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Effect of Synthetic Peptide LKEKK in Experimental Myocardial Infarction

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Abstract

The effect of the synthetic peptide LKEKK on the rat myocardium was studied in the norm and after experimental myocardial infarction (EMI) caused by ligation of the left coronary artery. It was found that [^{3}H]LKEKK binds with high affinity and specificity to the membranes of the rat myocardium before EMI (K_i 1.9 nM). One hour after the operation, a sharp decrease in binding affinity was observed (K_d 13.4 nM), after 24 hours it increased significantly (K_d 3.5 nM), and after 72 hours it was almost completely restored (K_d 2.1 nM). It was established that therapy of EMI with Riboxin (orally at a dose of 10 mg/kg once a day for 7 days after infarction) together with the peptide LKEKK (intranasally at a dose of 500 μg/rat according to the same scheme) has a pronounced anti-ischemic effect: improves coronary blood flow; increases myocardial contractility, promotes normalization of atrioventricular conduction disorders of the myocardium in the post-infarction period. A reliable decrease in the intensity of myocardial cytolysis and lipid peroxidation is also noted. All of the listed effects were more pronounced than in the case of Riboxin therapy without the peptide. Thus, the peptide can be used in complex therapy of ischemic heart disease and myocardial infarction.

***Keywords*:** proteins, peptides, receptors; myocardial infarction.

1. Introduction

Myocardial infarction (MI) is one of the important socially significant health problems, caused by high morbidity and mortality. Due to the accelerated rate of MI spread, the development and application of new effective and safe means and methods for the prevention and treatment of this disease is of crucial importance (Baghdasaryan et at., 2021; Salari et al., 2023).

Several years ago, we synthesized the LKEKK peptide, which corresponds to sequences 16–20 of human thymosin-α1 (TM-α_1) and 131–135 of human interferon-α2 (IFN-α_2). The study of the peptide activity showed that it is capable of exerting antimicrobial and anti-inflammatory effects *in vitro* and *in vivo* (Navolotskaya et al., 2019; 2021; 2023; 20250.

The aim of this work is to study the effect of the LKEKK peptide on the rat myocardium in normal conditions and after experimental myocardial infarction.

2. Materials and methods

2.1. Chemicals

The following reagents were used in the work: phenylmethylsulfonyl fluoride (PMSF), Tris (Fluka, USA), fetal calf serum, aluminum oxide (Al_2O_3) (Sigma, USA), *L*-glutamine, N-(2hydroxyethyl)piperazine-N'-2-ethylsulfonic acid (HEPES), Rh/Al_2O_3 (Flow, USA), sucrose, BSA, phenylmethylsulfonyl fluoride (PMSF), sodium azide (NaN_3) (Serva, Germany), N-methylpyrrolidone, diisopropylcar bodiimide, thioanisole (Merck, Germany), Unisolv 100 scintillator (Amersham, England), piboxin (9-β-D-ribofuranosylhypoxanthine, Nizhpharm LLC, Russia). Distilled water was additionally purified using the Mono-Q system (Millipore, USA).

2.2. Peptides

Peptides LKEKK and KKEKL were synthesized on automatic synthesizers (model 430A and Vega Coupler, model C250; Applied Biosystems, USA) using the Boc/Bzl peptide chain extension tactic and purified by preparative reversed-phase chromatography (Gilson chromatograph, France), Waters SymmetryPrep C18 column (19 × 300 mm) (Malva, Greece). The synthesized peptides were characterized by analytical reversed-phase HPLC (Gilson chromatograph, France; XTerra RP18 column, Malva, Greece), amino acid analysis (hydrolysis with 6 M HCl, 24 h, 110°C; 4151 Alpha Plus amino acid analyzer, LKB, Sweden) and mass spectral analysis (Finnigan mass spectrometer, USA.

2.3. Preparation of [^{3}H]LKEKK

To obtain [^{3}H]LKEKK, a high-temperature solid-phase catalytic isotope exchange (HTSCIE) reaction was used (Zolotarev et al., 2003) 20 mg of aluminum oxide were added to a solution of 1.0 mg of the peptide in 1 ml of water and evaporated on a rotary evaporator at 20°C. Aluminum oxide with the supported peptide was mixed with 10 mg of the catalyst (5% $Rh/A_{l2}O_3$). The resulting solid mixture was placed in a 10 ml ampoule. 20 min. The ampoule was evacuated, filled with gaseous tritium to a pressure of 250 mm Hg, heated to 170°C and kept at this temperature for 20 min. Then the ampoule was cooled, evacuated, purged with hydrogen and evacuated again. The labeled peptide was extracted from the solid reaction mixture with two portions of 3 ml of 50% aqueous ethanol, the resulting solution was combined and evaporated. To remove labile tritium, the procedure was repeated twice. The labeled peptide was purified by high-performance liquid chromatography (HPLC) with a Beckman spectrophotometer at wavelengths of 220 and 280 nm sequentially on Kromasil C18 (10 × 250 mm) and MN C8 (8 × 250 mm) columns at 20 °C in a concentration gradient of an aqueous acetonitrile solution in the presence of 0.1% trifluoroacetic acid. Tritium incorporation into the peptide was calculated using liquid scintillation counting.

