Experimental Studies

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Correlations of parameters of the oxidative-antioxidative system in the lens, aqueous humor and tear fluid with the grade of lens opacity in rabbits with cataract and/or bacterial keratitis: an experimental study

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Purpose: *To determine correlations of the parameters of the oxidative-antioxidative system in the lens, aqueous humor and tear fluid with the grade of lens opacity in rabbits with cataract and/or bacterial keratitis treated versus not treated with methyl-ethyl pyridinol hydrochloride (MH).*

Methods: *Fifty-four adult Chinchilla rabbits were used for all experiments. Superficial bacterial keratitis only was induced only in the right eye (groups 1 and 2), and each of animals in group 2 received five four-week cycles of four-times-a-day-treatment with MH in the right eye separated by four-week breaks. In animals of groups 3 and 4, cataract was induced by nine-hours-a-day total exposure to 350-1150 nm ultraviolet (UV) radiation from a mercury-arc lamp for a period 40 weeks. Each of animals in group 4 received MH treatment in both eyes using the same scheme as noted above. In animals of groups 5 and 6, cataract was induced in the same way as in animals of group 3, and keratitis was induced in the same way as in animals of group 1. Each of animals in group 6 received MH treatment in the right eye using the same scheme as noted above. Group 7 comprised intact control rabbits. The Spearmen rank correlation was used to assess associations between the grade of lens opacity, activities of glutathione peroxidase (GP) and catalase (CT), and levels of malondialdehyde (MDA) and diene conjugate (DC).*

Results: *The grade of lens opacity was negatively correlated with the activities of GP and CT, and positively correlated with the levels of lipid peroxidation products (MDA and DC) for rabbits with keratitis only, cataract only, and especially keratitis plus cataract, treated or not treated with MH. There was no substantial change in the values of correlation coefficients for rabbits treated with MH compared to those not treated with MH.*

Conclusion: *Our findings of correlations between the above-mentioned parameters indicate the important role the metabolic abnormalities have in the formation of structural and functional changes in the lens of animals with corneal inflammation. These findings also lay ground for introducing pathogenesis-targeted metabolic correction with MH for oxidative-antioxidative system imbalance in ocular tissues.*

Keywords:

cataract, keratitis, lipid peroxidation, antioxidant enzymes, methyl-ethyl pyridinol hydrochloride, lens, cornea, experiment

Introduction

Cataract is a common disease of multiple etiologies associated with lens opacity due to various exogenous factors, the presence of comorbidities and age-related degenerative processes in the body [1, 2], which may result in loss of vision and blindness. Currently, cataract removal is the only treatment option available; it, however, still bears the risk of postoperative complications [1, 3-5].

Oxidative stress has a substantial role in the pathogenesis and progression of cataract and is caused by and imbalance between the pro-oxidative and anti-oxidative systems (including enzymatic components like superoxide dismutase, catalase (CT), and glutathione peroxidase (GP), and non-enzymatic components like peroxiredoxins, selenoproteins, thiol compounds, etc.), which induces the degradation and aggregation of lens proteins and apoptosis of epithelial cells of the lens, and cellular functional abnormalities. Additionally, active oxygen species and cytotoxic peroxides are also involved in the pathogenesis of cataract and other disorders of the

anterior and posterior eye segments. Cataractogenesis complicates the course of such disorders [6-10].

Pathogenetic mechanisms of cataract and inflammatory corneal disorders have been intensely investigated in clinical and experimental studies recently [2, 111-14], especially given the fact that cataract and inflammatory corneal disorders share common pathogenetic features that result in metabolic abnormalities [4, 9, 15, 16], leading to an increase in lens opacity grade and substantial reduction in visual acuity [15, 16].

Therefore, further research on the association between the development of cataract and concomitant corneal inflammatory process is warranted. In our animal studies, bacterial keratitis contributed to light-induced cataract formation, whereas the anti-oxidant methyl-ethyl pyridinol hydrochloride (MH) improved the resistance of the lens against the pathogenic effect of light energy [17, 18]. Pathological changes in the lens of rabbits with keratitis only, cataract only or cataract plus keratitis were accompanied by increased levels of lipid peroxidation (LPO) products, malondialdehyde (MDA) and diene conjugate (DC), in the presence of decreased activities of enzymatic components of the antioxidative system, GP and CT, in the lens, aqueous humor and tear fluid [19].

