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Influence of drug lipoic acid on the thiol groups content of blood of patients with macular degeneration, in the presence of anterior uveitis

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Ключові слова: дегенерація маку-
ли, передній увеїт, ліпоєва кислота.

Вступ. Актуальність роботи полягає у визначенні дії ліпоату при лікуванні хворих на вікову макулодистрофію.

Мета дослідження: вивчити вплив препаратів ліпоєвої кислоти на тіоловий статус в крові хворих на вікову макулодистрофію за наявності запального процесу в увеальному тракті.

Матеріал і методи. Клінічні дослідження проведені у 66 хворих з початковою стадією дегенерації макули і хронічним запальним процесом увеального тракту. Було виділено дві групи хворих: основна (34 хворих) і контрольна (32 хворих). У крові хворих обох груп визначали вміст відновленого і окисленого глутатіону і тіолових і дисульфідних груп до і після лікування.

Результати. В організмі хворих на дегенерацію макули і увеїтом під впливом препарату ліпоєвої кислоти після курсу лікування зростає рівень відновленої форми глутатіону і тіолових груп білків (на 15,2 % і 12,4 % відповідно), знижується концентрація окисленого глутатіону на 25,4 % і рівень дисульфідних зв'язків на 20,8 %.

Висновки. Включення ліпоєвої кислоти до традиційного лікування свідчить про її сприятливий вплив на тіоловий статус в крові хворих на дегенерацію макули і увеїт.

Влияние препаратов липоевой кислоты на содержание тиоловых групп в крови больных дегенерацией макулы при переднем увеите

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макула, передний увеит, липоевая
кислота.

Введение. Актуальность работы состоит в выяснении действия липоата при лечении больных возрастной макулодистрофией.

Цель исследования: изучить влияние препаратов липоевой кислоты на тиоловый статус в крови больных возрастной макулодистрофией с наличием воспалительного процесса в увеальном тракте.

Материал и методы. Клинические исследования проведены у 66 больных с начальной стадией дегенерации макулы и хроническим воспалительным процессом в увеальном тракте, было выделено две группы больных: основная (34 больных) и контрольная (32 больных). В крови больных обеих групп производили определение восстановленного и окисленного глутатиона и тиоловых и дисульфидных групп до и после лечения.

Результаты. В организме больных макулярной дегенерацией и увеитом под влиянием препарата липоевой кислоты возрастает уровень восстановленной формы глутатиона и тиоловых групп белков после курса лечения (на 15,2 % и 12,4 %) и снижается концентрация окисленного глутатиона на 25,4 % и уровень дисульфидных связей на 20,8 %.

Выводы. Включение липоевой кислоты в традиционное лечение свидетельствует о благоприятном ее влиянии на тиоловый статус в крови больных макулярной дегенерацией и увеитом.

Introduction: Macular degeneration (MD) is currently one of the most common and severe eye diseases in the world [1].

It should be noted that besides the main risk factors—the age, there are a number of additional factors. These primarily include genetic predisposition, hypertension, atherosclerosis, deterioration of choroid perfusion and slowing blood flow in the posterior ciliary arteries, weakening the transport of oxygen in the blood, change in the rheological properties of blood and tendency to microthrombosis [7, 10, 16].

The main role of triggering mechanisms of the pathogenesis of AMD is an imbalance of processes of free-radical oxidation and anti-radical system of exogenous and endogenous in nature. As a result, the body sharply increases the concentration of free radicals and other reactive oxygen species decrease the level of functional groups of proteins (thiol, carboxyl groups and etc.) [6, 19].

By interacting with free radical compounds, antioxidants, transform them into stable molecular forms, thereby interrupting the continued reaction of peroxidation. In the hydrophilic condition active antioxidant that neutralizes free radicals and prevents oxidative change is ascorbic acid [22].

It should be paid attention to the fact that a significant percentage of uveitis among eye diseases, include chronic relapsing, not sufficiently effective treatment cause severe consequences of inflammatory diseases of the vascular tract of the eye and the high incidence of blindness and disability of vision due to uveitis. Paying close attention of Ophthalmologists to the issues of diagnose and treat of uveitis and its complications explained by the fact that in recent years there has been an increase in their number [4, 5, 17].

Currently, in order to delay progression of disease are used medication (vasodilator, antiplatelet, vitamins C and E, beta-carotene in combination with zinc preparations and compounds that improve tissue respiration — cocarboxylase, ATP) [8].

Of particular interest recently is lipoic acid.

Thioctic or α -lipoic acid, being inherently natural metabolite involved in many physiological processes, and is effective agent for metabolic pharmacotherapy. It has a wide range of biological and pharmacological effects [2, 9, 14, 20]. It has a high rate of penetration through biological membranes, and the presence of thiol groups in the molecule of lipoic acid gives it, antioxidant properties — free radical Oxygen absorber compounds which prevents damage to mitochondria and promotes more efficient DNA repair after damage caused by oxidative stress [11, 13, 15, 18, 21].

Objective: To study the effect of drugs lipoic acid on the thiol group in the blood of patients with age-related macular degeneration with the presence of inflammation in the uveal tract.