2.4. Animals

Male SD rats weighing 180–200 g was obtained from the nursery of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences. The cages with the animals were placed in separate rooms with a light regime of 12 h light, 12 h dark, air temperature within 19–25 °C and relative humidity of 50–70%. Air temperature and humidity were recorded daily. A ventilation regime was set to provide about 15 room volumes per hour, CO_2 concentration of no more than 0.15 vol.%, and ammonia concentration of no more than 0.001 mg/L. Each animal was examined daily during the study. The examination was carried out 1 hour after administration of the drugs and included an assessment of the behavior and general condition of the animals.

2.5. Binding of [^{3}H]LKEKK to myocardial membranes

Membranes from rat myocardium were isolated according to the method proposed by Dal Farra (Dal Farra et al., 2000), protein concentration was determined according to Lowry (Lowry et al., 1951), using bovine serum albumin as a standard.

The [^{3}H]LKEKK binding reaction with membranes was carried out in 50 mM Tris-HCI buffer containing PMSF (0.6 mg/mL), pH 7.5, according to the following scheme: 100 µl of the labeled peptide from the solution with the concentration of 10^{-10}–10^{-7} M (three parallel samples for each concentration), 100 µL of buffer (total binding) or 100 µL of a 10^{-3} M solution of unlabeled peptide in buffer (non-specific binding) and 800 µl of the suspension of freshly isolated membranes (2 mg of protein) were added to siliconized glass tubes. The tubes were incubated at 4°C for 1 h then the reaction mixture was filtered through GF/B glass fiber filters (Whatman, England). The filters were washed three times with 5 ml of ice-cold buffer solution. Radioactivity on the filters was counted using a LS 5801 liquid scintillation counter (Beckman, USA). The magnitude of specific binding of [^{3}H]LKEKK to membranes was determined by the difference between its total and non-specific binding. To determine the parameters of specific binding of [^{3}H]LKEKK to membranes (equilibrium dissociation constant K_d and receptor density B_{max} – maximum binding capacity per 1 mg of protein), a graph of the relationship between the molar concentrations of bound (B) and free (F) labeled peptide and the molar concentration of bound labeled peptide (B) (Scatchard plot) was plotted (Pennock, 1973).

To assess the binding specificity, the ability of unlabeled LKEKK and KKEKL peptides to inhibit the specific binding of [^{3}H]LKEKK to myocardial membranes was tested: the membrane suspension (1.5 mg protein, 800 µL) was incubated with [^{3}H]LKEKK (5 nM, 100 µL) and one of the potential inhibitors (concentration range 10^{-10}–10^{-4} M, three replicates for each concentration) in 50 mM Tris-HCl buffer containing PMSF (0.6 mg/mL), pH 7.5 at 4°C for 1 h. The reaction mixture was then filtered through GF/B glass fiber filters (Whatman, England). The filters were washed three times with 5 mL of ice-cold buffer solution. Radioactivity on the filters was counted using a LS 5801 liquid scintillation counter (Beckman, USA). The inhibition constant (K_i) was determined using the formula: $K_i = [IC]_{50}/(1 + [L]/K_d)$, where [L] is the molar concentration of [^{3}H]LKEKK; K_d is the equilibrium dissociation constant of the [^{3}H]LKEKK-receptor complex; $[IC]_{50}$ is the concentration of unlabeled ligand causing 50% inhibition of specific binding of labeled LKEKK (Cheng, 1973). The IC_{50} value was determined graphically based on the inhibition curve (a graph of the dependence of inhibition (%) on the molar concentration of the inhibitor).

2.6. Experimental myocardial infarction (EMI)

Modeling of myocardial infarction in rats was performed by ligation of the anterior descending branch of the left coronary artery. Under ether anesthesia, the skin and subcutaneous fat of the animal were dissected, and the pectoral muscles were separated along the course of the fibers. After opening the chest, the heart was brought out into the surgical wound, and the left coronary artery was stitched and tied with a ligature. The onset of myocardial ischemia was monitored electrocardiographically. After the operation, the wound was sutured layer by layer.

2.7. Electrocardiographic studies

Electrocardiogram (ECG) recording was performed on non-anesthetized rats using a polygraph RM-6000 (Japan). The animals were placed in special plastic cages. Thin steel needles inserted subcutaneously were used as electrodes. ECG was recorded in lead I. The electrocardiographic sign of MI in rats was considered to be the registration of ST segment elevation 1 h after the operation.

