivotal role in the regulation of systemic blood pressure, as well as fluid and electrolyte homeostasis. In addition to the systemic RAS, local RASs have been identified in various organs, where they regulate regional fluid balance. The eye also possesses its own local RAS, located in structures involved in aqueous humor dynamics [2]. This makes ocular RAS a promising pharmacological target for the development of novel antiglaucoma therapies.

Some studies suggest that drugs acting on the renin--angiotensin system – such as angiotensin – converting enzyme (ACE) inhibitors – could represent a new class of antiglaucoma agents. These drugs may reduce intraocular pressure (IOP) by modulating the dynamics of aqueous humor. Specifically, they are thought to lower Ang II levels in the aqueous humor, thereby enhancing uveoscleral outflow and reducing aqueous production via decreased ciliary body perfusion [3]. Several animal studies support these hypotheses, demonstrating a significant IOP-lowering effect that lasts more than four hours following the administration of enalapril, fosinopril, and ramipril in models of acute and chronic ocular hypertension [4].

Among ACE inhibitors, zofenopril stands out for its unique antioxidant and vasodilatory effects [5], attributed to the presence of two sulfhydryl (thiol) groups in its molecular structure. Clinical studies involving patients with arterial hypertension and myocardial ischemia have shown that zofenopril enhances the capacity of the vascular endothelium to induce vessel relaxation, increases the activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, and decreases the levels of lipid peroxidation products [6].

Oxidative stress is considered a critical pathogenic factor in glaucoma [7]. Thus, pharmacological agents that combine vasodilatory effects with modulation of intraocular fluid dynamics and antioxidant properties may hold therapeutic promise. Zofenopril is regarded as one such promising candidate.

This study aimed to investigate oxidative and antioxidative markers in the target ocular tissues of rabbits with experimentally induced glaucoma, treated with the ACE inhibitor zofenopril.

MATERIALS AND METHODS

Experimental studies were conducted on rabbits aged 2 to 2.5 years. All procedures complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Ukrainian Law "On the Protection of Animals from Cruelty" (No. 1759-VI of 15.12.2009). Ethical approval was obtained from the Local Bioethics Committee of the Institute (Protocol No. 1, dated 03.07.2025). Scientific research does not require informed consent.

Adrenaline-induced glaucoma (AIG) was modeled by intravenous administration of 0.1 ml of adrenaline solution (Pharmaceutical Company "Zdorovye" LLC, active ingredient content 1.80 mg/ml) [8] every other day for three months (a total of 40 injections) [9, 10]. Zofenopril (Zocardis, manufactured by Menarini, Italy, with 30.0 mg of zofenopril active ingredient) was administered orally as a 1 ml aqueous suspension at a dose of 1 mg/kg body weight once daily for three months. The drug was given 20–30 minutes before each adrenaline injection.

IOP in rabbits was measured using an applanation tonometer under local anesthesia with 0.5% Alcaine.

The animals were removed from the experiment under deep anesthesia (1 mL of 10% sodium thiopental solution per kg of body weight), using the air embolism method. The eyeballs were enucleated at a temperature of 0 °C to 5 °C. For biochemical studies, tissues from the ocular drainage area, retina, and optic nerve were used to prepare a homogenate with 0.9% sodium chloride solution at a ratio of 1:9 (w:v). The resulting extracts were centrifuged at 5 °C for 10 minutes at 10,000 rpm, and the supernatant was used for analysis.

The oxidative status of ocular tissues was assessed by measuring both pro-oxidant and antioxidant markers. Pro-oxidant indicators included hydroxyl [11] and superoxide radical [12] levels and malondialdehyde (MDA) concentration [13].

(The principle of the MDA assay is based on the reaction of malondialdehyde with 2-thiobarbituric acid (TBA) under acidic conditions at 100 °C, resulting in the formation of a colored trimethine complex with a maximum absorbance at 532 nm. MDA concentration was expressed as nmol/g of tissue.

The enzymatic arm of the antioxidant defense system was evaluated spectrophotometrically. GPx activity was determined by monitoring the rate of oxidized glutathione formation in the presence of the NADPH-dependent enzyme glutathione reductase, recording the decrease in optical density due to NADPH oxidation at 340 nm. GPx activity was expressed as μkat/g of tissue.

SOD activity was assessed by measuring the degree of inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals. The principle of this method is based on the enzyme's ability to inhibit the formation of formazan dye, resulting from the reduction of NBT by superoxide radicals. SOD activity was expressed in arbitrary units per gram of tissue.

CAT activity was determined, based on the ability of hydrogen peroxide to form a stable colored complex with molybdate salts. The absorbance of the resulting hydrogen peroxide-molybdate complex was measured at 410 nm. Enzyme activity was expressed as μkat/g of tissue.

