Influence of various algorithms of bioresonance therapy on repair processes in acute myocardial infarction in experiment

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Myocardial infarction (MI) is still one of the leading causes of death in the population worldwide. According to the forecasts of the World Health Organization, by 2020 mortality from cardiovascular diseases may reach 25 million cases per year, almost half of them will be mortality from coronary heart disease, primarily from myocardial infarction [1]. According to researchers, in the Russian Federation mortality from cardiovascular diseases is 8 times higher than in France, and is approximately 58% of the total mortality structure. The leading role in the structure of mortality from cardiovascular diseases belongs tocoronary heart disease

(Ischemic heart disease) - 35%. In recent years, there has been a clear trend towards its rejuvenation [2, 3].

Therefore, the search for the most effective measures for the treatment of acute myocardial infarction, as well as the prevention of ischemic disease and the development of optimal treatment algorithms is relevant at the present time. In this aspect, of interest are methods that allow to reduce the area of necrosis and improve the repair processes, reducing the level of atherogenicity of the main source of coronary heart disease [4].

Our earlier experimental studies found that BRT stimulates cell proliferation and accelerates the processes of liver, skin, and blood regeneration in white rats [5–7]. Based on this, it can be assumed that in acute myocardial infarction, the use of BRT sessions will stimulate the division of fibroblasts, accelerate the formation of a scar and stabilize the patient's condition, with the aim of further effective treatment. We did not find scientific studies on the study of the possibilities of the method of endogenous bioresonance therapy in the treatment of acute myocardial infarction in the experiment and clinic.

The purpose of this study: selection of the optimal mode of bioresonance effects, which affects the reparation processes in acute myocardial infarction.

Material and research methods

Research objects: The experiments were carried out on adult white rats of 160–180 years (107 pcs.). Research material: heart tissue and blood of white rats.

Research methods: a) modeling of experimental myocardial infarction; b) the method of bioresonance therapy; c) determination of troponin and enzyme activity in blood serum; d) assessment of the histoarchitectonics of heart tissue on paraffin sections - staining with hematoxylin-eosin; e) immunohistochemical assessment of the proliferation process by marker proteins (Ki-67 and vimentin). An experimental model of acute myocardial infarction was reproduced according to G. Selye [8]. The method of bioresonance therapy was carried out using the hardware-software complex "IMEDISEXPERT" manufactured by the firm "IMEDIS" (Russia).

Determination of troponin content and enzyme activity in serum. The most valuable for the diagnosis of acute myocardial infarction is the determination of the activity of several enzymes (biomarkers) in the blood serum. Based on this, after decapitation of the animals (euthanasia was carried out under ether anesthesia), 5 ml of blood was taken in heparin tubes. Blood tubes were left at room temperature in the for 20 minutes, after which it was centrifuged at 3000 rpm. IN supernatate was determined amount enzymes: creatine phosphokinase (KLF), MV-factions creatine phosphokinase (MB- CPK) and lactate dehydrogenase (LDH) (Vitrosdt 60 2 system. Ortho-clinical diagnosticsa Johnson-Johnson company). In addition, using or single-stage immunochromatographic test for the determination of troponin in plasma (HexagonTroponin, Human GmbH65205 Wiesbaden-Germany).

In order to identify morphological types of cells stained for the Ki-67 protein in our experiments, we used antibodies to vimentin (a marker protein of fibroblasts). Staining was performed on parallel sections.

The animals were divided into the following groups.

Control group - intact animals.

Experienced group I - animals that underwent ligation of the left coronary artery (model acute myocardial infarction).

Experienced group II - the BRT algorithm in group II was as follows. After 1 hour, after ligation of the left coronary artery, a session of endogenous bioresonance therapy was performed.

Experimental group III. After ligation of the left coronary artery, a session of endogenous bioresonance therapy was performed 1 hour later. From the beginning of the BRT session, the amplitude-frequency spectrum of the triton signal in the load was included from the drug selector.