Given the above experimental findings of pathological changes in the lens and levels of metabolic parameters in the lens, aqueous humor and tear fluid in cataractous animal eyes with corneal inflammation, it was important to determine correlations between the parameters under study.

The purpose of the study was to determine correlations of the parameters of the oxidative-anti-oxidative system in the lens, aqueous humor and tear fluid with the grade of lens opacity in rabbits with cataract and/or bacterial keratitis treated versus not treated with MH.

Material and Methods

All animal experiments were performed in compliance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), Regulations on Working with Experimental Animals approved by the Decree of the Ministry of Health of Ukraine and the Law of Ukraine on Protection of Animals from Cruel Treatment No. 1759-VI dated December 15, 2009.

Fifty-four adult Chinchilla rabbits (mean age, 4.5 months; mean weight, 2.6 kg; weight range, 2.0-3.2 kg) maintained under normal vivarium conditions and fed and watered ad libitum, were used for all experiments. Superficial bacterial keratitis was induced only in the right eye (group 1, n=8, 8 eyes). After epibulbar anesthesia with 0.25% dicaine, a scraper was used to split the cornea and create a 4-mm defect in it, and 0.05 ml suspension of one-day Staphylococcus aureus culture prepared to a dilution of 106 colony forming units (CFU)/ml was injected into the defect. Positive Staphylococcus culture was obtained from patients with purulent ulcerative keratitis at Microbiology laboratory of SI “The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine”.

Group 2 (n = 8, 8 eyes) of animals with superficial keratitis was treated with MH. Each of these animals obtained totally five four-week cycles of four-times-a-day-treatment with MH in the right eye separated by four-week breaks.

In ad libitum animals of group 3 (n = 8, 16 eyes), cataract was induced by nine-hours-a-day total exposure to 350-1150 nm ultraviolet (UV) radiation from a mercury-arc lamp for a period 40 weeks.

In animals of group 4 (n = 7, 14 eyes), cataract was induced in the same way as in animals of group 3. Each of these animals obtained totally five four-week cycles of four-times-a-day-treatment with MH in both eyes separated by four-week breaks.

In animals of group 5 (n = 7, 7 eyes under study), cataract was induced in the same way as in animals of group 3, and superficial bacterial keratitis was induced in the same way as in animals of group 1.

In animals of group 6 (n = 7, 7 eyes under study), light-induced cataract and superficial bacterial keratitis were treated with five four-week cycles of four-times-a-day-treatment with MH in the right eye separated by four-week breaks.

Group 7 (n = 9, 18 eyes) comprised intact control rabbits with normal anterior and posterior segments of the eye.

Severity of ulcerative keratitis was assessed through clinical manifestations which were determined using lateral illumination. Rabbit eyes with induced keratitis were treated with sodium sulfacetamide 30% and levomycetin 0.25% twice daily until complete corneal re-epithelialization.

Severity of lens opacity was assessed by slit lamp biomicroscopy (Carl Zeiss Meditec, Jena, Germany) and scored on a scale of 0 to 5 [20].

Upon completing the experiment, animals were deeply anesthetized with thiopental sodium 10% (1.0 mL/kg, intramuscularly) and euthanized by air embolism. Eyes were enucleated and immediately placed on ice at 0 to 5 degrees C.

In our previous studies [17-19], activities of GP and CT and levels of LPO products (MDA and DC) were determined in the lens, anterior chamber and aqueous. The results were statistically analyzed: parametric Student t-test was used for normally distributed data (biochemical parameters), and non-parametric tests (Kruskal-Wallis and Mann-Whitney tests) were used for the grade of lens opacity.

The novelty of the results of the present study lies in finding correlations for previously published data [17-19]. Spearman rank correlation was performed for correlation analysis. Correlation coefficient (R) values were determined and interpreted taking in account the P-values specified in Table 1 [21].

