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EXPERIMENTAL SUBSTANTIATION OF THE EFFECTIVENESS OF GLYCINE IN MYOCARDIAL INFARCTION

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Abstract: The aim of the study was to evaluate the effect of the amino acid glycine on the degree of damage to cardiomyocytes in myocardial infarction, which was assessed by the activity of cardiospecific enzymes in the blood. The experiments were carried out on male rabbits, in which experimental myocardial infarction was induced by ligation of the descending branch of the left coronary artery. Immediately after ligation, an aqueous solution of glycine (manufactured by MRPC Biotiki, RF) at a dose of 100 mg/kg of body weight was orally injected into the stomach of experimental rabbits through a tube. Further, the animals received glycine orally at the indicated dose every day. After 0.5, 1, 3, 6, 12, 24, 72 and 168 hours after coronary occlusion, the activity of MB-creatine phosphokinase (MB-CPK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) was determined in blood plasma. The results showed that glycine at a dose of 100 mg/kg of body weight led to a smaller increase in the activity of CF-CPK, LDH and AST in the blood plasma of rabbits in the dynamics of experimental myocardial infarction. Also, in the blood plasma of patients with myocardial infarction, the content of free amino acids was determined by high performance liquid chromatography. It was found that in acute myocardial infarction, the content of glycine in the blood is reduced by 39.7% compared with normal values. Based on the results, a conclusion was made about the cardioprotective property of glycine in necrotic myocardial damage.

Keywords: glycine, cardiospecific enzymes, experimental myocardial infarction, blood plasma, aminoacids.

Introduction. It is known that glycine has a sedative (calming), mild tranquilizing (anti-anxiety) and weak antidepressant effect, reduces feelings of anxiety, fear, psycho-emotional stress [1]. Glycine also has glycine- and GABA-ergic, ?1- adrenoblocking, antioxidant [2], and antitoxic effects. Based on this, we can assume that this medicine can have a positive effect on the course of myocardial infarction, since this disease is invariably accompanied by stress and fear of death.

Research aims. Evaluation of the effect of glycine on the activity of cardiospecific enzymes (MB-creatine phosphokinase - MB-CPK, lactate dehydrogenase - LDH, aspartate amino transferase - AST) in blood plasma in the dynamics of experimental myocardial infarction (EIM), as well as the content of free amino acids in the blood in patients with acute myocardial infarction.

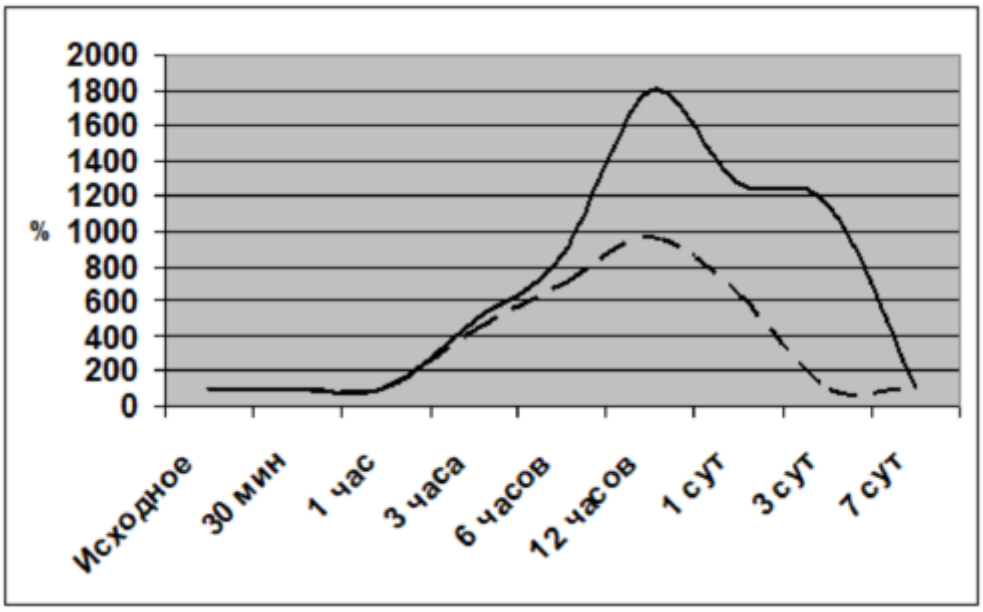
Materials and methods of research. In the experiments, 10 male chinchilla rabbits weighing 2.5-2.8 kg were used. All painful procedures and withdrawal of animals from the experiment were carried out on anesthetized animals according to the Guide for the Care and Use of Laboratory Animals: Eighth Edition National Research Council [3].