2.8. Tested drugs

Riboxin, widely used in the complex therapy of ischemic heart disease and myocardial infarction (Wu et al., 2022; Benak et al., 2024; Romodin et al., 2024), was administered orally at a dose of 10 mg/kg once a day for 7 days after EMI. The LKEKK peptide at doses of 200 and 500 µg per rat in 100 µL of distilled water was administered intranasally simultaneously with Riboxin according to the same scheme. Animals of the control groups received a solvent (distilled water). Comparative

effectiveness of the drugs was assessed by several parameters: mortality and clinical picture; functional - ECG (1 h, 3 and 7 days), biochemical (blood and heart tissue examination 24 h after EMI), and histological (24 h, 3 and 7 days).

2.9. Experimental groups

Five experimental groups of 15 animals each were formed: group I sham-operated animals (negative control), group II animals with EMI without treatment (positive control), group III animals with EIM, therapy with Riboxin (10 mg/kg orally), group IV animals with EMI, therapy with Riboxin 10 mg/kg orally + peptide 200 µg per rat intranasally, group V animals with EMI, therapy with Riboxin 10 mg/kg orally + peptide 500 µg per rat intranasally.

2.10. Biochemical and histological studies

Blood for biochemical studies was obtained by tail vein puncture. After decapitation, pathological dissection and histological examination of the animals' heart tissue were performed. The tissue was fixed in 10-15% formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin-eosin.

The content of reduced glutathione in the liver was determined iodometrically (Ellouk-Achard et al., 1995) the content of glycogen in the liver was determined by the Samogyi method (Arenas et al., 2022), and the level of glucose in the blood and organ homogenates was measured by the orthotoluidine method (Viner y al., 1993). The activity of ALT, AST, ALP, CP, and LDH in the blood serum was determined using Bio-Lat-Test kits (Czech Republic).

The intensity of tissue respiration in organ homogenates was determined using the Warburg manometric method (Johnson et al., 1982). The content of lactic acid in the blood was determined using the Barker method (Freitas et al., 2020), and the activity of succinate dehydrogenase was determined using the Vogels method (Bell et al., 2017).

2.11. Statistical Analysis

The data were evaluated using the Mann-Whitney test. The results are presented as mea ± SEM or as median (min-max).

3. Results and discussion

The main characteristics of the LKEKK and KKEKL peptides ((purity, amino acid content, and molecular mass) are shown in Table 1.

Table 1: Main characteristics of the peptides.

Peptide	Purity, %	Amino acid analysis data	Molecular mass, D
LKEKK	>98	Glu 1.08, Leu 1.00, Lys 3.32	645.4 (calculated value - 644.8)
KKEKL	>97	Glu 1.14 (1), Leu 1.05 (1), Lys 3.30 (3)	648.65 (642.7)

The high-temperature solid-phase catalytic isotope exchange reaction yielded 2 mCi of tritium-labeled LKEKK peptide ([^{3}H]LKEKK, 2 mCi, the specific activity 42 Ci/mol). The retention times of [^{3}H]LKEKK and unlabeled LKEKK on a Kromasil C18 column (chromatographic conditions are given in the Experimental Section) were the same; the extinction ratios at 220 and 280 nm for the labeled and unlabeled peptide were also the same, indicating that the chemical structure of LKEKK is preserved when hydrogen is replaced by tritium.

Study of [^{3}H]LKEKK binding to rat myocardial membranes at 4°C showed that dynamic equilibrium in the labeled peptide–receptor system was established after approximately 1 h and was maintained for 2 h. Therefore, to determine the value of the equilibrium dissociation constant (K_d), the [^{3}H]LKEKK binding to membranes was carried out for 1 h. Nonspecific binding of [^{3}H]LKEKK in these was 7.1 ± 1.3% of the value of its total binding.

Figure 1 shows the Scatchard plot (1) characterizing the specific binding of [^{3}H]LKEKK to rat myocardial membranes before EMI. The linearity of the plot indicates the presence of one type of receptor to the peptide on the myocardial membranes, and the value of K_{d1} = 1.9 ± 0.2 nM indicates a high affinity of the peptide to the receptor. The receptor density B_{max} was 0.3 ± 0.1 pmol/mg protein.

Graphs 2, 3 and 4 in Fig. 1 characterize the binding of [^{3}H]LKEKK to membranes isolated from rat myocardium 1, 24 and 72 h after EMI, respectively. It is evident that the receptor density remained virtually unchanged, while the binding affinity sharply decreased 1 h after the operation (K_{d2} = 13.4 ± 0.3 nM, graph 2). 24 h after EMI, the affinity of [^{3}H]LKEKK to the receptor increased (K_{d3} = 3.5 ± 0.3 nM, graph 3), and after 72 h it was almost completely restored (K_{d4} = 2.1 ± 0.3 nM, graph 4).