Table 1. Rule of Thumb for Interpreting the Size of a Correlation Coefficient [21]

Size of Correlation	Interpretation
0.90 to 1.00 (−0.90 to −1.00)	Very strong positive (negative) correlation
0.70 to 0.90 (−0.70 to −0.90)	Strong positive (negative) correlation
0.50 to 0.70 (−0.50 to −0.70)	Moderate positive (negative) correlation
0.30 to 0.50 (−0.30 to −0.50)	Weak positive (negative) correlation
00 to 0.30 (0.00 to −0.30)	Negligible correlation
R=0	Кореляція відсутня абсолютно

Results

For intact animals, there were strong or moderate negative correlations of the activities of antioxidant enzymes and levels of LOP products in the lens (for GP and MDA, R = -0.63, $p < 0.05$; for GP and DC, R = -0.52, $p > 0.05$; for CT and MDA, R = -0.52, $p > 0.05$; for CT and DC, R = -0.50, $p > 0.05$), aqueous humor (for GP and MDA, R = -0.58, $p < 0.05$; for GP and DC, R = -0.50, $p > 0.05$; for CT and MDA, R = -0.48, $p > 0.05$; for CT and DC, R = -0.46, $p > 0.05$), and tear fluid (for GP and MDA, R = -0.53, $p < 0.05$; for GP and DC, R = -0.46, $p > 0.05$; for CT and MDA, R = -0.44, $p > 0.05$; for CT and DC, R = -0.42, $p > 0.05$). Statistically significant correlations were found only between GP activity and MDA levels in the lens, aqueous humor and tear fluid.

Table 2 shows correlations of the grade of lens opacity, activities of anti-oxidative enzymes, and levels of LPO products in the lens of rabbits with cataract only or bacterial keratitis only treated versus not treated with MH. Significant correlations between the grade of lens opacity and activities of anti-oxidative enzymes in the ocular tissues in rabbits with light-induced cataract were statistically significantly higher compared to the correlations between these variables for rabbits with keratitis. In addition, moderate or strong correlations between the metabolic parameters in the lens in rabbits with light-induced cataract were significantly stronger compared to the correlations between these variables for rabbits with keratitis.

For rabbits with bacterial keratitis treated with MH, there was a statistically significant increase in the strength of moderate negative correlations between the grade of lens opacity and GP and CT activities, and in the strength of positive correlations between the grade of lens opacity and MDA levels in the lens. Of note that a significant negative strong correlation was identified only between GP activity and MDA levels.

For rabbits with light-induced cataract treated with MH, correlations between the grade of lens opacity, levels of LPO products and activities of antioxidant enzymes in the lens were statistically significant and stronger compared to the correlations between these variables for rabbits with keratitis treated with MH.

Table 2. Correlations of the grade of lens opacity, activities of anti-oxidative enzymes, and levels of lipid peroxidation products in the lens of rabbits with light-induced cataract only or bacterial keratitis only treated versus not treated with methyl-ethyl pyridinol hydrochloride (MH)

Grade of lens opacity / GP	Keratitis only	Keratitis only plus MH	Light-induced cataract only	Light-induced cataract only plus MH
	Grade of lens opacity / CT	2	3	4
Grade of lens opacity / MDA	-0.57 (p<0.05)	-0.59 (p<0.05)	-0.78 (p<0.05)	-0.82 (p<0.05)
Grade of lens opacity / DC	-0.52 (p<0.05)	-0.53 (p<0.05)	-0.64 (p<0.05)	-0.67 (p<0.05)
GP / MDA	0.54 (p<0.05)	0.57 (p<0.05)	0.68 (p<0.05)	0.73 (p<0.05)
GP /DC	0.42 (p>0.05)	0.41 (p>0.05)	0.53 (p<0.05)	0.54 (p<0.05)
CT / MDA	-0.65 (p<0.05)	-0.69 (p<0.05)	-0.77 (p<0.05)	-0.80 (p<0.05)
CT / DC	-0.58 (p>0.05)	-0.56 (p>0.05)	-0.68 (p<0.05)	-0.65 (p<0.05)
КТ /МДА	-0.53 (p>0.05)	-0.51 (p>0.05)	-0.63 (p<0.05)	-0.62 (p<0.05)
КТ /ДК	-0.52 (p>0.05)	-0.50 (p>0.05)	-0.56 (p<0.05)	-0.54 (p<0.05)

Note: R, Spearman correlation coefficient; p, correlation p-value; DC, diene conjugate; CT, catalase; GP, glutathione peroxidase; MDA, malondialdehyde

Table 3. Correlations of the grade of lens opacity, activities of anti-oxidative enzymes, and levels of lipid peroxidation products in the lens of rabbits with light-induced cataract plus bacterial keratitis treated versus not treated with methyl-ethyl pyridinol hydrochloride (MH)