The obtained IOP data in animals were statistically processed using non-parametric analysis methods, namely the Kruskal-Wallis and Mann-Whitney tests. Biochemical parameters were analyzed using the parametric Student's t-test method. All methods' significance was set at $p\text{-value} < 0.05$.)

Table 1. IOP in rabbits with AIG under the influence of the angiotensin-converting enzyme blocker zofenopril (in mm Hg)

Animal groups	Statistical indicators	Exposure time, days			
		Input data	30	60	90
Control (n = 10)	M ±m	14.6 ±0,8	15.1 ±0.7	14.3 ±0.9	14.7 ±0.7
	p	-	> 0.05	> 0.05	> 0.05
	%	100.0	103.4	97.9	100.7
	p_1	-	-	-	-
	$\%_1$	100.0	100.0	100.0	100.0
Glaucoma (n = 12)	M ±m	15.2 ±0.9	19. 5±1.2	20.4 ±1.3	22. 3±0.9
	p	-	< 0,01	< 0,01	< 0,01
	%	100.0	128.3	134.2	146.7
	p_1	> 0.05	< 0.01	< 0.01	< 0.001
	$\%_1$	104.1	129.1	142.7	151.7
	p_2	-	-	-	-
	$\%_2$	100.0	100.0	100.0	100.0
Glaucoma + Zofenopril (n = 14)	M ±m	14.9 ±0.7	17.5 ±0.9	16.4 ±0.8	15.7 ±1.2
	p	-	< 0.05	> 0.05	> 0.05
	%	100.0	117.4	110.1	105.4
	p_1	> 0.05	> 0.05	> 0.05	> 0.05
	$\%_1$	102.1	115.9	114.7	106.8
	p_2	> 0.05	> 0.05	< 0.05	< 0.001
	$\%_2$	98.0	89.7	80.4	70.4

n – number of eyes, p – level of significance of differences in data in relation to the baseline data, p1 – level of significance of differences in data in relation to the control group, p2 – level of significance of differences in data in relation to the group of animals with glaucoma, IOP – intraocular pressure, AIG – adrenaline-induced glaucoma

RESULTS

In the AIG modeling group, a dynamic increase in IOP was observed (Table 1): by 28.3% after 30 days, by 34.2% and 46.7% after 60 and 90 days, respectively, compared with baseline data. Oral administration of zofenopril suspension during AIG modeling resulted in a milder elevation of IOP – by 17.4% on day 30 ($p < 0.05$) – followed by a gradual decline to 110.1% and 105.4% of baseline values on days 60 and 90, respectively. When compared to the control group, these differences were not statistically significant at any time point. However, a significant reduction in IOP was observed in the zofenopril-treated group compared to untreated AIG animals – by 19.6% on day 60 ($p < 0.05$) and by 29.6% on day 90 ($p < 0.001$).

The first stage of the study involved measuring MDA, a terminal product of lipid peroxidation, in the ocular tissues of rabbits across all experimental groups: control, AIG (adrenaline-induced glaucoma), and AIG with zofenopril treatment (Table 2).

In the group with experimentally induced glaucoma, MDA levels were significantly elevated in all examined ocular tissues, compared to the control group. Specifically, MDA content increased by 54.1% in the retina, 39.9% in the optic nerve, and 70.1% in the drainage zone tissues.

In the group with experimentally induced glaucoma, MDA levels were significantly elevated in all examined ocular tissues, compared to the control group. Specifically, MDA content increased by 54.1% in the retina, 39.9% in the optic nerve, and 70.1% in the drainage zone tissues.

Zofenopril administration during AIG modeling led to a reduction in MDA levels in all studied tissues. Compared to the control group, MDA levels in the zofenopril-treated group were 124.4% in the retina, 117.3% in the optic nerve, and 122.3% in the drainage zone. When compared to untreated AIG animals, the zofenopril-treated group demonstrated a decrease in MDA levels by 19.3% in the retina, 16.1% in the optic nerve, and 28.1% in the drainage zone (Graph 1).

The results of other oxidative stress markers – namely, superoxide and hydroxyl radical levels in the ocular drainage zone, retina, and optic nerve of AIG animals treated with zofenopril – are presented in Table 2, Graph 1.

Hydroxyl radical generation on day 90 of AIG modeling was significantly elevated: by 71.3% in the retina, 58.9% in the optic nerve, and 81.8% in the drainage zone, compared to the control group. Zofenopril treatment resulted in a marked reduction in hydroxyl radical generation.