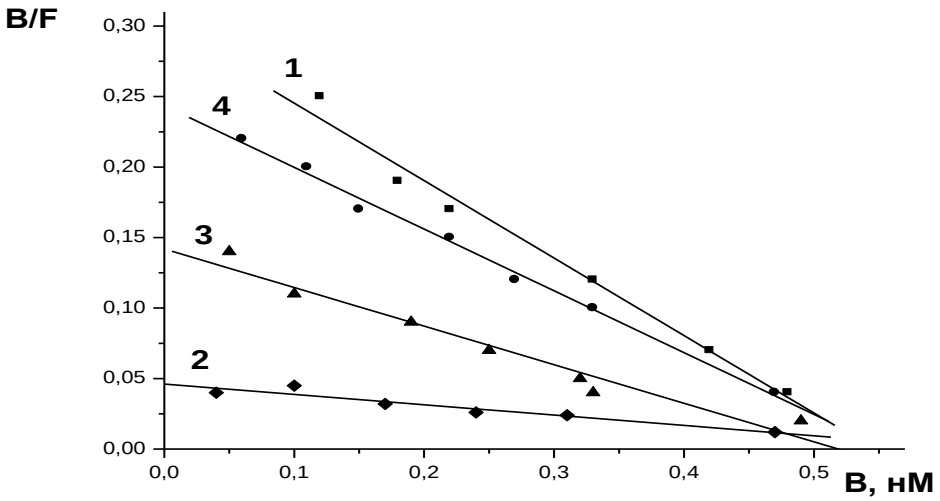

Fig. 1. Scatchard analysis of specific binding of [^{3}H]LKEKK to rat myocardial membranes under normal conditions (1) and at 1) h (2), 24) h (3), and 72 h (4) after EMI. B and F are molar concentrations of bound and free labeled peptide, respectively.

To characterize the binding specificity, unlabeled peptides LKEKK and KKEKL were tested as potential competitors of [^{3}H]LKEKK. According to the results obtained, the specific binding of [^{3}H]LKEKK to membranes was inhibited by the LKEKK peptide (K_i 2.4 ± 0.3 nM), while the peptide with the inverted sequence KKEKL was inactive (K_i > 10 μM), confirming the high binding specificity of the labeled peptide.

Numerous studies show that myocardial infarction (MI) is characterized by the formation of a necrotic focus, insufficient coronary blood flow, inhibition of myocardial contractile activity, impaired atrioventricular and intraventricular conduction, tissue respiration and metabolism, activation of lipid peroxidation and decreased antioxidant protection (Borst et al., 2011; Lu et al., 2015; Reed et al., 2017; Frampton et al., 2023; Salari et al., 2023].

The experimental modeling of MI was accompanied by approximately 20-26% mortality; thus, 11-12 animals from each group were observed in the study. The results of the study of the ECG dynamics did not reveal virtually any differences between the groups before the start of the modeling of MI (Table 2). According to the ECG data (registration of the ST segment elevation, characterizing the presence of myocardial infarction), after the modeling of MI, no reliable differences in the frequency of MI occurrence in the experimental groups were observed: the frequency of MI averaged 83%.

Table 2: Heart rate (HR) and ECG parameters of experimental animals before EMI, m $M \pm m$.

Parameter	Experimental group (*n**)				
	I (15)	II (15)	III (15)	IV (15)	V (15)
HR, beats/minute	445±30	463±30	452±28	427±31	441±31
P, mV	0.29±0.06	0.30±0.06	0.26±0.05	0.31±0.06	0.28±0.06
R, mV	1.71±0.19	1.83±0.17	1.86±0.18	1.71±0.20	1.65±0.19
S, mV	0.45±0.12	0.44±0.11	0.46±0.12	0.42±0.11	0.47±0.12
T, mV	0.21±0.05	0.23±0.05	0.22±0.04	0.21±0.05	0.23±0.05
PQ, ms	36±10	33±10	37±9	36±9	33±10
QT, ms	48±5	45±4	46±4	46±5	52±4

**n* — number of animals in the group; p<0.05 compared to group I.

Arrhythmia was not registered in the experimental groups.

Table 3 shows the data on the effect of the studied drugs on the ST segment value in the ECG of rats with MI (the registration of ST segment elevation is the main diagnostic sign of infarction). It is evident that 1 h after the modeling of MI, the ST segment elevation value did not differ significantly between the groups.

A decrease in the ST segment below the isoline (depression) characterizes post-infarction ischemic changes and indicates insufficient blood supply and myocardial hypoxia. The results presented in Table 3 show that the magnitude of ST segment depression 3 days after EMI in groups III (Riboxin therapy) and IV (Riboxin + peptide at a dose of 200 μg per rat) was 1.5, and in group V (Riboxin + peptide at a dose of 500 μg per rat) 2 times less than in group II (EMI without therapy).