Correlation pairs	Light-induced cataract plus keratitis	Light-induced cataract plus keratitis plus MH
	5	6
Grade of lens opacity / GP	-0.82 (p<0.05)	-0.84 (p<0.05)
Grade of lens opacity / CT	-0.69 (p<0.05)	-0.72 (p<0.05)
Grade of lens opacity / MDA	0.76 (p<0.05)	0.75 (p<0.05)
Grade of lens opacity / DC	0.58 (p<0.05)	0.62 (p<0.05)
GP / MDA	-0.84 (p<0.05)	-0.86 (p<0.05)
GP / DC	-0.74 (p<0.05)	-0.72 (p<0.05)
CT / MDA	-0.72 (p<0.05)	-0.70 (p<0.05)
CT / DC	-0.68 (p<0.05)	-0.65 (p<0.05)

Note: R, Spearman correlation coefficient; p, correlation p-value; DC, diene conjugate; CT, catalase; GP, glutathione peroxidase; MDA, malondialdehyde

We also assessed correlations of the grade of lens opacity, activities of anti-oxidative enzymes, and levels of LPO products in the lens of rabbits with cataract plus keratitis treated versus not treated with MH (Table 3). For these rabbits, correlations were moderate or strong, and significantly stronger compared to the correlations between these variables for rabbits with keratitis only or cataract only (Table 2).

Table 4 shows correlations of the grade of lens opacity, activities of anti-oxidative enzymes, and levels of LPO products in the aqueous humor of rabbits with cataract only or bacterial keratitis only treated versus not treated with MH.

We found negative moderate correlations between the status of the lens and the activities of anti-oxidative enzymes, positive moderate correlations between the status

Table 4. Correlations of the grade of lens opacity, activities of anti-oxidative enzymes, and levels of lipid peroxidation products in the aqueous humor of rabbits with light-induced cataract only or bacterial keratitis only treated versus not treated with methyl-ethyl pyridinol hydrochloride (MH)

Correlation pairs	Keratitis only	Keratitis plus MH	Light-induced cataract only	Light-induced cataract plus MH
	1	2	3	4
Grade of lens opacity / GP	-0.64 (p<0.05)	-0.65 (p<0.05)	-0.84 (p<0.05)	-0.88 (p<0.05)
Grade of lens opacity / CT	-0.58 (p<0.05)	-0.56(p<0.05)	-0.77 (p<0.05)	-0.74 (p<0.05)
Grade of lens opacity / MDA	0.62 (p<0.05)	0.64 (p<0.05)	0.76 (p<0.05)	0.79 (p<0.05)
Grade of lens opacity / DC	0.47 (p>0.05)	0.48 (p>0.05)	0.59 (p<0.05)	0.61 (p<0.05)
GP / MDA	-0.72(p<0.05)	-0.74 (p<0.05)	-0.82 (p<0.05)	-0.79 (p<0.05)
GP / DC	-0.67 (p<0.05)	-0.66 (p<0.05)	-0.76 (p<0.05)	-0.74 (p<0.05)
CT / MDA	-0.65 (p<0.05)	-0.63 (p<0.05)	-0.73 (p<0.05)	-0.70 (p<0.05)
CT / DC	-0.62(p<0.05)	-0.60 (p<0.05)	-0.65 (p<0.05)	-0.62 (p<0.05)

Note: R, Spearman correlation coefficient; p, correlation p-value; DC, diene conjugate; CT, catalase; GP, glutathione peroxidase; MDA, malondialdehyde

Table 5. Correlations of the grade of lens opacity, activities of anti-oxidative enzymes, and levels of lipid peroxidation products in the aqueous humor of rabbits with light-induced cataract plus bacterial keratitis treated versus not treated with methyl-ethyl pyridinol hydrochloride (MH)