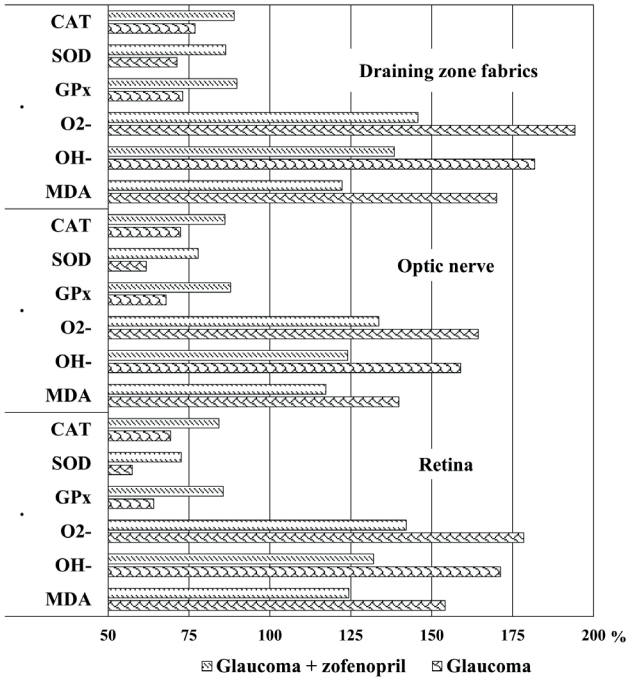
Zofenopril treatment led to a decrease in hydroxyl radical levels in the retina, optic nerve, and drainage zone, compared to the untreated glaucoma group. However, the values in the treatment group remained higher than in the control group, indicating partial compensation of oxidative stress.

Zofenopril administration during AIG modeling resulted in a reduction of hydroxyl radical generation to 132.0% in the retina ($p < 0.05$), 124.1% in the optic nerve ($p > 0.05$), and 138.4% in the drainage zone tissues ($p < 0.05$), relative to the control group. Compared to the untreated glaucoma group, hydroxyl radical generation decreased by 23.0% in the retina, 21.9% in the optic nerve, and 23.9% in the drainage zone tissues.

Table 2. Indicators of oxidative stress in the eye tissues of rabbits with AIG and with the use of the ACE inhibitor zofenopril

Biochemical Indicators	Tisseues	Experimental Conditions		
		Control (n = 10)	Glaucoma (n = 12)	Glaucoma + Zofenopril (n = 14)
Gpx (µkcat/g tissue)	Retina	514.52 ±37.28	329.56 ±23.64*	440.37 ±33.24
	Optic nerve	413.82 ±32.14	280.98 ±16.82*	363.59 ±26.42
	Drainage zone tissues	321.12 ±21.98	234.76 ±16.78*	288.29 ±18.54
SOD (units/g tissue)	Retina	39.26 ±2.14	22.57 ±1.25*	28.49 ±1.38*
	Optic nerve	32.19 ±2.16	19.89 ±1.14*	25.04 ±1.62*
	Drainage zone tissues	23.48 ±1.24	16.74 ±1.25*	20.29 ±1.15
CAT (µkcat/g tissue)	Retina	48.57 ±3.29	33.68 ±2.23*	40.94 ±2.65
	Optic nerve	39.52 ±2.60	28.61 ±1.58*	34.0 ±2.40
	Drainage zone tissues	26.85 ±1.64	20.64 ±1.15*	23.90 ±1.36
MDA (nmol/g tissue)	Retina	858.14 ±65.42	1322.34 ±90.26*	1067.52 ±82.13
	Optic nerve	638.26 ±47.14	892.73 ±65.37*	748.67 ±46.16
	Drainage zone tissues	380.12 ±23.80	646.73 ±42.24*	465.02 ±34.27
OH^- (absorbance units/hr/mg protein)	Retina	132.17 ±10.56	226.40 ±19.56*	174.42 ±14.92*
	Optic nerve	114.08 ±8.72	181.28 ±15.34*	141.63 ±11.07
	Drainage zone tissues	123.74 ±9.82	224.95 ±20.42*	171.29 ±14.30*
O_2^- (absorbance units/hr/mg protein)	Retina	2.18 ±0.20	3.89 ±0.28*	3.10 ±0.24*
	Optic nerve	1.49 ±0.12	2.45 ±0.20*	1.99 ±0.16*
	Drainage zone tissues	1.90 ±0.16	3.69 ±0.32*	2.77 ±0.22*

Gpx – Glutathione peroxidas, SOD – Superoxide dismutase, CAT – Catalase, MDA – Malondialdehyde, OH^- – hydroxide ion, $O2^-$ – superoxide anion, AIG – adrenaline-induced glaucoma, ACE – angeotensin converting enzyme
*p-value < 0.05