Seven days after EMI, the magnitude of ST segment depression in the EEG of animals in group II significantly increased by 1.2 times compared to the period of 3 days (Table 3). Against the background of therapy, the magnitude of ST segment depression in rats in groups III and IV did not change, and in group V it significantly decreased by 2 times and was 4 times less pronounced than in the control (group II). The obtained results indicate the ability of the peptide to increase myocardial resistance to hypoxia.

Table 3: The effect of the studied drugs on the ST segment value in rats with EMI.

Time after surgery	ST segment magnitude, mm ($M \pm m$)			
	Группа II	Группа III	Группа IV	Группа V
1 h	1.42±0.14	1.46±0.16	1.39±0.15	1.48±0.15
3 days	-0.93±0.11	-0.54±0.12*	-0.64±0.13*	-0.49±0.15*
7 days	-1.24±0.11	-0.65±0.13*	-0.77±0.16*	-0.23±0.11*

* $p<0.05$ compared to group II.

Table 4 presents the data on the effect of Riboxin and the LKEKK peptide on the dynamics of the heart rate (HR) and ECG parameters in rats after EMI. It is evident that in Group II (EMI without treatment) a pronounced persistent negative chronotropic effect was observed (a reliable decrease in HR by 30-40% after 1 h and 7 days compared to Group I). In addition, the rats of this group showed the following reliable ECG changes after 7 days: a 2-fold decrease in the amplitude of the R peak compared to the observation period of 1 h and Group I, which characterizes the suppression of myocardial contractile activity in the post-infarction period; as well as an elongation of the PQ and QT peaks by 2.4 and 2 times compared to the observation period before EMI, indicating a persistent progressive disorder of atrioventricular (PQ) and intraventricular (QT) conduction.

During therapy with Riboxin (group III), a reliable decrease in heart rate, a decrease in the amplitude of the R peak and an extension of the QT peak were maintained after 7 days compared to the norm (group I) and with an observation period of 1 h. At the same time, there was no reliable extension of the PQ wave, which indicates normalization of atrioventricular conduction (Table 4).

The peptide (groups IV and V) also did not affect the heart rate; bradycardia was at the same level as in groups II and III (Table 4). At the same time, after 7 days, there was no reliable decrease in the amplitude of the R peak and a reliable (2-fold) increase in the amplitude of the T peak was observed, indicating an increase in contractile activity. As in the case of Riboxin therapy, in groups IV and V there were reliable disturbances of intraventricular conduction (prolongation of the QTpeak by 1.3 and 1.5 times after 1 h and 7 days compared to the observation period before EMI) and normalization of atrioventricular conduction.

Table 4: The effect of the studied drugs on the dynamics of heart rate (HR) and ECG parameters in rats with EMI, $M \pm m$.

	Parameter						
Time after surgery	ЧСС	P	R	S	T	PQ	QT
Group II							
1 h	323±27•	0.022±0.001	2.01±0.43	0.048±0.02	0.26±0.13	30±5	64±9•
7 days	274±20*•	0.020±0.008	0.80±0.19*•	0.038±0.01	0.22±0.17	80±8*•	90±15*•
Group III							
1 h	315±36•	0.022±0.001	2.02±0.45	0.049±0.01	0.27±0.13	39±5	61±9•
7 days	334±14•	0.017±0.003	0.97±0..0*•	0.046±0.01	0.28±0.10	43±7	70±13•
Group IV							
1 h	316±16•	0.022±0.003	2.02±0.42	0.054±0.02	0.26±0.13•	35±7	60±9•
7 days	277±14*•	0.018±0.002	1.94±0.40	0.043±0.01	0.57±0.21•	28±10	72±7•
Group V							
1 h	320±34•	0.022±0.0009	1.93±0.46	0.047±0.009	0.25±0.12•	28±5	63±8•
7 days	314±21•	0.033±0.002	1.81±0.41	0.039±0.01	0.58±0.20*•	55±1*	70±6•

* $p<0.05$ compared to the observation period of 1 h.

• $p<0.05$ compared to the norm - before EMI

The results of morphometric and biochemical studies against the background of EIM are presented in Table 5. It turned out that EMI is characterized by a reliable increase in the relative mass of the heart. In addition, reliable signs of cytolysis in the myocardium were observed: an increase in the activity of alanine aminotransferase (AAT), aspartate aminotransferase {AsAT), acid phosphatase (AP) and lactate dehydrogenase (LDG) in the blood plasma. Disturbances in the processes of tissue respiration and metabolism in the myocardium were recorded: ATP and glycogen reserves decreased; the content of underoxidized metabolic products - lactic acid - increased; activation of free-radical reactions (an increase in the concentration of malondialdehyde (MDA) - the end product of lipid peroxidation) and a decrease in the level of antioxidant protection (catalase, succinate dehydrogenase SDG) were observed. Combined therapy with Riboxin and peptide (groups IV and V) reliably reduced the activity of AsAT, CP and LDG in the blood; normalized the relative mass of the heart, as well as the content of lactic acid and MDA and increased the content of glycogen and ATP in the myocardium.