EIM in rabbits under Nembutal anesthesia (40 mg/kg body weight) was induced by ligation of the descending branch of the left coronary artery. 1 rabbit died after bandaging, an hour after bandaging, another 1 rabbit died. So, the mortality rate equalled 20%. Immediately after bandaging, 5 rabbits were orally injected into the stomach with an aqueous solution of glycine (manufactured by the Biotiki Medical Research and Production Complex, Russian Federation) at a dose of 100 mg/kg of body weight. Further, the animals received glycine orally at the indicated dose every day. 3 animals that did not receive glycine constituted the control group.

Blood from the ear vein of the animals was obtained before ligation (initial) and after 30 minutes, 1, 3, 6, 12 hours and on days 1, 3, and 7 of the courses of EMI into test tubes with heparin. Blood was centrifuged at 3000 rpm for 15 min. The activity of cardiospecific enzymes - MB-CPK, LDH and AST in blood plasma was determined on a "DAYTONA" autoanalyzer manufactured by Randox (Great Britain).

To isolate free amino acids from the blood plasma of patients, 1 ml of 20% TCA was added to 1 ml of the test sample. After 10 min, the precipitate was separated by centrifugation at 8000 rpm for 15 min. After separating 0.1 ml of the supernatant, freeze-dried. The hydrolyzate was evaporated, the dry residue was dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This operation was repeated twice to neutralize the acid. Reaction with phenylthioisocyanate gave phenylthiocarbamyl derivatives (FTC) of amino acids according to the method of Steven A., Cohen Daviel. Identification of amino acid derivatives was carried out by HPLC. HPLC conditions: Agilent Technologies 1200 chromatograph with DAD detector, 75x4.6 mm Discovery HS C18 column. Solution A: 0.14 M CH3COONa + 0.05% TEA pH 6.4, B: CH3CN. Flow rate 1.2 ml/min, absorbance 269 nm. Gradient %B/min: 1-6%/0-2.5 min; 6-30%/2.51-40 min; 30-60%/40.1-45 min; 60-60%/45.1-50 min; 60-0%/50.1-55 min. Numerical data were statistically processed using Student's t-test.

Research results and discussion. The obtained results showed that during EIM, a statistically significant increase in the activity of CF-CPK by 386% is observed starting from the 3rd hour after ligation (Fig.1).



Rice. Fig. 1. The activity of MB-CPK in the blood plasma of rabbits in the dynamics of EMI (solid line) and during treatment with glycine (stroke line)

At 6 and 12 hours after occlusion, there was an increase in the activity of MV-CFC by 758 and 981% compared to the initial indicator. The maximum increase in enzyme

activity (by 1172% of the initial value) was observed on the 1st day of EIM. On the 3rd day of pathology, the activity of MV-CFC was higher than the initial indicator by 1046%, and on the 7th day it was at the level of the initial indicator. Glycine in the early stages of the disease (30 min, 1, 3, 6, 12 h) did not significantly affect the dynamics of MV-CFC activity. However, for 1 day, the activity of the enzyme was significantly lower compared to the control (by 50.4% of the control). Normalization of MV-CFC activity, in contrast to the control, was observed already on the 3rd day of the disease.

The study of LDH activity showed that with EIM, its increase is observed starting from 6 hours after occlusion (by 66.9% of the initial indicator) and remains elevated for up to 7 days (Fig. 2).

The maximum increase in LDH activity in EIM was observed on the 3rd day of the disease (by 157.8% of the initial indicator). When glycine was administered to rabbits with EIM on days 1, 3, and 7, significantly lower LDH activity was observed compared to control parameters. Thus, on day 1, LDH activity was lower than control by 25.9%, and on days 3 and 7 - by 35.3 and 38.6%, respectively.

The activity of AsAT in blood plasma during EIM in rabbits was increased by 20.4% already at 1 hour of occlusion (Fig. 3). Further, a progressive increase in the activity of AST was observed, and the maximum increase (by 207.9% of the initial indicator) was recorded for 1 day of EIM. By the 3rd and 7th day, AsAT activity was at the level of the initial indicator. When glycine was administered for up to 1 day, no statistically significant changes from control values were found in AsAT activity. On the 1st day of EIM, AST activity was 54.4% lower than the control numbers.

