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The role of lipid peroxidation in the pathogenesis of experimental diabetic retinopathy

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Abstract

The aim of work – to analysis of changes in the level of primary products of lipid peroxidation in experimental animals were simulated diabetic retinopathy and against the background of its correction.

The obtained results indicate an increase in the content of lipid peroxidation, starting from the 30th and with further progression on the 60th and 180th days of experimental diabetic retinopathy, which is confirmed by an increase in the level of diene conjugates in the 2nd group, the maximum of which is observed at the 3rd stage. Correction with hypoglycemic agents in the 3rd group had a positive effect, but was not able to reduce the level of primary products of lipid peroxidation and restore antioxidant activity, so the use of additional agents became necessary. The use of aflibercept and a nitric oxide donor in the 4th group to correct the development of diabetic retinopathy significantly suppressed oxidative stress, the maximum of which occurred on the 180th day of the experiment, but did not reach control indicators. It was proven that the combined administration of bromfenac and aflibercept in the

5th group significantly reduced the number of primary LPO products, but not as significantly as in the 4th group. It was proved that the administration of aflibercept, L-carnitine and bromfenac to the animals of the 6th group reduced the content of diene conjugates already on the 30th and continued on the 60th and 180th days of the study, but it also did not reach the control indicators. The combination of metformin, aflibercept, L-arginine and citicoline in rats of the 7th group proved to be the most effective correction, as evidenced by the normalization of the level of the studied indicators on the 30th and 60th days of the experiment, and on the 180th, a decrease in the content of markers was detected oxidative stress to control indicators.

Introduction. According to the WHO, diabetic retinopathy (DR) is the main cause of vision loss and blindness in diabetes. This pathology is the main cause of visual impairment in the population of economically developed countries [1-4] and is diagnosed in 40-85 % of patients suffering from diabetes. It should be noted that even with the compensation of carbohydrate metabolism, the development of DR continues [1-5].

One of the key links in the pathogenesis of membrane destabilization and further development of pathology is the strengthening of lipid peroxidation processes (LPO) [6, 7]. At the initial stage of this process, diene conjugates of fatty acids are formed. Lipid hydroperoxides are formed from the latter when interacting with hydroxyl radicals. It should be noted that the formed LPO compounds cause disturbances in the phospholipid complex, which, in turn, leads to a functional imbalance in cells, organs, and subsequently in the whole body. Upon contact with peroxide radicals, fatty acids disintegrate into fragments, on the periphery of which there are aldehyde groups with high reactivity [6]. Therefore, lipid peroxidation plays a key role in the dysregulation of membrane lipid metabolism, the permeability of biological membranes, and the impact on their physicochemical properties in both physiological and pathological conditions [7].

The aim of work – to analysis of changes in the level of primary products of lipid peroxidation in experimental animals were simulated diabetic retinopathy and against the background of its correction.

Materials and methods. The study was conducted on white Wistar rats weighing 180-200 g. According to the tasks, the animals were divided into 7 groups:

1st group – intact animals;

2nd group – 60 animals with modelling of DR without correction (control pathology);

3rd group –60 animals with modelling of DR with correction of hyperglycemia;

4th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept and L-arginine solution;

5th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept and bromfenak;

6th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept, L-carnitine solution and bromfenak;

7th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept, L-arginine solution and citicoline.

Type 2 diabetes and DR were modeled by intraperitoneal administration of streptozotocin (Sigma, USA) dissolved in 0.1 M citrate buffer with pH 4.5 [14, 15]. Dose of streptozocin of 55 mg/kg of animal weight was divided into two administrations. Administration of streptozocin was preceded by a high-fat diet for 28 days [10].

Animals were subjected to research by decapitation in accordance with the "Rules for the performance of work using experimental animals", approved by the Order of the Ministry of Health of Ukraine No. 249 of 01.03.2012 and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (as amended on 15.12.2009 and 16.10.2012).

The hypoglycemic medicine – metformin (Merck Sante, manufactured in France) - at at a dose of 300 mg/kg body weight [11] in a 0.9 % sodium chloride solution through a syringe with an intragastric probe daily, during the entire experiment.

The introduction of L-arginine solution, which is NO donor, (SIMESTA, manufactured in China, quality standard USP32) was carried out by intragastric administration of L-arginine solution in 0.9 % sodium chloride solution at a dose of 500 mg/kg [12] through a syringe with an intragastric probe.

Aflibercept (anti-VEGF therapy) was administered in the form of subconjunctival injections at a dose of 0.08 ml (25 mg/ml) [13] with an interval of 1 injection every 30 days.

Citicoline – 81.8 mg/kg (0.33 ml/kg) was administered intramuscularly once per a day [14].