Table 5: The influence of the studied drugs on the morphometric and biochemical parameters of experimental animals, $M \pm m$.

Parameter	Experimental group				
	I	II	III	IV	V
Body weight, g	184 ± 10	182 ± 10	194 ± 10	205 ± 10	190 ± 5
Relative heart mass, mg/100 g body weight	3.5 ± 0.1	4.9 ± 0.3	3.5±0.1*	3.2 ± 0.1*	3.3± 0.2*
AAT, blood, µE/l	0.22±0.03	2.46±0.45	1.19±0.12*	0.88±0.26	0.55±0.22
AsAT, blood, µE/l	0.65±0.05	2.85±0.12	1.17±0.19*	0.99±0.21*	0.72±0.12*
AP, blood, µE/l	0..1±0.10	1.96±0.16	0.95±0.11*	0.90±0.11*	0.73±0.13*
LDG, blood, mmol/h/l	4.90± 0.32	8.92± 0.36	6.77± 0.27	6.80± 0.25	4.92± 0.33*
Catalase, blood, mg/ml/min	445 ± 20	252 ± 28	263 ± 35	327 ± 43	312 ± 47
Serum MDA, nmol/mg protein	1.65± 0.25	4.82± 0.33	4.83± 0.32	3.11± 0.24	3.17± 0.20
Reduced glutathione, heart, mg%	85 ± 10	40 ± 5	45 ± 6	57 ± 12	64 ± 13*
Glycogen, heart, mg%	2450± 110	850±90	1350± 120*	1990± 110*	2160± 140*
Lactic acid, heart, mg%	60±10	185 ± 15	170 ±13	93 ± 7*	65 ± 11*
ATP, heart, µmol/g	2.30± 0.11	0.55± 0.10	1.23± 0.14*	1.51± 0.12*	2.23± 0.15*
SDG, heart, µg formazan/g protein/h	145± 15	75 ± 10	55± 13	80 ± 12	98 ± 10
Tissue respiration rate, heart, µl O_2/100 mg/h	61 ± 6	38 ± 4	38 ± 5	37 ± 5	52 ± 6
MDA, heart, nmol/mg protein	3.57± 0.35	7.50± 0.45	4.52± 0.34*	4.11± 0.23*	3.49± 0.31*
Catalase, heart, mgml/min	475 ± 20	260 ± 35	256 ± 39	315 ± 42	331 ± 46

*- the differences are significant compared to group II ($p < 0.05$). AAT – alanine aminotransferase; AsAT – aspartate aminotransferase; AC – acid phosphatase; LDG – lactate dehydrogenase; MDA – malondialdehyde; SDG - succinate dehydrogenase.

The results of the histological studies are shown in Fig. 2 and 3. Fig. 1a shows a typical histological picture of the left ventricular myocardium of the intact rat. The transverse striation of the myofibrils and the nuclei of the cardiomyocytes are clearly visible. Twenty-four hours after the operation, general circulatory disorders in the form of plethora, minor hemorrhages, blood stasis in the capillaries, and edema were observed in the left ventricular myocardium of the rats in Group II. Muscle fibers, intensely stained with eosin or picric acid, are visible under the epicardium (Fig. 2b). Three days later (Fig. 2c), the circulatory disorder was no longer general, but local. Fig. 2c shows that the cardiomyocytes are homogeneously stained with eosin, i.e., lacking transverse striation and nuclei. Most of the necrotic fibers are in a state of lysis and are stained very pale. Hemolyzed erythrocytes and polymorphonuclear leukocytes are visible in the necrotic zone. The myocardial stroma outside the necrotic zone was edematous. On the 7th day (Fig. 2d), resorption of necrotic fibers and replacement of necrotic areas with young connective tissue rich in cellular elements, mainly fibroblastic, were observed. At later stages, a fibrous scar formed at the site of these foci of proliferation.