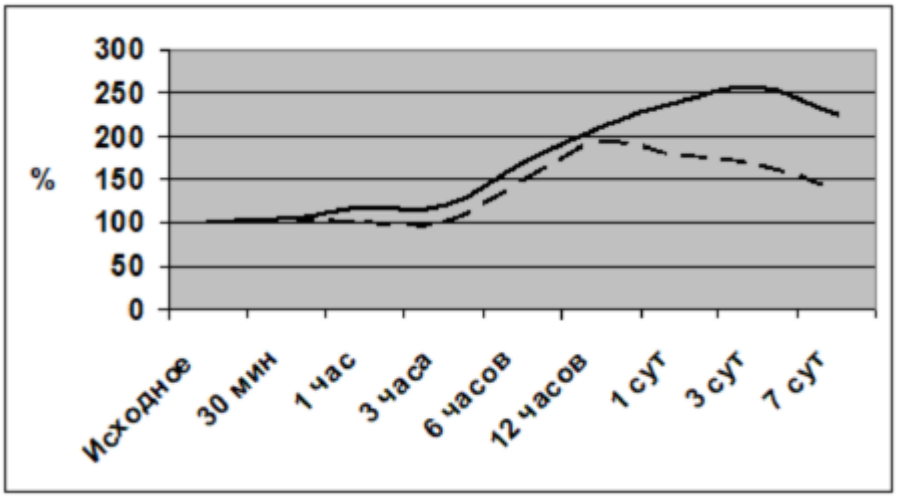


Fig. 2. LDH activity in blood plasma of rabbits in the dynamics of EIM (solid line) and against the background of treatment with glycine (stroke line)

On the 3rd and 7th days of the disease, the enzyme activity was at the level of the initial indicator.

The above data indicate the effectiveness of glycine in experimental myocardial infarction. At the same time, there is a question about the content of glycine, as well as other amino acids in the blood during a myocardial infarction in a person, since with a normal level of glycine in the blood, its effectiveness during a myocardial infarction cannot be explained. Taking this into account, we conducted research on the amino acid spectrum of the blood of patients with myocardial infarction. For this purpose, blood was obtained from 8 patients admitted to the Cardioresuscitation department of the Republican Specialized Scientific and Practical Medical Center of Cardiology with a diagnosis of acute myocardial infarction.

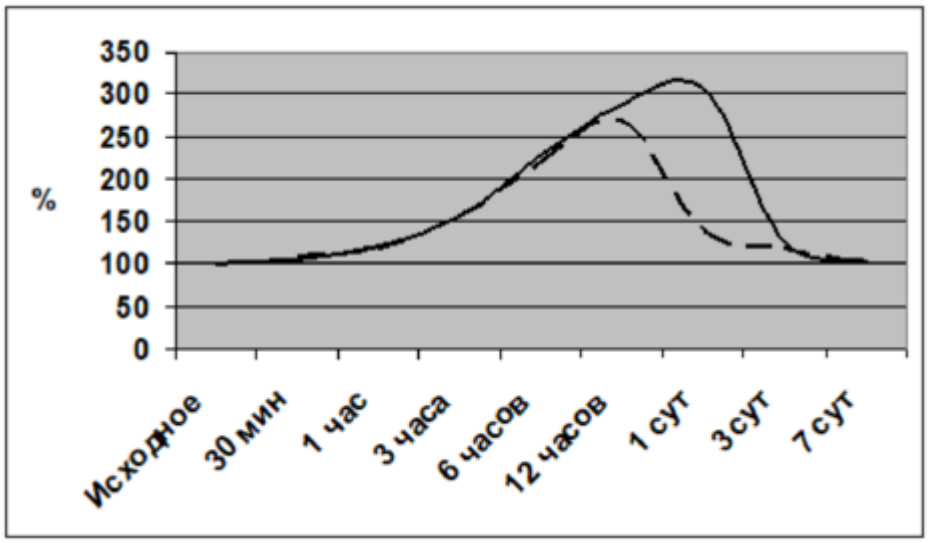


Fig. 3. Activity of AST in the blood plasma of rabbits in the dynamics of EIM (solid line) and against the background of treatment with glycine (stroke line)

The latter was diagnosed based on determination of troponin I content in the blood. The average value of troponin I in the blood of the patients we studied was 27.55 ± 5.90 ng/ml with fluctuations from 8.09 to 50.00 ng/ml. At the same time, reference values are less than 0.29 ng/ml. The blood of 8 male volunteers was used as a control.

Our results showed that myocardial infarction is accompanied by changes in the amino acid spectrum of blood. Thus, out of 18 amino acids studied by us, statistically significant changes were revealed in 8 (table). No statistically significant changes were found in the content of serine, threonine, proline, tyrosine, valine, histidine, isoleucine, leucine, tryptophan, and phenylalanine compared to normal values. The content of glycine turned out to be reduced by 39.7% of the normal indicator. At the same time, if the fluctuation of normal values was in the range of 69.80÷125.48 mmol/l, then with myocardial infarction it was equal to 28.37÷92.18 mmol/l.