Bromfenak – was introduced of 0,09 % eyes drop solution once per a day [15].

L-carnitine (manufacturing by "Sigma", USA) was administrated in the form of an aqueous solution through a syringe with an intragastric probe at a dose of 25 mg/100 g of animal weight [16, 17].

Determination of the content of diene conjugates in blood serum was carried out by the spectrophotometric method [18]. It was considered that primary products of free radical oxidation, one of which are diene conjugates, are characterized by absorption in the ultraviolet region of the spectrum with a maximum at 220-230 nm. The millimolar extinction coefficient of diene conjugates is 24.4 mmol1 cm1 [19].

To identify changes in the studied indicators between different groups and at different stages, we used parametric statistical methods, which are based on operating with the parameters of the statistical distribution (mean and variance). The methods used are designed for normally distributed data, so we checked all data for normality using E.I. Pustilnyk's asymmetry and kurtosis criterion. All the data that we are considering turned out to be normally distributed, so we can pairwise compare the mean values of the samples. Note that in the following comparisons we perform comparisons in independent samples. These will be comparisons between different groups of animals or comparisons between the same group of animals (but since there is no correspondence between animals in the samples, they will also be independent). The value $p<0.05$ was chosen as the reliability criterion. An analysis was performed to see if the means differed. The results of determining the t-test give an answer about the equality or difference of the mean values, but they do not provide an opportunity to accurately measure the difference between the mean values. Note that this difference is quite conditional. This difference was calculated as a percentage. Thus, we demonstrated a comparison of mean values between different groups of animals.

Results of study and their discussion:

The dynamics of the diene conjugates level in the blood of experimental animals with simulated diabetic retinopathy and with different methods of its correction on the 30th, 60th, and 180th day are presented in Table 1.

It was established that at the first stage in the 2nd group, the level of primary products of lipid peroxidation is 40.3 % ($p<0.001$) higher compared to the intact group, at the second stage it is increased on 42.5 % ($п<0.001$). At the third stage, the value of the indicator is 43.7 % ($p<0.001$) higher compared to group 1 and on 5.6 % ($p<0.05$) more compared to the first stage.

In the third group at the first stage, the content of primary LPO products is 30.7 % ($p<0.001$) higher than the obtained values during the study of intact animals. Compared to group No. 2, the level is lower on 6.2 % ($p<0.001$). At the second stage, the level of the marker is higher on 33.3 % ($p<0.001$), and compared to group 2, it is higher on 15.8 %

(p<0.001). At the third stage, the level is higher on 33.5 % (p<0.001) compared to the 1st group, and on 18 % (p<0.001) lower compared to group 2.

Table 1. – The diene conjugates level in the blood of experimental animals with simulated diabetic retinopathy and with different methods of its correction on the 30th, 60th, and 180th day (M±m), (µM/l)

Stages Group	I stage		II stage		III stage	
1 group	50,19±1,5		50,18±2,02		50,18±1,56	
			I-II	p>0,05	I-III	p>0,05
					II-III	p>0,05
2 group	84,12±1,91		87,24±1,63		89,07±2,03	
	1-2	p<0,001	I-II	p>0,05	I-III	p<0,05
			1-2	p<0,001	II-III	p>0,05
					1-2	p<0,001
3 group	72,4±2,1		75,3±2,04		75,48±1,88	
	1-3	p<0,001	I-II	p>0,05	I-III	p>0,05
	2-3	p<0,001	1-3	p<0,001	II-III	p>0,05
			2-3	p<0,001	1-3	p<0,001
					2-3	p<0,001
4 group	68,12±1,94		66,14±1,77		63,24±1,58	
	1-4	p<0,001	I-II	p>0,05	I-III	p<0,05
	2-4	p<0,001	1-4	p<0,001	II-III	p>0,05
	3-4	p>0,05	2-4	p<0,001	1-4	p<0,001
			3-4	p<0,001	2-4	p<0,001
					3-4	p<0,001
5 group	68,74±1,87		68,42±1,7		65,01±1,96	
	1-5	p<0,001	I-II	p>0,05	I-III	p>0,05
	2-5	p<0,001	1-5	p<0,001	II-III	p>0,05
	3-5	p>0,05	2-5	p<0,001	1-5	p<0,001
	4-5	p>0,05	3-5	p<0,01	2-5	p<0,001
			4-5	p>0,05	3-5	p<0,001
					4-5	p>0,05
6 group	64,12±1,42		64,01±1,69		62,12±1,76	
	1-6	p<0,001	I-II	p>0,05	I-III	p>0,05
	2-6	p<0,001	1-6	p<0,001	II-III	p>0,05
	3-6	p<0,01	2-6	p<0,001	1-6	p<0,001
	4-6	p>0,05	3-6	p<0,001	2-6	p<0,001
	5-6	p<0,05	4-6	p>0,05	3-6	p<0,001
			5-6	p<0,05	4-6	p>0,05
					5-6	p>0,05
7 group	66,03±1,72		59,53±1,65		51,07±1,52	
	1-7	p<0,001	I-II	p<0,01	I-III	p<0,001
	2-7	p<0,001	1-7	p<0,001	II-III	p<0,001
	3-7	p<0,05	2-7	p<0,001	1-7	p>0,05
	4-7	p>0,05	3-7	p<0,001	2-7	p<0,001
	5-7	p>0,05	4-7	p<0,01	3-7	p<0,001
	6-7	p>0,05	5-7	p<0,001	4-7	p<0,001
			6-7	p<0,05	5-7	p<0,001
				..	6-7	p<0,001