Graph 1. Relative changes in the oxidative state (% of control) of rabbit eye tissues with AIG and with the use of the ACE inhibitor zofenopril
Gpx – Glutathione peroxidas, SOD – Superoxide dismutase, CAT – Catalase, MDA – Malondialdehyde, OH- – hydroxide ion, O2- – superoxide anion ACE – angeotensin converting enzyme, AIG – adrenaline-induced glaucoma

The experiment also evaluated the generation of superoxide radicals in the ocular tissues of rabbits under various conditions. The control group demonstrated baseline radical levels, while a significant increase was observed in the glaucoma group, indicating elevated oxidative stress. Specifically, superoxide radical levels increased to 178.4% in the retina, 164.4% in the optic nerve, and 194.2% in the drainage zone, compared to the control group.

Zofenopril treatment resulted in a substantial reduction in superoxide radical generation across all examined tissues. In the retina, the level decreased to 142.2%, in the optic nerve to 133.6%, and in the drainage zone to 145.8%, relative to the controls. Thus, although superoxide radical generation remained elevated under zofenopril treatment during AIG modeling, a statistically significant reduction was noted in comparison with untreated glaucoma animals: by 20.3% in the retina and 24.9% in the drainage zone. In the optic nerve, the reduction reached 18.8%, but due to a broader range of values, the result did not reach statistical significance ($p > 0.05$).

The drainage zone consistently exhibited the highest levels of radical generation under all experimental conditions, indicating a high vulnerability of this ocular structure to oxidative stress.

The increase in MDA levels confirms the activation of lipid peroxidation processes in ocular tissues under AIG conditions, which is a recognized mechanism contributing to the pathogenesis of primary glaucoma.

The free-radical degradation of peroxynitrite drives the accumulation of hydroxyl radicals. It is also known that superoxide radical generation in the body is associated with the activation of xanthine oxidase, NADPH oxidase, and lipid-related cyclooxygenase and lipoxygenase pathways.

The status of antioxidant enzymes in the ocular tissues of rabbits with experimental glaucoma and under zofenopril treatment changed as follows: (Table 2).

By day 90 of AIG modeling, the activity of antioxidant enzymes in the retinal tissue showed a significant decrease: glutathione peroxidase activity dropped by 35.9%, superoxide dismutase by 42.4%, and catalase by 30.7%, compared to the control group.

Similarly, antioxidant enzyme activity in the optic nerve was significantly reduced in rabbits with AIG: GPx by 32.1%, SOD by 38.2%, and CAT by 27.6% versus the controls.

In the drainage zone tissues, AIG led to a significant decrease in antioxidant activity as well: GPx by 26.9%, SOD by 28.7%, and CAT by 23.1%.

A comparative analysis of enzyme activity across tissues revealed more pronounced changes in neural structures – retina and optic nerve – than in the drainage zone, highlighting the higher susceptibility of nervous tissue to oxidative damage under glaucomatous conditions.

Zofenopril administration during AIG modeling led to a significant improvement in the enzymatic component of the eye's antioxidant defense system. In the retina, statistically significant increases in enzyme activity were observed: GPx by 33.6%, SOD by 26.2%, and CAT by 21.6%, compared to untreated AIG animals. In the optic nerve, GPx activity increased by 29.4%, SOD by 25.9%, and CAT by 18.9% ($p > 0.05$). In the drainage zone, the increases were 22.8%, 21.2%, and 15.8%, respectively ($p > 0.05$), compared to untreated AIG animals (Graph 1).

These findings confirm that AIG induces oxidative stress, and the reactive components of this process negatively affect the retina, optic nerve, and drainage zone tissues of experimental animals. Zofenopril treatment attenuated oxidative stress markers, suggesting a slowing of glaucomatous progression.

DISCUSSION

Adrenaline-induced glaucoma (AIG) reproduces the chronic glaucomatous process, which is triggered by β-adrenergic activation with increased cAMP and Ca^{2+} influx, and then leads to a gradual disruption of hydrodynamics: after a short increase in intraocular fluid production and outflow, a predominant outflow deficit, retention, and a persistent increase in intraocular pressure are formed. In parallel, vascular and ischemic shifts with endothelial dysfunction and oxidative stress develop, which exacerbate damage to neurosensory structures. Morphologically, this is manifested by damage to the drainage system, thinning of the nerve fiber layer, death of ganglion cells and progressive optic disc excavation – changes identical to primary open-angle glaucoma. Due to its chronic course, high reproducibility, and controllability, AIG induction allows us to trace the complete sequence "trigger → hemodynamic/metabolic shifts → neurodegeneration" and to correctly test pathogenetic approaches (β-blockade, limitation of Ca^{2+}-overload, antioxidant and vascular strategies). The combination of these factors makes AIG an adequate and most appropriate experimental model of glaucoma [8]

Our study demonstrated that rabbits with AIG develop pronounced oxidative stress, as evidenced by increased lipid peroxidation in target ocular tissues, elevated MDA levels, intensified free radical generation, and reduced activity of antioxidant enzymes – GPx, SOD and CAT. These findings reflect a disruption of the balance between pro-oxidant and antioxidant systems, which is a key mechanism in the progression of neurodegenerative changes in glaucoma-affected ocular tissues [14].