Table.
Aminoacid spectrum of normal human blood and in myocardial infarction

Aminoacides	Normal, mmol/l		MI patients, mmol/l		P <
	M ± m	Max ÷ Min	M ± m	Max ÷ Min	
Serin	109,04±6,83	77,08÷138,55	100,08±26,51	62,80÷282,62	нд.
Glycine	92,20±6,62	69,80÷125,48	55,61±7,45	28,37÷92,18	0,001
Asparagine	105,15±7,43	79,40÷142,14	53,69±8,58	29,52÷102,48	0,001
Glutamine	266,81±29,98	112,01÷355,73	51,72±12,41	30,11÷135,48	0,001
Cysteine	244,85±56,48	103,75÷497,06	96,57±10,44	44,24÷129,42	0,05
Threonine	85,70±12,10	34,34÷134,99	92,31±12,42	41,97÷134,74	нд.
Arginine	142,41±16,83	63,89÷195,58	101,13±11,31	55,74÷164,52	0,05
Alanine	37,34±4,26	18,18÷55,34	55,69±4,18	40,63÷69,14	0,001
Proline	119,95±20,12	6,64÷180,52	98,88±14,51	37,55÷176,00	нд.
Tyrosine	125,93±24,16	35,60÷210,06	91,19±15,46	23,73÷141,73	нд.
Valyn	124,31±14,79	52,67÷179,77	120,81±17,77	80,58÷210,59	нд.
Methionine	155,56±42,70	38,07÷411,10	66,22±10,87	33,24÷124,79	0,05
Histidine	90,05±33,93	10,18÷258,77	83,99±12,34	25,14÷129,16	нд.
Isoleucine	40,62±4,98	20,20÷58,85	52,46±10,01	20,66÷110,00	нд.
Leucine	72,74±6,02	44,75÷101,23	84,07±9,31	46,96÷125,40	нд.
Tryptophan	53,84±17,97	21,54÷177,94	40,67±7,43	19,14÷79,47	нд.
Phenylalanine	39,26±6,01	15,38÷64,23	56,06±10,47	21,97÷123,86	нд.
Lysine	89,69±20,70	30,30÷201,18	45,22±4,64	30,99÷70,93	0,05

Amount of asparagine turned out to be below the normal value by 48.9%. Fluctuations in values were equal to 79.40÷142.14 mmol/l in normal and 29.52÷102.48 mmol/l in myocardial infarction. The content of glutamine in the blood during a myocardial infarction was 80.6% lower than the normal value. Fluctuations in normal values were within 112.01÷355.73 mmol/l, and in myocardial infarction 30.11÷135.48 mmol/l. The cysteine content also turned out to be below the norm (by 60.6%). If at the same time the fluctuation of the values in the norm was equal to 103.75÷497.06 mmol/l, then with a heart attack it was equal to 44.24÷129.42 mmol/l. The content of arginine in the blood during myocardial infarction was also lower than the normal value by 29%.

Fluctuation of normal values was equal to 63.89÷195.58 mmol/l, while with myocardial infarction it was equal to 55.74÷164.52 mmol/l. The content of alanine in the blood, unlike all other amino acids, in myocardial infarction, on the contrary, turned out to be higher than 49.1%. Fluctuations in values were normally 18.18÷55.34 mmol/l, and with myocardial infarction 40.63÷69.14 mmol/l. The content of methionine in the blood during myocardial infarction also turned out to be below the normal value by 57.4%, with fluctuations in the values for the norm in the range of 38.07÷411.10 mmol/l and for myocardial infarction - 33.24÷124.79 mmol/l. In myocardial infarction, a significant decrease compared to normal values was also observed in the content of lysine (by 49.6%). Fluctuations of values in this case were equal to 30.30÷201.18 mmol/l in the norm and 30.99÷70.93 mmol/l in case of myocardial infarction.

Discussion. Glycine is a precursor of several important compounds, such as creatine, glutathione, purines, and glucose [4], and is involved in a wide range of metabolic pathways [5]. Circulating glycine has shown many positive effects in various diseases, including cardiovascular diseases [6]. Circulating concentrations of glycine have anti-inflammatory and antioxidant effects and have been shown to be inversely associated with acute myocardial infarction [7], as well as with some traditional cardiovascular risk factors, including obesity [8], hypertension [9], and type 2 diabetes [10].

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