UDC 617.7-007.681:616.379-008.64-092.9:617.72-07+577.11

Effect of ocular hypertension on the levels of lipid peroxidation products in anterior eye tissues in experimental diabetes

V.R. Yurevych, Cand. Sc. (Med), Ass. Prof.

Danylo Halytsky Lviv National Medical University

Lviv (Ukraine)

E-mail: yurevych@yahoo.com

Background: *Even though there has been significant advance in the care of ocular complications of diabetes mellitus, the issue related to the prevention of and therapy for this pathology is still an important area that needs to be addressed.*

Purpose: *To investigate the effect of ocular hypertension on the levels of lipid peroxidation products in anterior eye tissues in experimental diabetes.*

Materials and Methods: Thirty-two rabbits were divided into four groups: group 1 (controls; 10 rabbits), group 2 (diabetes and ocular hypertension (D+OH) group; 8 rabbits), group 3 (diabetes-only group; 7 rabbits) and group 4 (ocular hypertension-only group; 7 rabbits). In these animals, the levels of malondialdehyde and diene conjugate in the anterior chamber angle tissue and aqueous humor were determined.

Results: *The activation of lipid peroxidation was seen as increased diene conjugate and malondialdehyde levels in the anterior chamber angle tissue and aqueous humor in animals with diabetes. In D+OH group, the levels of malondialdehyde and diene conjugates in the anterior chamber angle tissue, and the levels of malondialdehyde and diene conjugates in the aqueous humor were 23.8%, 21.1%, 31.9% and 28.1%, respectively, higher than those in diabetic animals without ocular hypertension.*

Conclusion: *Increased lipid peroxidation imbalance found in anterior eye tissues and aqueous humor in animals with diabetes mellitus and experimental ocular hypertension is a key element of the mechanism of the accelerated destruction in the aqueous humor outflow pathways.*

Key words: ocular hypertension, experimental diabetes, anterior eye, lipid peroxidation

Introduction

The issues related to the efficacy of treatment of glaucomatous optic neuropathy are important for the ophthalmologist, especially when the glaucomatous process develops in the presence of diabetes mellitus [1, 2].

Although the pathogenesis of the former disease has some elements in common with that of the latter, the question remains open as to what are the risks of glaucomatous damage to the eye in diabetic patients as compared to healthy individuals [3, 4].

The aqueous humor provides cell nutrition and metabolism of the avascular drainage system of the eye, and, since it contains free radicals and lipid peroxidation products, this can result in a cytotoxic effect, especially when antioxidative system components are deficient; therefore, lipid peroxidation process is an important pathogenetic factor in glaucoma [5-11].

Oxidative stress and metabolic abnormalities also play a role in the mechanisms of damage to neural eye structures in glaucoma.

Thus, in experimental glaucoma, the levels of toxic lipid peroxidation products (malondialdehyde and diene conjugates) in the retina and optic nerve have increased by 32.5% and 19.8% at week 3, by 69.8% and 27% at week 5, and were at maximum (110.7% and 54.9%) by week 10. This resulted in imbalance in the thiol-disulphide system of proteins (reduction in protein thiol levels by 25%, and an increase in disulphide levels by 44.8%) by the end of the experiment [12-14].

At the same time, it is known that in diabetes mellitus, oxidative stress and glycolysation processes

play a key role in pathogenetic mechanisms of ocular damage.

Thus, evidence has been reported on the role of free radical processes (and lipid peroxidation, in particular) in diabetic damage to retinal vessels and other intraocular structures. In diabetes, free radicals generated additionally in glucose autooxidation and protein glycolysation can induce lipid peroxidation not only in the retinal vascular system, but also in the membranes of cellular and subcellular structures of the retina [15, 16].

Therefore, we believe that investigation of pathogenetic features of ocular damage in glaucomatous process developing in diabetes mellitus is of scientific and practical importance.

The purpose of the study was to investigate the effect of ocular hypertension on the levels of lipid peroxidation products in anterior eye tissues in experimental diabetes.

Materials and Methods

A total of 32 Chinchilla adult male rabbits (weight, 2.5–3.2 kg) were used in the study. All care and use of animals followed guidelines stipulated by the 2012 International Guiding Principles for Biomedical Research Involving Animals issued by the Council for the International Organizations of Medical Sciences.

Animals were divided into four groups: group 1 (controls; 10 rabbits), group 2 (diabetes and ocular hypertension (D+OH) group; 8 rabbits), group 3 (diabetes-only group; 7 rabbits) and group 4 (ocular hypertension-only (OH) group; 7 rabbits). Each group was subdivided into two subgroups, depending on the study time points used (subgroup 1, three weeks; subgroup 2, six weeks).

At the baseline and at the end of the experiment, animals underwent ophthalmic examination and intraocular pressure (IOP) measurement with a non-contact tonometer Topcon CT–80 (Topcon Corp., Tokyo, Japan).