In group No. 4, the content of the studied indicator at the first stage is higher on 26.3 % (p<0.001) compared to the intact group. In comparison with the data of group No. 2, the level is less pronounced on 23.5 % (p<0.001). Compared with group No. 3, no statistically significant differences were found. At the second stage, the level of the marker is 24.1 % (p<0.001) higher compared to the group of intact rats. Compared to the 2nd group, it is lower on 31.9 % (p<0.001) and compared to the 3rd group on 13.8 % (p<0.001). At the third stage, the content of primary LPO products is on 7.7 % (p<0.05) less elevated compared to the 30th day of the experiment. In comparison with the 1st group, the level of the indicator is higher on 20.6 % (p<0.001). Compared to the 2nd and 3rd groups, it is lower on 40.8 % (p<0.001) and on 19.4 % (p<0.001), respectively.

In the fifth group, the level of diene conjugates is in 26.9 % (p<0.001) higher compared to the intact group. Compared to the group without correction, the content of the marker is lower on 22.4 % (p<0.001), and no statistically significant differences were found in the 3rd and 4th groups. At the second stage, the level of secondary LPO products is on 26.6 % (p<0.001) higher compared to group 1, on 27.5 % (p<0.001) lower compared to group 2, and on 10 % (p<0 .01) lower relative to the 3rd group. In comparison with group 4, no statistically significant differences were found. At the third stage, the DC content is higher on 22.8 % (p<0.001) compared to group No. 1. Relative to group No. 2, it is lower on 37 % (p<0.001), relative to group No. 3 it is lower on 16.1 % (p<0.001), and compared to group No. 4, no statistically significant differences were found. There were also no statistically significant stepwise differences.

In the sixth group at the first stage, the level of the studied indicator is on 21.7 % (p<0.001) higher than the data of the intact group. The marker content is lower compared to group 2 on 31.2 % (p<0.001), on 12.9 % (p<0.01) compared to group 3, on 7.2 % (p<0.05) compared to the 5th group. According to the group No. 4, no statistically significant differences were found. At the second stage, the level of the indicator is on 21.6 % (p<0.001) higher compared to the 1st group, compared to group 2 the content is lower on 36.3 % (p<0.001), compared to group 3 - lower on 17.6 % (p<0.001). According to the 4th group, no statistically significant differences were found. Compared with the 5th group, the marker level is lower on 6.9 % (p<0.05). At the third stage, the level of the indicator is higher on 19.2 % (p<0.001) relative to the intact group. In comparison with group No. 2, the level is lower on 43.4 % (p<0.001), in comparison with the 3rd group – on 21.5 % (p<0.001). According to the 4th and 5th groups, no statistical differences were found. Stage-by-stage differences were also not established.

In the seventh group at the first stage, the level of the investigated marker is higher on 23.9 % (p<0.001) compared to the intact group; compared to the 2nd and 3rd groups, the content of the indicator is lower on 27.4 % (p<0.001) and on 9.6 % (p<0.05), respectively. Compared with groups 4, 5 and 6, no statistically significant differences were found. At the second stage, the DC level is lower on 10.9 % (p<0.01) compared to the first stage. Compared to group No. 1, the content of the marker is higher on 15.7 % (p<0.001), and compared to all subsequent groups, it is lower: on 46.5 % (p<0.001) compared to the 2nd group, on 26.5 % (p<0.001) compared to the 3rd group, on 11.1 % (p<0.01) compared to the 4th group, on 14.9 % (p<0.001) compared to the 5th group, and on 7.7 % (p<0.05) is less than the 6th group. At the third stage, there is a positive trend in the form of normalization of the increased level of DC – on 29.3 % (p<0.001) compared to the 1st stage and on 16.6 % (p<0.001) compared to the 2nd stage. According to the 1st group, no statistical differences were found, which indicates the normalization of the level of secondary lipid peroxidation products under the influence of the method of correction analyzed in the seventh group. According to the 2nd group, the level is lower on 74.4 % (p<0.001), relative to the 3rd – on 47.8 % (p<0.001), compared to the 4th it is lower on 23.8 % (p< 0.001), compared to the 5th on 27.3% (p<0.001) and compared to the 6th it is lower on 21.6 % (p<0.001). Analyzing the changes in the levels of primary LPO products, we can claim a positive effect of correction in the 6th and 7th groups. The method of therapy proposed in group No. 7 is more effective in normalizing the processes of lipid peroxidation.