Correlation pairs	Light-induced cataract plus keratitis	Light-induced cataract plus keratitis plus MH
	5	6
Grade of lens opacity / GP	-0.87 (p<0.05)	-0.86 (p<0.05)
Grade of lens opacity / CT	-0.82 (p<0.05)	-0.78 (p<0.05)
Grade of lens opacity / MDA	0.83 (p<0.05)	0.82 (p<0.05)
Grade of lens opacity / DC	0.67 (p<0.05)	0.70 (p<0.05)
GP / MDA	-0.89 (p<0.05)	-0.87(p<0.05)
GP / DC	-0.82 (p<0.05)	-0.79 (p<0.05)
CT / MDA	-0.78 (p<0.05)	-0.75 (p<0.05)
CT / DC	-0.70 (p<0.05)	-0.68 (p<0.05)

Note: R, Spearman correlation coefficient; p, correlation p-value; DC, diene conjugate; CT, catalase; GP, glutathione peroxidase; MDA, malondialdehyde

of the lens and MDA levels, and negative moderate and strong correlations between the activities of antioxidant enzymes and LPO products in the aqueous humor for rabbits with keratitis not treated with MH, and these correlations were found to increase in strength for rabbits with keratitis treated with MH compared to rabbits with keratitis not treated with MH.

For rabbits with light-induced cataract, we found negative strong correlations between the grade of lens opacity and activities of anti-oxidative enzymes, positive correlation between the grade of lens opacity and MDA levels and moderate correlation between the grade of lens opacity and DC levels in the aqueous humor.

With regard to correlations between the activities of anti-oxidative enzymes and the levels of LPO products in the aqueous of cataractous rabbits not treated with MH, there were statistically significant strong correlations between GP activities and MDA levels, GP activities and DC levels, and CT activities and DC levels, and moderate correlation between CT activities and DC levels in the aqueous.

Table 6. Correlations of the grade of lens opacity, activities of anti-oxidative enzymes, and levels of lipid peroxidation products in the tear fluid of rabbits with light-induced cataract only or bacterial keratitis only treated versus not treated with methyl-ethyl pyridinol hydrochloride (MH)

Correlation pairs	Keratitis only	Keratitis plus MH	Light-induced cataract only	Light-induced cataract only plus MH
	1	2	3	4
Grade of lens opacity / GP	-0.62 (p<0.05)	-0.59 (p<0.05)	-0.82 (p<0.05)	-0.80 (p<0.05)
Grade of lens opacity / CT	-0.55 (p<0.05)	-0.53 (p<0.05)	-0.72 (p<0.05)	-0.73 (p<0.05)
Grade of lens opacity / MDA	0.59 (p<0.05)	0.56 (p<0.05)	0.73 (p<0.05)	0.70 (p<0.05)
Grade of lens opacity / DC	0.44 (p>0.05)	0.42 (p>0.05)	0.57 (p<0.05)	0.58 (p<0.05)
GP / MDA	-0.76 (p<0.05)	-0.74 (p<0.05)	-0.85 (p<0.05)	-0.81 (p<0.05)
GP / DC	-0.73 (p<0.05)	-0.71 (p<0.05)	-0.78 (p<0.05)	-0.74 (p<0.05)
CT / MDA	-0.69 (p<0.05)	-0.66 (p<0.05)	-0.76 (p<0.05)	-0.74 (p<0.05)
CT / DC	-0.64 (p<0.05)	-0.63 (p<0.05)	-0.67 (p<0.05)	-0.64 (p<0.05)

Note: R, Spearman correlation coefficient; p, correlation p-value; DC, diene conjugate; CT, catalase; GP, glutathione peroxidase; MDA, malondialdehyde

Table 7. Correlations of the grade of lens opacity, activities of anti-oxidative enzymes, and levels of lipid peroxidation products in the tear fluid of rabbits with light-induced cataract plus bacterial keratitis treated versus not treated with methyl-ethyl pyridinol hydrochloride (MH)

Correlation pairs	Light-induced cataract plus keratitis	Light-induced cataract plus keratitis plus MH
	5	6
Grade of lens opacity / GP	-0.85 (p<0.05)	-0.83 (p<0.05)
Grade of lens opacity / CT	-0.78 (p<0.05)	-0.75 (p<0.05)
Grade of lens opacity / MDA	0.80 (p<0.05)	0.78 (p<0.05)
Grade of lens opacity / DC	0.64 (p<0.05)	0.63 (p<0.05)
GP / MDA	-0.92 (p<0.05)	-0.89 (p<0.05)
GP / DC	-0.84 (p<0.05)	-0.81 (p<0.05)
CT / MDA	-0.83 (p<0.05)	-0.80 (p<0.05)
CT / DC	-0.73 (p<0.05)	-0.71 (p<0.05)

Note: R, Spearman correlation coefficient; p, correlation p-value; DC, diene conjugate; CT, catalase; GP, glutathione peroxidase; MDA, malondialdehyde

For cataractous rabbits treated with MH, there were statistically significant negative strong correlations between the status of the lens and the activities of anti-oxidative enzymes, positive strong correlation between the status of the lens and MDA levels, and moderate correlation between the status of the lens and DC levels in the aqueous.