Diabetes was induced with a single intravenous injection of streptozotocin, 65 mg/kg body wt [17, 18].

At day 2 after streptozotocin injection, animals groups 2 and 4 were anesthetized generally with ketamine (50 mg/kg), and locally with 0.5% procaine hydrochloride instilled into the conjunctival sac. Thereafter, 0.2% methylcellulose was administered into the anterior chamber of these animals to model ocular hypertension. Immediately after injection, the rabbit eye was examined biomicroscopically for injection-related trauma. The IOP was measured every few hours [19].

At the conclusion of the study, rabbits were euthanized by 100 mg/kg pentobarbital sodium injection administered intravenously via the marginal ear vein.

The malondialdehyde and diene conjugate levels in the anterior chamber angle tissue and aqueous humor were determined using the methodologies slightly modified to perform spectrophotometry with wedge cells for measurements of 0.2-mL of saline samples.

The technique to determine the malondialdehyde level is based on the fact that, in acid medium at 100°C, malondialdehyde reacts with 2-thiobarbituric acid to form a pink-coloured trimethine complex that exhibits an absorption maximum at 532 nm. Tissue homogenate was prepared in a ratio of 1 g of wet tissue to 10 times (w/v) homogenization medium. 0.1 mL of the fluid under investigation was taken into a test tube (10 mL), and 3 mL of 1% orthophosphoric acid (pH 2.0), 1 mL of 0.6% thiobarbituric acid solution, and 0.1 mL of 0.28% ferrous sulphate solution were added. The sample was vortexed and heated in a 100 °C water bath for 60 min. After cooling in a 0°-2°C cold water bath, 4 mL of butanol solution was added. The sample was mixed thoroughly, and centrifuged at 3,000 Ч g for 10 min. The optical density reading of upper layer was taken with a spectral colorimeter Specol–210 at the 535-nm wavelength against butanol. The levels of products reacting with thiobarbituric acid were calculated taking into account the molar extinction coefficient of malondialdehyde (1.56 x 105 M-1•cm-1) and were expressed in nM/g of the tissue. The coefficient of variation of the technique [20] has been reported to be 5.2%.

The technique to determine the level of diene conjugates is based on the fact that, in the course of lipid peroxidation in the stage of formation of free radicals, a system of double conjugated bonds is established in molecules of polyunsaturated fatty acids, resulting in the appearance of an absorption maximum at 233 nm. Tissue homogenate was prepared in a ratio of 1 g of wet tissue to 10 times (w/v) homogenization medium. 0.1 mL of the fluid under investigation was taken into a test tube, and 4.5 mL of heptane-isopropyl alcohol (1:1, v/v) extraction mixture were added. After extraction, 0.5 mL of distilled water was added to the mixture, and 0.5 mL of the separated heptanes phase was taken and mixed with 2.5 mL of ethanol. The optical density was read with a spectrophotometer SF–26 at 233 nm against ethanol. The levels of diene conjugates were expressed either in nM/mL of the liquid under study or nM/g of the tissue, based on the molar extinction coefficient (2.2 x 105 M-1•cm-1).

The coefficient of variation of the technique [20] has been reported to be 4%.

The data obtained were statistically processed with SPSS 11.0 software [21].

Results and Discussion

Table 1 summarizes the data on the effect of ocular hypertension on peroxidation processes in the anterior chamber angle tissue in experimental diabetes in rabbits.

The table shows that, the malondialdehyde level in the anterior chamber angle tissue in rabbits of D+OH group increased to 792.51±50.36 nM/g (176.6%) ($P < 0.001$) and to 966.62±68.20 nM/g (215.4%) at the first time point and at the second time point, respectively, compared to the norm of 448.76±30.40 nM/g ($P < 0.001$).

As can be seen from the comparison of the data of D+OH and diabetes-only groups, the increase in the malondialdehyde level in the anterior chamber angle tissue in the former was higher than that in the latter. Thus, at the first time point and at the second time point, the difference was equal to 13.8% and 23.8%, respectively ($P < 0.05$).

The malondialdehyde level in the anterior chamber angle tissue in rabbits with ocular hypertension only increased to 606.58±50.24 nM/g (134.0%) at the first time point, and to 680.36 ± 54.70 nM/g (170.3%) at the second time point, compared to the norm of 452.67±38.40 nM/g ($P < 0.001$).