Zofenopril administration led to a substantial decrease in MDA levels in the retina, optic nerve, and drainage zone, accompanied by normalization of antioxidant enzyme activity. These effects demonstrate zofenopril's ability to mitigate oxidative stress by targeting several key pathogenic mechanisms of glaucoma. Notably, increased activity of GPx, SOD, and CAT suggests restoration of antioxidant defenses, which can inhibit lipid peroxidation and reduce damage to cell membranes.

Primary glaucoma is a multifactorial condition, marked by progressive optic nerve degeneration and retinal ganglion cell atrophy. Impaired ocular hydrodynamics and elevated IOP are considered primary risk factors. Among its underlying pathophysiological mechanisms, oxidative stress plays a pivotal role, significantly contributing to disease onset and progression [1,14,15].

Oxidative stress in glaucoma leads to trabecular meshwork damage, impaired aqueous humor outflow, elevated IOP, and mitochondrial dysfunction in retinal ganglion cells, ultimately resulting in apoptosis and disrupted axonal transport [15,16].

Sulfur-containing ACE inhibitors play a crucial role in the treatment of arterial hypertension, due to their ability to modulate the RAS and promote vasodilation. They enhance nitric oxide (NO) production by endothelial cells, which – along with prostaglandins – suppresses the synthesis of the potent vasoconstrictor endothelin-1 (ET-1) [17].

Animal studies have shown that ACE inhibitors such as enalapril, fosinopril, and ramipril can lower IOP by enhancing uveoscleral outflow and reducing aqueous humor production through modulation of ciliary body blood flow. In addition, they decrease angiotensin II levels in the aqueous humor, promoting vasodilation via increased endothelial NO production. Dysregulation of vascular tone modulators (ET-1 and NO) correlates with changes in IOP [18]. Zofenopril is of particular interest due to its thiol-containing molecular structure. It has been shown to elevate levels of hydrogen sulfide (H_2S) metabolites,

which exert vasodilatory effects and improve vascular function – an effect not observed with non-thiol ACE inhibitors such as enalapril. Several studies have confirmed that zofenopril administration increased plasma H_2S metabolite levels in both mice and pigs [19,20].

Our work has shown that zofenopril reduced IOP in a rabbit model of glaucoma, and one of the mechanisms of this effect is the effect on the pro-oxidant-antioxidant balance.

From our perspective, zofenopril represents the most promising ACE inhibitor for investigating therapeutic effects in primary glaucoma, owing to its combined vasodilatory and antioxidant actions mediated through its unique thiol group. Zofenopril reduces oxidative stress, as evidenced by the increased activity of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase. It also decreases the levels of lipid peroxidation products. In addition, zofenopril serves as a donor of hydrogen sulfide (H_2S), further enhancing its antioxidant and vasodilatory effects. This pharmacological profile makes zofenopril effective in reducing oxidative stress, which plays a key role in the progression of glaucoma and other ocular neurodegenerative diseases [21,22].

The renin-angiotensin system is responsible for regulating blood pressure and maintaining fluid and electrolyte homeostasis. In addition to the systemic RAS, local RASs exist in various organs, including the eye, where they contribute to the regulation of intraocular fluid dynamics [2]. This positions the ocular RAS as a potential therapeutic target for the development of novel antiglaucoma agents. Several studies suggest that RAS-modulating drugs, particularly ACE inhibitors, may emerge as future candidates for glaucoma therapy [23].

ACE inhibitors may lower IOP by modulating the dynamics of aqueous humor. They reduce Ang II levels in the aqueous humor, thereby enhancing uveoscleral outflow and decreasing aqueous production through a reduction in ciliary body perfusion [4,24].

By inhibiting bradykinin degradation, ACE inhibitors also promote vasodilation by enhancing the synthesis of nitric oxide in endothelial cells. Alongside prostaglandins, NO acts as a vasodilator and suppresses the production of endothelin-1 (ET-1), a potent vasoconstrictor [25].

A study by Bucci et al. reported that zofenopril improved vascular function in spontaneously hypertensive rats through mechanisms associated with H_2S release [20]. In contrast, enalapril – which lacks a thiol group – did not demonstrate similar vascular benefits in the same study.