Material and methods

Clinical studies were conducted on 66 patients with MD (Macular Degeneration) and chronic inflammation in the uveal tract.

In the treatment process divided patients into two groups: the main (34 patients) and control group (32 patients). In the complex treatment of all patients were: corticosteroids, antibiotics and mydriatics eye drops, desensitizing and tissue preparations and vitamins.

For main group Patients, additionally prescribed lipoic acid.

The Ophthalmological examination was performed using visual acuity, perimetry, tonometry, biomicroscopy, ophthalmoscopy.

The patients in main and control group were compared by sex, age, duration of the disease.

In the blood of patients in both groups were determined reduced and oxidized glutathione and also thiol and disulfide groups before and after treatment.

The principle method for determining glutathione based on the reaction between glutathione and methylglyoxal in the presence of a glyoxalase enzyme to form a conjugated S-lactol glutathione having a maximum absorption at wavelength of 240 nm [12].

The principle method for determining the oxidized form of glutathione is that as a result of enzymatic reduction of glutathione by glutathione reductase enzyme, oxidation of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) occurs, its subsidence is recorded spectrophotometrically at wave length of 340 nm.

The range of determined concentrations of reduced and oxidized forms is — from 5 to 200 mcg / ml of the appropriate solution. Mean coefficient of variation of the method for the specified range of the reduced form — 4.0 %, the oxidized form — 5.0 %. For the measurement used the spectrophotometer (Sph-26) [12].

The amount of sulfhydryl groups are judged by the number of theo nitrophenyl anion liberated by the interaction of 5.5 -dithiobis (2-nitrobenzoic acid) with free SH-groups of proteins. Protein disulfide group reduced with dithiothreitol to sulfhydryl groups. Comparing the free anion theo nitrophenyl before and after the addition of dithiothreitol, calculated number of disulfide groups in the protein.

The Optical density of solutions was measured at a wavelength of 412 nm Spectrocolorimetry «Specol-210.» The average value of the coefficient of variation of the method — 1.02 %. The content of sulfhydryl and disulfide groups is expressed in micromol / l [12].

The results of experimental study were processed using appropriate methods of statistical analysis using the SPSS 11. [3].

Results and discussion

Data about content of reduced and oxidized glutathione in the blood of patients in the control and main groups before and after treatment are shown in Table 1.

As seen from the data, in patients with MD (Macular Degeneration) on the background of uveitis in the control group (conventional treatment), before treatment level of reduced glutathione was reduced to (534.36 ± 38.32) micromol / L, which was — 68.3 %, compared to normal — (782.32 ± 62.40) micromol / l. After treatment, the level of reduced glutathione was — (573.38 ± 37.20) micromol / L — 73.3 % compared with

Table 1. The content of reduced and oxidized glutathione in the blood of patients in the control and main group before and after treatment

The investigated index	The statistical index	Normal	The control group		The main group	
			Before treatment	After treatment	Before treatment	After treatment
Reduced glutathione (micromol/l)	n	38	32	32	34	34
	M	782.32	534.36	573.38	545.08	627.93
	m	62.40	38.32	37.20	39.50	48.32
	P	–	<0.01	<0.01	<0.01	>0.05
	%	100.0	68.3	73.3	69.8	80.3
	P1	–	–	>0.05	–	<0.05
	%1	–	100.0	107.3	100.0	115.2
	P2	–	–	–	>0.05	>0.05
	2%	–	100.0	100.0	102.0	109.5
Oxidized glutathione (micromol/l)	n	38	32	32	34	34
	M	140.46	213.92	190.83	207.52	154.82
	m	10.24	15.30	13.20	16.04	11.36
	P	–	<0.001	<0.01	<0.001	>0.05
	%	100.0	152.3	135.9	147.7	110.2
	P1	–	–	>0.05	–	<0.01
	%1	–	100.0	89.2	100.0	74.6
	P2	–	–	–	>0.05	<0.05
	%2	–	100.0	100.0	97.0	81.1

Note: p — the level of significance of differences the data in relation to normal; p1 — comparison the level of significance of differences the data before and after the treatment in the control and main groups, p2 — the level of significance of differences in the comparison of data between the two groups before and after treatment.

the normal, but compared with those before treatment increasing the level of reduced glutathione was — 7.3 %.

In the main group patients (conventional treatment and lipoate, i.e. A salt or ester of lipoic acid) the level of reduced glutathione before treatment was reduced to (545.08±3.50) micromol / L, which was — 69.8 % compared to the normal. When using lipoate glutathione level was — (627.93±48.32) micromol / l — 80.3 % relative to the normal level, but with respect to data before treatment, level of reduced glutathione after application of lipoate increased by 15.2 %.

By comparing these data, it should be noted that the use of lipoate increased level of reduced glutathione in the main group up to 109.5 % compared with the control group after conventional treatment.