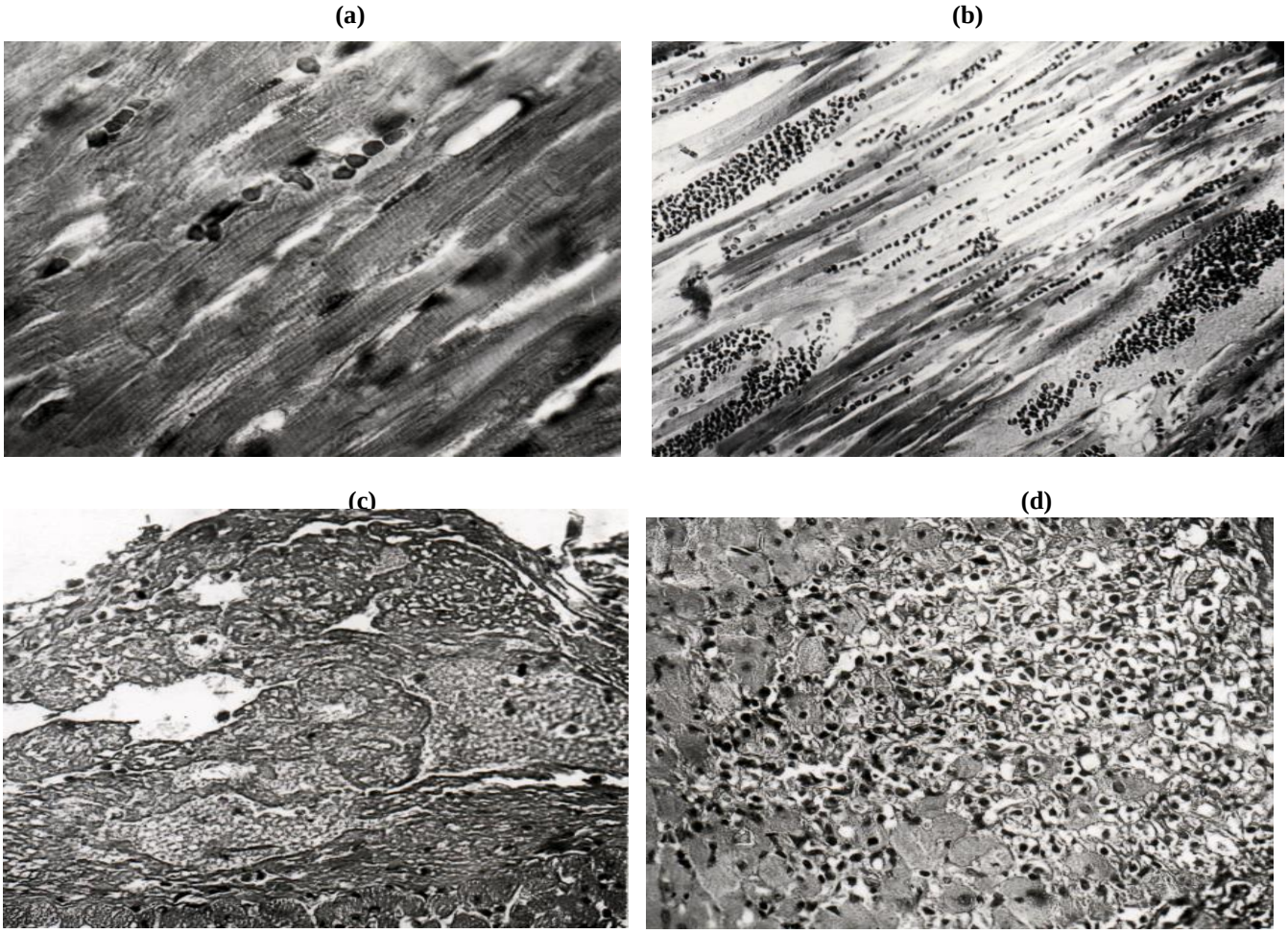

Fig. 2. Histological picture of the myocardium of the left ventricle of the rat heart: intact (a), group II after 24 h (b), after 3 (c) and 7 (d) days after EMI. Van Gieson staining, magnification 40×7.

In rats of experimental groups (III–V) 24 h after EMI, the general disorder of blood circulation in the myocardium of the left ventricle of the heart was less pronounced compared to group II. In the ischemic area, accumulation of leukocytes in muscle fibers (eosinophilia of muscle fibers) and slight edema were observed. Three days later (Fig. 3a, b, c), formation of myocardial infarction was observed, mainly under the epicardium of the anterior wall of the left ventricle. The size of the necrotic focus was significantly smaller than in the control rats. In Fig. 3c (group V), transverse striation of myofibrils is discernible. Leukocyte infiltration around the necrotic focus was weakly expressed. Interstitial edema was absent outside the ischemic zone.

On the 7th day, a proliferative reaction of the stroma with a large number of fibroblastic cells was observed at the site of necrotic fibers. The size of the proliferation area was smaller compared to the control (group III). In Fig. 3d, young connective tissue with a large number of fibroblasts is visible in the infarction zone (group V).