Zofenopril's metabolites act as H_2S donors, releasing the molecule into the solution. Moreover, zofenopril increases endothelial nitric oxide synthase (eNOS) activity, promoting the synthesis of physiological levels of NO. Thus, the drug functions as a dual donor of H_2S and NO, contributing to its vasodilatory efficacy.

Beyond these pharmacological effects, zofenopril demonstrates significant antioxidant activity, primarily due to the presence of two sulfhydryl groups in its molecular structure. Clinical studies in patients with arterial hypertension and myocardial ischemia have shown that zofenopril enhances endothelial vasodilation, accompanied by increased activity of antioxidant enzymes [8].

As shown earlier, endothelial dysfunction – characterized by imbalances in ET-1 and NO signaling, disrupted oxidative-antioxidative homeostasis, and possible dysregulation of H_2S production – is a pathogenic basis of glaucomatous neurodegeneration [23].

The influence of oxidative stress on the development and progression of the glaucomatous process has been extensively investigated [13,14,15]. According to the existing metabolic theory of primary open-angle glaucoma (POAG) pathogenesis, one of the key mechanisms underlying glaucomatous optic neuropathy (GON) is the activation of free radical processes in cellular membranes. Under physiological conditions, free radical oxidation reactions are continuously occurring at a controlled level. However, under pathological conditions, their activation leads to the accumulation of reactive oxygen species (ROS) in ocular tissues and biological fluids. As shown by Korelina [26], the intensification of lipid peroxidation and oxidative stress plays a critical role in the experimental model of glaucoma.

Reactive oxygen species initiate structural damage to mitochondria, promote calcium influx into retinal ganglion cells (RGCs), and trigger apoptotic pathways [27,28]. Elevated oxidative stress also contributes to an increase in the concentration of glutamate – a highly toxic excitatory neurotransmitter in the retina and central nervous system – thereby inducing neuronal excitotoxicity and accelerating RGC apoptosis [29,30]. Salt and Cordeiro [29] demonstrated that glutamate excitotoxicity is a key mechanism of neuronal loss in glaucoma, while Goyal et al. [30] confirmed elevated oxidative stress markers in the aqueous humor of patients with both primary open-angle and primary angle-closure glaucoma.

Experimental studies by Korelina [31], using an adrenaline-induced glaucoma model, further support these findings. Intravenous administration of adrenaline to laboratory animals led to retinal ganglion cell layer atrophy and thinning of the nerve fiber layer. Over a 100-day experimental period, RGC loss reached 47.3%, while the rate of apoptosis increased by approximately 100-fold compared with control animals.

Adrenaline is known as a "stress hormone", and chronic psychological stress has been identified as a significant trigger for glaucoma development. There are studies that show the additional contribution of anxiety to the worsening of glaucoma. Rezapour et al. [32] reported that patients with glaucoma often exhibit comorbid anxiety and depression, suggesting a link between psychological stress and disease progression. Similarly, Mendez-Ulrich et al. [33] demonstrated that anxiety-related IOP may affect the diagnostic accuracy of glaucoma, while Otori et al. [34] assessed the quality of life of glaucoma patients using the State-Trait Anxiety Inventory, confirming that emotional stress is closely related to disease burden. Persistent stress

not only increases ROS production but also suppresses the activity of endogenous antioxidant defense systems, creating a condition known as metabolic stress syndrome [30,35]. This imbalance between oxidative and antioxidant processes plays a central role in the progression of glaucomatous optic neuropathy.

For the above reasons, in-depth investigation of these pathogenic mechanisms and pharmacological interventions targeting them remains a relevant and fundamental research objective in ophthalmology. Our results support the effectiveness of zofenopril as a neuroprotective agent that modulates key mechanisms in the glaucomatous process. Its antioxidant effect may play a crucial role in preventing damage to the retina and optic nerve, which is essential for preserving visual function in patients with glaucoma.

These findings underscore the importance of further studies evaluating the clinical potential of zofenopril and its long-term effects on structural and functional changes in the glaucomatous eye.

CONCLUSION

In the glaucoma model, a marked increase in oxidative stress markers was observed, particularly MDA, hydroxyl, and superoxide radicals in the retina, optic nerve, and ocular drainage zone. Administration of the ACE inhibitor zofenopril resulted in a reduction of these markers, compared to untreated AIG animals, although the values remained above those of the control group. A decrease in free radical generation was documented: superoxide radicals by 20.3% in the retina and 24.9% in the drainage zone; hydroxyl radicals by 23% in the retina, 21.9% in the optic nerve, and 23.9% in the drainage zone.