Experimental group IV. After ligation of the left coronary artery, after 1 hour, a session of endogenous bioresonance therapy was performed, lasting 30 minutes. From the beginning of the BRT session, the blood of the infarcted animal was placed in the inverse container. From the drug selector, the trepang signal in the load, organ preparations of the heart, hypothalamus, and nervous tissue were included in the therapy process.

In the first 2 weeks, endogenous BRT sessions were performed every day, lasting 30 minutes, for 2 weeks. In the future, the sessions were carried out every other day, until the 30th day.

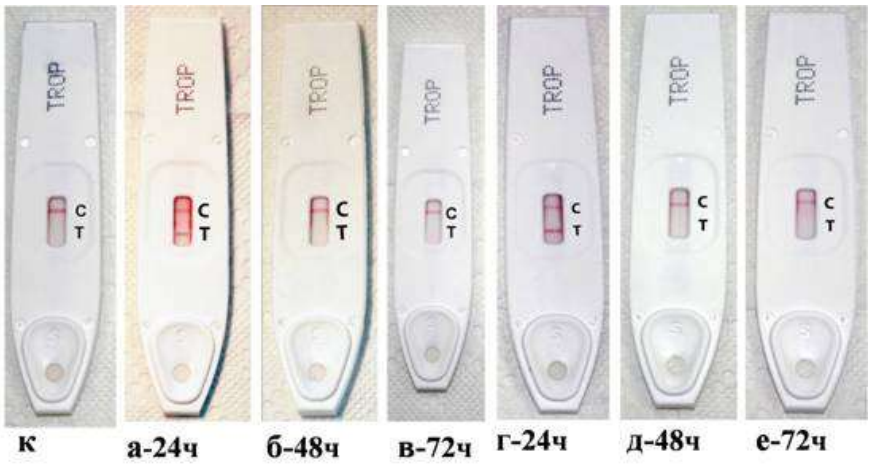
Material for research was taken at 24 h, 48 h, 72 h, 7, 14, 21, 30 days after ligation of the left coronary artery. The reliability of the data obtained was assessed by the Student's test ($p < 0.001$).

Results and discussion

WITHaim establishing occurrence of acute heart attack myocardium, using immunochromatographic test we determined the change in the concentration of troponin in the blood plasma of experimental animals (groups II, III and IV) in dynamics (24, 48 and 72 hours after the operation) and in the control group.

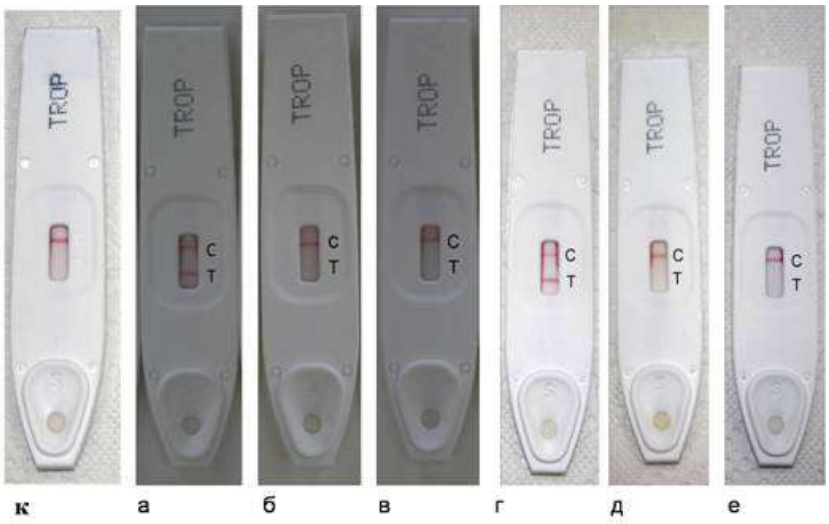
According to the literature, 4–5 hours after the death of cardiomyocytes, due to the development of irreversible necrotic changes, troponin enters the peripheral bloodstream and is determined in the venous blood. However, the peak troponin concentration is reached in the first 12-24 hours after the onset of acute myocardial infarction [10]. We found that 24 hours after ligation of the left coronary artery, in contrast to intact rats, in the blood plasma of animals as

The presence of troponin is found in groups I and II (Fig. 1a). It also follows from the figure that at the 48th and 72nd hours after the operation, the troponin fraction is not detected in the blood of animals of both experimental groups [9, 10].