With regard to correlations of the pathological changes in the lens with the activities of antioxidant enzymes and with LPO products in the aqueous of cataractous rabbits, Spearman correlation coefficients were larger for rabbits with cataract plus keratitis (Table 5) than for rabbits with cataract only or keratitis only (Table 4).

For rabbits with cataract plus keratitis treated with MH, there were statistically significant negative strong correlations between the status of the lens and the activities of anti-oxidative enzymes and positive strong correlations of the status of the lens with MDA levels, and with DC levels in the aqueous. In addition, for rabbits with cataract plus keratitis treated with MH, there were statistically significant negative strong correlation and moderate correlation of the activities of anti-oxidative enzymes and the levels of LPO products in the aqueous.

Table 6 shows correlations of the status of the lens, activities of anti-oxidative enzymes, and levels of LPO products in the tear fluid of rabbits with cataract only or bacterial keratitis only treated versus not treated with MH.

For rabbits with keratitis, there were statistically significant negative moderate correlations between the status of the lens and the activities of anti-oxidative enzymes, positive moderate correlation between the status of the lens and MDA levels, and statistically significant negative moderate correlation and strong correlation between the activities of anti-oxidative enzymes and the levels of LPO products in the tear fluid.

For rabbits with light-induced cataract, Spearman correlation coefficients were larger than for rabbits with keratitis, and there were negative correlation between the status of the lens and the activities of anti-oxidative enzymes, positive strong correlation between the status of the lens and MDA levels and moderate correlation between the status of the lens and DC levels in the tear fluid. In addition, there were statistically significant negative strong correlation and moderate correlation between the activities of anti-oxidative enzymes and the levels of LPO products in the tear fluid.

For rabbits with cataract plus keratitis, there stronger statistically significant moderate and strong correlations of the status of the lens with the activities of anti-oxidative enzymes and the levels of LPO products in the tear fluid, compared to rabbits with cataract only or rabbits with keratitis only (Table 7).

Moreover, the correlations found between the parameters of the oxidative and anti-oxidative system in the tear fluid of rabbits with cataract plus keratitis were statistically significant and stronger than the correlations between these parameters for rabbits with cataract only or rabbits with keratitis only.

There were statistically significant negative strong correlations between the grade of lens opacity and characteristics of the enzymatic anti-oxidative system in the tear fluid for rabbits with cataract plus keratitis treated with MH. In addition, for these rabbits, the grade of lens opacity was significantly and strongly positively correlated with MDA activities, and moderately positively correlated with DC activities in the tear fluid. Moreover, for rabbits

with cataract plus keratitis treated with MH, activities of anti-oxidative enzymes were significantly and strongly negatively correlated with levels of LPO products in the tear fluid.

Discussion

Recent studies on the biochemical and biophysical mechanisms mediating the longevity of the transparent optics of the eye lens indicated the importance of the spatial arrangement of (1) collagen fibrils in the stroma and (2) lens crystallins in the cytoplasm of lens fiber cells. However, while corneal collagen is remodeled continuously and replaced, lens crystallins are very long-lived and are not replaced and so accumulate posttranslational modifications (PTMs) over a lifetime. Eventually, a tipping point is reached when protein aggregation results in increased light scatter, inevitably leading to the iconic protein condensation–based disease, age-related cataract [22].

Aging is a major risk factor for age-related ocular diseases including those of the cornea and lens [23].

Oxidative stress is a major factor of damage to the epithelial cells of the cornea and lens, resulting in lens opacity. Additionally, it may result in mitochondrial dysfunction, imbalance between the oxidative and anti-oxidative systems, apoptosis and other metabolic and cellular abnormalities in the eye [2, 6, 9].