Antioxidant activity in ocular tissues under glaucoma conditions was significantly reduced, particularly the activities of glutathione peroxidase, superoxide dismutase, and catalase. Zofenopril treatment contributed to the normalization of these values, especially in the retina and optic nerve. In the retina, glutathione peroxidase activity increased by 33.6%, superoxide dismutase by 26.2%, and catalase by 21.6%. In the optic nerve, glutathione peroxidase activity rose by 29.4%, superoxide dismutase by 25.9%, and catalase by 18.9%, compared to untreated AIG animals. These data indicate a significant restoration of the enzymatic component of the antioxidant defense system in the eyes of experimental animals with glaucoma following zofenopril administration.

This study demonstrated that zofenopril effectively reduces oxidative stress in experimental glaucoma by enhancing antioxidant enzyme activity and reducing the burden of cytotoxic free radicals and pro-oxidant metabolites on ocular tissues.

REFERENCES

1. Ban N, Siegfried CJ, Apte RS. Monitoring neurodegeneration in glaucoma: Therapeutic implications. Trends Mol Med. 2018;24(1):7-17.
2. Kurihara T, Ozawa Y, Ishida S, Okano H, Tsubota K. Renin-angiotensin system hyperactivation can induce inflammation and retinal neural dysfunction. Int J Inflamm. 2012;2012:581695. doi:10.1155/2012/581695
3. Holappa M, Vapaatalo H, Vaajanen A. Local ocular renin-angiotensin-aldosterone system: Any connection with intraocular pressure? A comprehensive review. Ann Med. 2020;52(5):191-206. doi:10.1080/07853890.2020.1758341
4. Shah GB, Sharma S, Mehta AA, Goyal RK. Oculohypotensive effect of angiotensin-converting enzyme inhibitors in acute and chronic models of glaucoma. J Cardiovasc Pharmacol. 2000;36(2):169-175. doi:10.1097/00005344-200008000-00005
5. Borghi C, Omboni S. Angiotensin-converting enzyme inhibition: Beyond blood pressure control – the role of zofenopril. Adv Ther. 2020;37(10):4068-4085. doi:10.1007/s12325-020-01455-2
6. Palić B, Brizić I, Sher EK, et al. Effects of zofenopril on arterial stiffness in hypertension patients. Mol Biotechnol. 2025;67:3454–3466. doi:10.1007/s12033-023-00861-5.
7. Hurley DJ, Normile C, Irnaten M, O'Brien C. The intertwined roles of oxidative stress and endoplasmic reticulum stress in glaucoma. Antioxidants (Basel). 2022;11(5):886. doi:10.3390/antiox11050886
8. Mikheytseva I. Modeli glaukomy, preimushchestva i nedostatki. Adrenalin-indutsirovannaya glaukoma kak adekvatnaya model glaukomnogo protsessa cheloveka. Oftalmol Zh. 2011;3:89-92. doi:10.31288/oftalmolzh201138992
9. Mikheitseva IM. Method for modelling adrenaline-induced glaucoma in rabbits. Patent of Ukraine for utility model No. 61478. Bulletin. 2011;(14). Avaible from: https://sis.nipo.gov.ua/uk/search/detail/246925/
10. Fedorov KN, Koivanvladimir N, et al. Modeling experimental glaucoma for screening studies of antiglaucomatous activity. Res Results Pharmacol. 2023;9(4):13-19. doi:10.18413/rrpharmacology.9.10043
11. Conte D, Narindrasorasa KS, Sarkar B. In vivo and in vitro iron-replaced zinc finger generates free radicals and causes DNA damage. Eur J Biochem. 1996;9:5125-5130.
12. Kuthan H, Ullrich U, Estabrook RW. A quantitative test for superoxide radicals produced in biological systems. Biochem J. 1982;203(3):551-558.
13. Orekhovich VN. Sovremennye metody v biokhimii. Moscow: Meditsina; 1977. 392 p.
14. Bou Ghanem GO, Wareham LK, Calkins DJ. Addressing neurodegeneration in glaucoma: Mechanisms, challenges, and treatments. Prog Retin Eye Res. 2024;100:101261. doi:10.1016/j.preteyeres.2024.101261
15. Nita M, Grzybowski A. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid Med Cell Longev. 2016;2016:3164734. doi:10.1155/2016/3164734
16. Hurley DJ, Normile C, Irnaten M, O'Brien C. The intertwined roles of oxidative stress and endoplasmic reticulum stress in glaucoma. Antioxidants (Basel). 2022;11(5):886. doi:10.3390/antiox11050886
17. Nathanson JA, McKee M. Alterations of ocular nitric oxide synthase in human glaucoma. Invest Ophthalmol Vis Sci. 1995;36(9):1774-1784.
18. Mikheytseva I. Patogeneticheskoe znachenie endotelialnoj disfunkcii pri pervichnoj glaukome. Dosyagnennya biolohiyi medytsyny. 2009;14(2):17-20.
19. Donnarumma E, Ali M, Rushing A, Scarborough AJ. Zofenopril protects against myocardial ischemia-reperfusion injury by increasing nitric oxide and hydrogen sulfide bioavailability. J Am Heart Assoc. 2016;5(3)\:e003531. doi:10.1161/JAHA.116.003531
20. Bucci M, Vellecco V, Cantalupo A, et al. Hydrogen sulfide accounts for the peripheral vascular effects of zofenopril independently of ACE inhibition. Cardiovasc Res. 2014;102(1):138-147. doi:10.1093/cvr/cvu026