Table 1. Effect of ocular hypertension on lipid peroxidation processes in anterior eye tissues in experimental diabetes in rabbits (n=7-10)

Characteristic under study	Statistic indices	Experimental conditions		
		Norm (i.e., controls)	First time point (day 7)	Second time point (day 14)
Malondialdehyde (nM/g tissue)	**Diabetes + hypertension**			
	M±m	448,76±30,40	792,51±50,36	966.62±68.20
	P	-	<0.001	<0.001
	%	100,0	176.6	215.4
	P1	>0.05	>0.05	<0.05
	%1	97,9	113.8	123.8
	P2	>0,05	<0.05	<0.01
	%2	99.1	130.7	142.1
	Diabetes			
	M±m	458.30±34.78	696.62±45.20	780.48±50.12
	p	-	<0.001	<0.001
	%	100.0	152.0	170.3
	Hypertension			
	M±m	452.67±38.40	606.58±50.24	680.36±54.70
	p	-	<0.05	<0.01
	%	100.0	134.0	150.3
Diene conjugates (nM/g tissue)	**Diabetes + hypertension**			
	M±m	101.26±6.72	145.81±10.40	177.31±10.80
	P	-	<0.01	<0.001
	%	100.0	144.0	175.1
	P1	>0.05	>0.05	>0.05
	%1	102.9	111.8	121.1
	P2	>0.05	>0.05	>0.05
	%2	96.1	111.6	124.5
	Diabetes			
	M±m	98.45±6.20	130.45±9.56	146.40±9.78
	p	-	<0.05	<0.001
	%	100.0	132.5	148.7
	Hypertension			
	M±m	105.36±7.56	130.65±8.24	142.43±12.50
	p	-	<0.05	<0.05
	%	100.0	124.0	135.2

Note: P, significance of difference versus controls; P1, significance of difference versus diabetes-only animals; P2, significance of difference versus hypertension-only animals

As can be seen from the comparison of the data of D+OH and hepertension-only groups, the increase in the malondialdehyde level in the anterior chamber angle tissue in the former was higher than that in the latter. Thus, at the first time point and at the second time point, the difference was equal to 30.7% (P<0.05), and 42.1% (P<0.01), respectively.

Table 1 demonstrates that, the diene conjugate level in the anterior chamber angle tissue in rabbits of D+OH group increased to 145.81±0.40 nM/g (144.0%) (P<0.01) and to 177.31±10.80 nM/g (175.1%) at day 7 and day 14, respectively, compared to the norm of 101.26±6.72 nM/g (P < 0.001).

The diene conjugate level in the anterior chamber angle tissue in rabbits of diabetes-only group increased to 130.45±9.56 nM/g (132.5%) (P<0.05) and to 146.40±9.78 nM/g (148.7%) at the first time point and at the second time point, respectively, compared to the norm of 98.45±6.20 nM/g (P < 0.001).

It must be pointed out that, at both time points of the study, the increase in the diene conjugate level in the anterior chamber angle tissue in D+OH

Table 2. Effect of ocular hypertension on lipid peroxidation processes in the aqueous humor in experimental diabetes in rabbits (n=7-10)

Characteristic under study	Statistic indices	Experimental conditions		
		Norm (i.e., controls)	First time point (day 7)	Second time point (day 14)
Malondialdehyde (nM/g tissue)	**Diabetes + hypertension**			
	M±m	54.73±3.40	87.65±5.75	112.92±7.43
	P	-	<0.001	<0.001
	%	100.0	160.1	206.3
	P1	>0.05	<0.05	<0.05
	%1	102.4	123.8	131.9
	P2	>0.05	<0.05	<0.01
	%2	101.7	130.1	138.0
	Diabetes			
	M±m	53.45±3.24	70.82±5.30	85.62±5.92
	p	-	<0.05	<0.001
	%	100.0	132.5	160.2
	Hypertension			
	M±m	53.80±4.07	67.35±4.40	81.78±6.92
	p	-	<0.05	<0.01
	%	100.0	125.2	152.0
Diene conjugates (nM/g tissue)	**Diabetes + hypertension**			
	M±m	21.94±1.26	28.15±1.30	33.72±1.64
	P	-	<0.01	<0.001
	%	100.0	128.3	153.7
	P1	>0.05	>0.05	<0.01
	%1	105.3	114.1	128.1
	P2	>0.05	>0.05	<0.05
	%2	104.1	115.8	130.6
	Diabetes			
	M±m	20.82±1.30	24.67±1.52	26.32±1.60
	p	-	>0.05	<0.05
	%	100.0	118.5	126.4
	Hypertension			
	M±m	21.07±1.54	24.30±1.52	25.81±1.43
	p	-	>0.05	<0.05
	%	100.0	115.3	122.5

Note: P, significance of difference versus controls; P1, significance of difference versus diabetes-only animals; P2, significance of difference versus hypertension-only animals

group was higher than that in the diabetes-only group. Thus, at the first time point and at the second time point, the difference was equal to 11.8% and 21.1%, respectively.

The diene conjugate level in the anterior chamber angle tissue in rabbits of ocular hypertension-only group increased to 130.65±8.24 nM/g (124.0%) ($P < 0.05$) and to 142.43±12.50 nM/g (135.2%) at the first time point and at the second time point, respectively, compared to the norm of 105.36 ±7.56 nM/g ($P < 0.05$).