Studying the level of oxidized glutathione, it should be noted that in the group of patients with traditional treatment, the investigated index was raised to (213.92±15.30) micromol / L, which was 152.3 % compare to the normal (140.46±10.24) micromol / l. After treatment, the level of oxidized glutathione in this group was- (190.83±13.20) micromol / L, i.e. — 135.9 % relative to the normal, where in, the difference between the data before and after the treatment not confirmed statistically (p> 0.05).

In patients with conventional treatment (main group), the level of oxidized glutathione before treatment was raised to (207.52±16.04) micromol / L, which was — 147.7 % compared to the normal. When lipoate included in treatment, the level of oxidized glutathione was (154.82±11.36) micromol / l — 110.2 %. In this case, after treatment the level of oxidized glutathione

decreased compared with its contents before treatment up to 25.4 %.

Use of lipoate in patients AMD on the background of uveitis together with conventional treatments led to reduction of oxidized glutathione up to 81.1 % as compared with the group without lipoate.

The data about the content of protein thiol and disulfide groups in the blood of patients in the control and main groups before and after treatment shown in Table 2.

As seen from the data presented in AMD patients on the background of uveitis in the control group (conventional treatments) before treatment, the level of thiol groups was reduced to (667.38±35.24) micromol / L, which was — 76.3 %, compared to the normal — (874.52±52.30) micromol / l. After treatment, the level of thiol groups was (704.70±38.64) micromol / L — 80.6 % compared with the normal, but in relation to the data before treatment the level of thiol groups increased by 5.6 %.

In the main group patients (conventional treatment and lipoate) the level of thiol groups before treatment was reduced to (687.42±34.50) micromol / L, which was 78.6 % compared to normal. After applying lipoate, level of thiol groups was — (772.67±35.43) micromol / L — 88.4 % or 88.4 % compared to normal but in relation to the data before treatment the level of thiol groups with drug treatment and use of lipoate increased by 12.4 %.

Thus the use of lipoate increased level of thiol groups in the main group up to 109.6 % compared with the control group after conventional treatment.

The original amount of disulfide groups in the control group was increased to (316.24±16.50) micromol / l, i.e- 140.3 % compared the normal — (225.40±12.38)

Table 2. The content of protein thiol and disulfide groups in the blood of patients in the control and main group before and after treatment.

The investigated index	The statistical index	Normal	The control group		The main group	
			Before treatment	After treatment	Before treatment	After treatment
Thiol groups (micromol/l)	n	38	32	32	34	34
	M	874.52	667.38	704.70	687.42	772.67
	m	52.30	35.24	38.64	34.50	35.43
	p	–	<0.01	<0.05	<0.01	>0.05
	%	100.0	76.3	80.6	78.6	88.4
	P1	–	–	>0.05	–	<0.05
	%1	–	100.0	105.6	100.0	112.4
	P2	–	–	–	>0.05	>0.05
Disulfide groups (micromol/l)	n	38	32	32	34	34
	M	225.40	316.24	290.94	310.55	245.96
	m	12.38	16.50	13.32	14.30	12.25
	p	–	<0.001	<0.001	<0.001	>0.05
	%	100.0	140.3	129.1	137.8	109.1
	P1	–	–	>0.05	–	<0.01
	%1	–	100.0	92.0	100.0	79.2
	P2	–	–	–	>0.05	<0.05
%2	–	100.0	100.0	98.2	84.5	

Note: p — the level of significance of differences the data in relation to normal; p1 — comparison the level of significance of differences the data before and after the treatment in the control and main groups, p2 — the level of significance of differences in the comparison of data between the two groups before and after treatment.

micromol / l. After treatment in this group ,the investigated index was (290.94±13.32) micromol / l — 129.1 % compared to the normal. In this case, the differences were not statistically significant.

In the main group of patients, the amount of disulfide groups before treatment was raised to (310.55±14.30) micromol / L, which was — 137.8 % compared to the normal. After applying the lipoate, it increased to (245.96±12.25) micromol / l — 109.1 % as compared to the normal. Therefore, the content of disulfide groups after the treatment decreased by 20.8 % compared to the data before treatment.

The Comparison of results of treatment in both groups showed that the use of lipoate reduces the level of disulfide groups up to 84.5 %.

The Summarizing the results of clinical and biochemical studies on the effect of lipoic acid on the status of the thiol-disulfide exchange in patients with age-related macular degeneration and chronic uveitis, it should be noted that on the background of increasing the

level of reduced glutathione and thiol groups of proteins under conditions of using drug lipoate in complex the treatment is observed a more pronounced relative reduction of oxidized thiol (oxidized glutathione and disulfide bonds in proteins). The latter occurs, most likely due to reduction of oxidative stress, characteristic of, degenerative and inflammatory diseases.

Conclusions

1. In the body of patients with MD and uveitis under the influence of drugs lipoic acid significantly reduced the degree of disturbance of the thiol-disulfide exchange, which results in a distinct increase in the levels of reduced glutathione and thiol groups of proteins after treatment (up to 15.2 and 12.4 %, respectively).

2. Of particular significant was relative reduction in the process of oxidation of thiol compounds, manifested in a decrease in the concentration of oxidized glutathione up to 25.4 % and the level of disulfide bonds up to 20.8 %.

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