21. Nebbioso M, Franzone F, Lambiase A, et al. Oxidative stress implication in retinal diseases–a review. Antioxidants (Basel). 2022;11:1790. doi:10.3390/antiox11091790
22. Monti M, Terzuoli E, Ziche M, Morbidelli L. H2S dependent and independent anti-inflammatory activity of zofenoprilat in cells of the vascular wall. Pharmacol Res. 2016;113(Pt A):426-437. doi:10.1016/j.phrs.2016.09.017
23. Vaajanen A, Vapaatalo H. Local ocular renin-angiotensin systém–a target for glaucoma therapy? Basic Clin Pharmacol Toxicol. 2011;109(4):217-224. doi:10.1111/j.1742-7843.2011.00729.x
24. Holappa M, Vapaatalo H, Vaajanen A. Many faces of renin-angiotensin system—focus on eye. Open Ophthalmol J. 2017;11:122-142. doi:10.2174/1874364101711010122
25. Mitchell J, Ali F, Bailey L, Moreno L, Mackenzie L. Role of nitric oxide and prostacyclin as vasoactive hormones released by endothelium. Exp Physiol. 2008;93:141-147. doi:10.1113/expphysiol.2007.038588
26. Korelina V, Nagornova ZM, Seleznev A, et al. Review of herbal remedies in alternative antihypertensive and neuroprotective treatment of glaucoma. Med Biol Probl Life Act. 2024;1(31):136-148. doi:10.58708/2074-2088.2024-1(31)-136-148
27. Nakajima E, Otsugu-Kobayashi M, Uchida T, Orihara K, Shearer T, Azuma M. Calcium-dependent pathway as a primary cause of hypoxic RGC damage in monkey retinal explants. PLoS One. 2025;20:e0327246. doi:10.1371/journal.pone.0327246
28. Kwak S, Hwang S-K, Kwon M, Chun B. L-Serine protects murine retinal ganglion cells from oxidative stress via modulation of mitochondrial dysfunction. Curr Eye Res. 2024;49(7):1123-1130. doi:10.1080/02713683.2024.2355661
29. Salt T, Cordeiro M. Glutamate excitotoxicity in glaucoma. Eye (Lond). 2006;20(6):730-732. doi:10.1038/sj.eye.6701967
30. Goyal A, Srivastava A, Sihota R, Kaur J. Evaluation of oxidative stress markers in aqueous humor of primary open-angle glaucoma and primary angle-closure glaucoma patients. Curr Eye Res. 2014;39(8):823-829. doi:10.3109/02713683.2011.556299
31. Korelina VE. Study of the correction of lipid peroxidation by antioxidants in experimental glaucoma (experimental study): thesis. 1999. (in Russ.)
32. Rezapour J, Nickels S, Schuster AK, et al. Prevalence of depression and anxiety among participants with glaucoma in a population-based cohort study: The Gutenberg Health Study. BMC Ophthalmol. 2018;18(1):157. doi:10.1186/s12886-018-0831-1
33. Mendez-Ulrich JL, Sanz A, Feliu-Soler A, et al. Could White Coat Ocular Hypertension Affect the Accuracy of the Diagnosis of Glaucoma? Relationships Between Anxiety and Intraocular Pressure in a Simulated Clinical Setting. Appl Psychophysiol Biofeedback. 2018;43(1):49-56. doi:10.1007/s10484-017-9385-x
34. Otori Y, Takahashi G, Urashima M, Kuwayama Y. Evaluating the Quality of Life of Glaucoma Patients Using the State-Trait Anxiety Inventory. J Glaucoma. 2017;26(11):1025-1029. doi:10.1097/IJG.0000000000000761
35. Korelina VE, Gazizova I, Kuroyedov A, Didur MD. Glaucoma progression during the COVID-19 pandemics. Russ J Clin Ophthalmol. 2021;21:147-152. doi:10.32364/2311-7729-2021-21-3-147-152