According to obtained results, it is relevant to study the dynamics of the level of primary LPO products in the conditions of the studied pathology and to select optimal methods of its correction. Analyzing the changes in the levels of primary LPO products, we can claim a positive effect of correction in the 6th and 7th groups. The method of therapy proposed in group No. 7 is more effective in normalizing the processes of lipid peroxidation (Fig. 1).
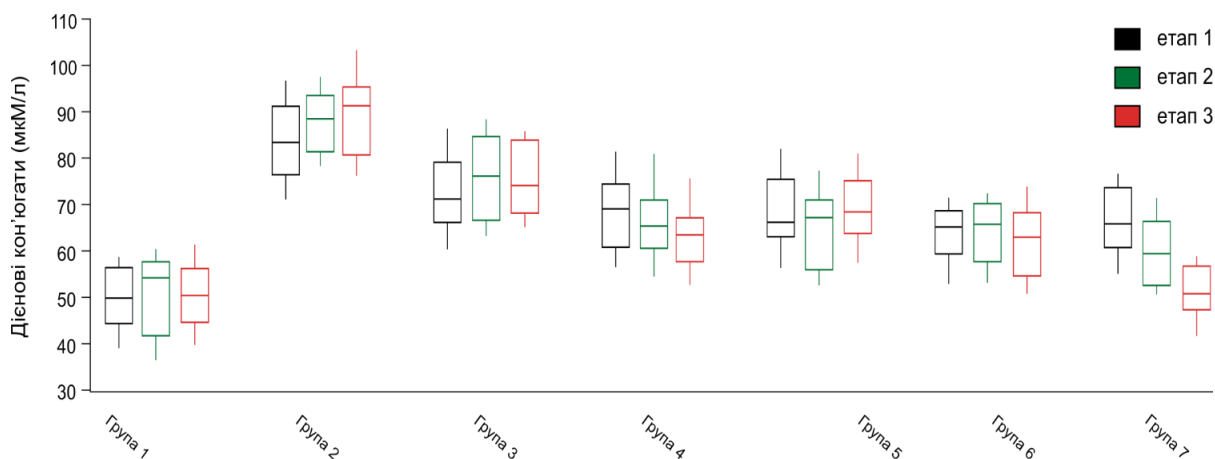
Fig. 1 – The diene conjugates level in the blood of experimental animals with simulated diabetic retinopathy and with different methods of its correction on the 30th, 60th, and 180th day. Box plots illustrate the distribution of values of the level of the studied indicator in all experimental groups at each of the stages of the study (n=60)

Conclusions:

1. The obtained results indicate an increase in the content of lipid peroxidation, starting from the 30th and with further progression on the 60th and 180th days of experimental diabetic retinopathy, which is confirmed by an increase in the level of diene conjugates in the 2nd group, the maximum of which is observed at the 3rd stage.

2. Correction with hypoglycemic agents in the 3rd group had a positive effect, but was not able to reduce the level of primary products of lipid peroxidation and restore antioxidant activity, so the use of additional agents became necessary.

3. The use of aflibercept and a nitric oxide donor in the 4th group to correct the development of diabetic retinopathy significantly suppressed oxidative stress, the maximum of which occurred on the 180th day of the experiment, but did not reach control indicators.

4. It was proven that the combined administration of bromfenac and aflibercept in the 5th group significantly reduced the number of primary LPO products, but not as significantly as in the 4th group.

5. It was proved that the administration of aflibercept, L-carnitine and bromfenac to the animals of the 6th group reduced the content of diene conjugates already on the 30th and continued on the 60th and 180th days of the study, but it also did not reach the control indicators.

6. The combination of metformin, aflibercept, L-arginine and citicoline in rats of the 7th group proved to be the most effective correction, as evidenced by the normalization of the

level of the studied indicators on the 30th and 60th days of the experiment, and on the 180th, a decrease in the content of markers was detected oxidative stress to control indicators.

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