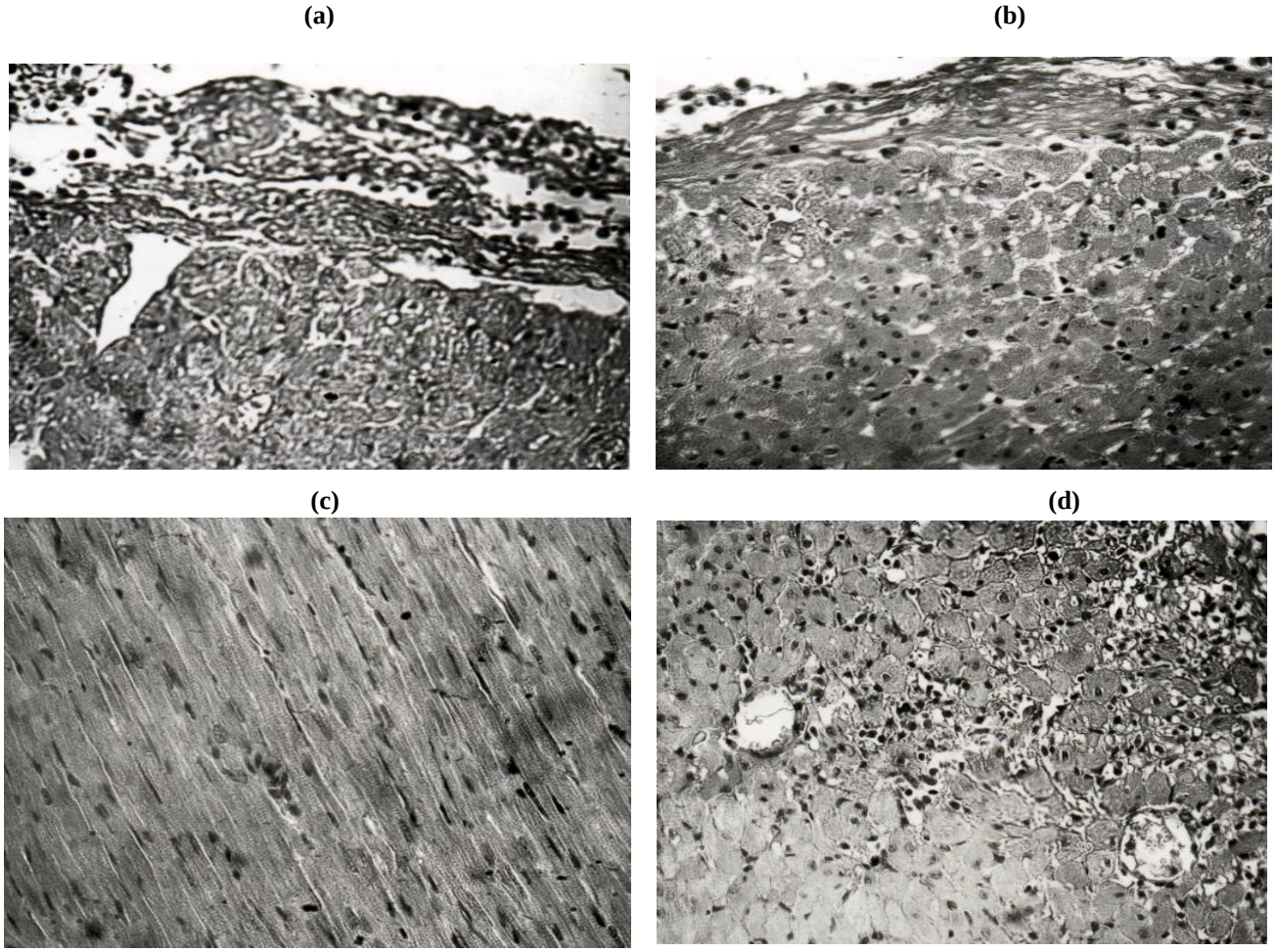

Fig. 3. Histological picture of the myocardium of the left ventricle of the rat heart in groups III (a), IV (b), V (c) after 3 days and in group V (d) after 7 days after EMI. Van Gieson staining, magnification 40×7.

Conclusion

summarizing the obtained results, the following conclusions can be made: the LKEKK peptide is able to bind to rat myocardial membranes with high affinity and high specificity. Combined seven-day therapy of EMI with the standard drug Riboxin and the peptide has a pronounced anti-ischemic effect: improves coronary blood flow; increases myocardial contractility (at a peptide dose of 200 µg per rat by 1.5, at a dose of 500 µg per rat by 4 times), doubles myocardial contractility, promotes normalization of myocardial atrioventricular conduction disorders, and this effect is achieved already at a peptide dose of 200 µg per rat; In addition, a reliable decrease in the intensity of myocardial cytolysis and lipid peroxidation, as well as an increase in the level of antioxidant protection are noted. All of the listed effects were more pronounced than in the case of Riboxin therapy without the peptide. Thus, the synthetic peptide LKEKK has significant anti-ischemic and cardioprotective activity, it has a simple structure, is non-toxic and non-allergenic. All these effects potentially suitable for use in the prevention and treatment of cardiovascular diseases,

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Authors contributions

Navolotskaya Elena V - Management of work, systematization and analysis of results, writing of the article.

Zinchenko Dmitry V - Working with labeled peptide, binding experiments.

Kolobov Alexander A - Animal experiments, biochemical and electrocardiographic studies.

Murashev Arcady N - Work with animals, histological and biochemical studies, analysis of results.

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