It should be noted that, at the first time point and at the second time of the study, the increase in the diene conjugate level in the anterior chamber angle tissue in diabetic rabbits with ocular hypertension was 11.6% and 24.5% higher than that in animals with ocular hypertension only.

Table 2 summarizes the data on the effect of ocular hypertension on peroxidation processes in the aqueous humor in experimental diabetes in rabbits.

In rabbits of D+OH group, the malondialdehyde level in the aqueous humor increased to 87.65±5.75 nM/g (160.1%) ($P < 0.001$) and to 112.92±7.43 nM/g (206.3%) at day 7 and at day 14, respectively, compared to the norm of 54.73±3.40 nM/g ($P< 0.001$).

Table 2 shows that, in the diabetic rabbits without ocular hypertension, the malondialdehyde level in the aqueous humor increased to 70.82±5.30 nM/g (132.5%) ($p<0.05$) and to 85.62±5.92 nM/g (160.2%) at the first time point and at the second time point, respectively, compared to the norm of 53.45±3.24 nM/g ($P < 0.001$).

In the hypertensive rabbits with diabetes, the malondialdehyde level in the aqueous humor increased 23.8% ($P < 0.05$) more at day 7, and 31.9% ($P < 0.05$) more at day 14, compared to that in diabetic animals without hypertension.

In animals of D+OH group, the malondialdehyde level in the aqueous humor at the first time point and at the second time point was 30.1% ($P<0.05$) and 38.0% ($P<0.01$), respectively, higher compared to that in animals of ocular hypertension-only group.

In the hypertensive rabbits with diabetes, the diene conjugate level in the aqueous humor increased to 28.14±1.30 nM/g (128.3%) ($P < 0.01$) and to 33.72±1.64 nM/g (153.70%) at the first time point and at the second time point, respectively, compared to the norm of 21.94±1.26 nM/g ($P < 0.001$).

In the diabetic rabbits without ocular hypertension, the diene conjugate level in the aqueous humor increased to 24.67±1.52 nM/g (118.5%) at the first time point, and to 26.32±1.60 nM/g (126.4%) at the second time point, compared to the norm of 20.82±1.30 nM/g ($p<0.05$).

Therefore, within the study period, the diene conjugate level in the aqueous humor in diabetic animals with ocular hypertension was higher than in those without hypertension. In particular, at the first time point and at the second time point, it was 14.1% and 28.1%, respectively ($p<0,001$), higher than that in diabetic animals without hypertension.

In the rabbits with hypertension only, the diene conjugate level in the aqueous humor increased to 24.30±1.52 nM/g (115.3%) at the first time point, and to 25.81±1.43 nM/g (122.5%) at the second time point, compared to the norm of 21.07±1.54 nM/g ($p<0.05$).

The comparison of the data reveals that, at day 7 and day 14, the diene conjugate level in the aqueous humor in diabetic animals with ocular hypertension was 15.8% and 30.6% ($p<0.05$), respectively, higher than in animals with hypertension only.

Our data on activation of lipid peroxidation and reduction in antioxidant activity in diabetes-only and ocular hypertension-only models agree with the findings of other investigators. Thus, the activation of lipid peroxidation (i.e., increased plasma diene conjugates and malondialdehyde, and reduced total plasma antioxidant activity and peroxidation resistance of erythrocyte membranes) has been found in animals with glaucoma [22]. This can explain our findings of the increased lipid peroxidation levels in aqueous humor in ocular hypertension.

It should be noted that, in general, the analysis of the results of our study showed that the development of ocular hypertension in animals with streptozotocin-induced diabetes was accompanied by accelerated oxidation processes in the anterior chamber angle tissue.

Under these conditions, at the last time point of the study, the levels of final products of lipid peroxidation in these animals were 23.8% and 42.1%, respectively, higher than those in diabetes-only and ocular hypertension-only groups.

Therefore, this is the evidence that, in the presence of experimental diabetes, ocular hypertension results in abnormally high oxidative stress, with significantly increased levels of lipid peroxidation, followed by accelerated destruction of the membrane structures of the ocular outflow system.

Conclusion

Increased lipid peroxidation imbalance found in anterior eye tissues and aqueous humor in animals with diabetes mellitus and experimental ocular hypertension is a key element of the mechanism of the accelerated destruction in the aqueous humor outflow pathways under these conditions. Thus, in D+OH group, the levels of malondialdehyde and diene conjugates in the anterior chamber angle tissue, and the levels of malondialdehyde and diene conjugates in the aqueous humor were 23.8%, 21.1%, 31.9% and 28.1%, respectively, higher than those in diabetic animals without ocular hypertension.

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Received 05.05.2015