Studies have demonstrated metabolic changes in the tear fluid and aqueous humor in the course of keratitis. Thus, we have reported previously on increased peroxidation and decreased activities of the enzymatic anti-oxidative system in the tear fluid and aqueous humor in animals with experimental bacterial keratitis [19]. Shrestha and colleagues [24] concluded that (1) bacterial keratitis significantly changes the tear metabolite profile and (2) tear fluids can be used to map the metabolic pathways and uncover metabolic markers associated with bacterial keratitis. The aqueous humor, in turn, plays an important role in the regulation of the homeostasis of the ocular tissues. Different eye pathologies influence its composition, modifying its physiological properties, and cause pathological conditions in the anterior segment [25]. Increased concentrations of IL-1β, IL-6, IL-8, and IFN-γ in the aqueous humor have been demonstrated in patients with keratitis.

Substantial abnormalities in the antioxidant status and other biochemical parameters in the aqueous humor in cataractogenesis have been reported by studies [19, 27, 28]. There were, however, controversial reports on metabolic changes in the tear fluid in cataractogenesis. Currently, there are no reliable studies regarding tear film proteomic changes in the course of cataract formation [29].

A large-scale randomized clinical trial would be of great value in finding potential tear film biomarkers for cataract development [29]. We should also take in account the presence of publications on cataractogenic effects of excessive sunlight on the lens, anterior segment and eye in general [30, 31]. Additionally, inducing cataract in animals by subjecting them to 350-1150 nm UV radiation from a mercury-arc lamp can result in certain changes in biochemistry of the tear fluid, which was demonstrated in our studies [19]. Thus, in animals subjected to polychromatic light, the aqueous humor levels of LPO products, MDA and DC, were found to be increased by 57.4% and 47.8%, respectively, whereas the activities of anti-oxidative enzymes, GP and CT, were found to be statistically significantly decreased by 48.0% and 40.1%, respectively.

Therefore, given the presence of statistically significant correlations between the grade of lens opacity and characteristics of oxidative-antioxidative homeostasis in the eye, and findings of previous studies [19], exhaustion of the anti-oxidative system with the development of oxidative stress (and especially in the presence of concomitant corneal inflammation) plays an important role in the progression of pathological changes in the lens.

We found the grade of lens opacity to be negatively correlated with the activities of anti-oxidative enzymes, and positively correlated with the levels of LPO products in rabbits with cataract plus keratitis. This finding indicates the important role these metabolic abnormalities have in the formation of structural and functional changes in the lens of cataractous animals with corneal inflammation.

Given that oxidative stress is involved in the pathogenesis of various types of cataract, studies investigated the anti-cataract potential of various compounds with anti-oxidative and other properties [2, 3, 6, 32].

Of note, randomized trials have failed to find effects of antioxidant vitamin supplementation for cataract [33].

Braakhuis and colleagues [34] proposed that the lens utilizes a unique internal microcirculation system to actively deliver antioxidants to these different regions, and that selecting antioxidants that can utilize this system is the key to developing novel nutritional therapies to delay the onset and progression of lens cataract.

Some natural antioxidant compounds prevent cataracts by inhibiting protein glycation and aldose reductase and preventing apoptosis of the eye lens [35]. Other reviews underscored the importance of comprehensive consideration of pharmaceutical preparations that target cataractogenesis to promote clinical anti-cataract drug research. The development of a nanosystem that incorporates both antioxidants and protein aggregation inhibitors may enhance the overall effectiveness of cataract prevention and treatment [36-38].

The current literature on the use of antioxidant therapies to prevent cataract formation is sparse. There is a lack of evidence-based conclusions; further clinical studies are needed to endorse the use of antioxidant strategies in patients to prevent cataractogenesis [32].

Our findings of strong correlations between pathological changes in the lens, activities of antioxidant enzymes and levels of LPO products in the lens, aqueous humor and tear fluid in animals with cataract plus keratitis

treated with MH indicate the efficacy of this exposure to the medication targeting the etiopathogenesis in preclinical research.

Therefore, molecular and cellular studies on the key compounds required for maintaining the transparency and optical characteristics of the lens and cornea will facilitate the development of novel therapeutic agents [22].

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Abbreviations: *CT, catalase; DC, diene conjugate; GP, glutathione peroxidase; LPO, lipid peroxidation; MDA, malondialdehyde; MH, methyl-ethyl pyridinol hydrochloride; TAO, total antioxidant activity*