Rice. 1a.The presence of troponin in the plasma of white rats in normal conditions and after surgeries in dynamics (24, 48 and 72 h): K - control group; a, b, c - the first group; d, e, f - the second group.

By enzyme immunoassay, the presence of troponin was detected in the blood of animals of groups III and IV. As in the previous groups, troponin appears only during the first day after ligation of the left coronary artery (Fig. 1b).



Rice. 1b.The presence of troponin in the plasma of white rats in normal conditions and after surgeries in dynamics (24, 48 and 72 h): K - control group; a, b, c - group III; d, e, f - group IV.

The presence of troponin in the blood of animals from the experimental groups is a reliable indicator of the development of acute myocardial infarction in animals. However, the priority of determining troponin in the blood does not diminish the importance of studying some other biomarkers, in particular, the quantitative determination of creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and the isoenzyme MB-CPK.

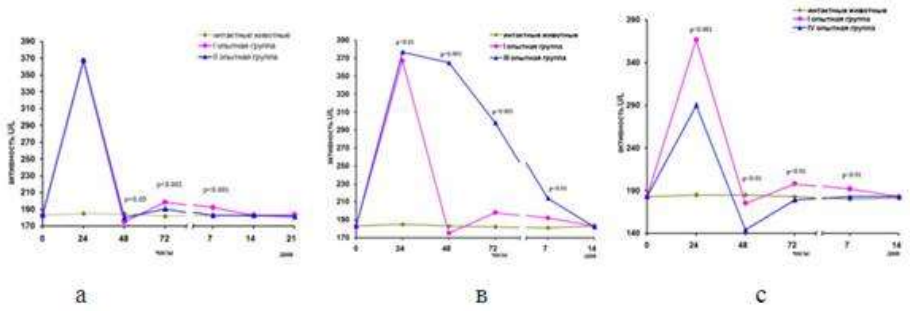
By enzyme immunoassay, the presence of troponin was detected in the blood of animals of groups III and IV. As in the previous groups, troponin appeared only during the first day after ligation of the left coronary artery.

The absence of a significant concentration of CPK in other internal organs, except for the heart, sharply increases the specificity of this assay in comparison with the determination of the activity of other biomarkers. An increase in the activity of CPK in the blood is determined in almost all patients with acute myocardial infarction, according to the literature [9]. CPK begins to rise in 6-12 hours, reaching its maximum within 18-24 hours. The duration of hyperenzymemia is 3-4 days. The normalization of CPK activity usually occurs on the 4th day.

Figure 2 shows the dynamics of changes in the activity of creatine phosphokinase (CPK) in the blood of intact and experimental animals with experimental acute myocardial infarction.

In fig. 2a shows the results of changes in the activity of CPK in the blood of intact animals and experimental animals of groups I and II. In fig. 2c - the results of studies of CPK in the blood of intact and experimental animals of groups II and III are presented. In fig. 2c shows the results of studies of CPK in the blood of intact and experimental animals of groups II and IV.

As shown by the results of our studies (Fig. 2), in the first 24 hours after



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Rice. 2.Changes in the activity of creatine phosphokinase (CPK) in the blood of intact and experimental animals with experimental acute myocardial infarction: a - group II; c - group III; c - IV group

the occurrence of acute myocardial infarction in the blood of animals of groups I and II, there is a sharp increase in the activity of CPK (367 ± 1.5 U / L and 368 1.1 U / L, respectively).

48 hours after the reproduction of the model of acute myocardial infarction, there was a sharp decrease in CPK activity in both groups, and even lower than in the control group (K - 183 ± 1 U / L, I group - 175 ± 1.3 U / L and II group - 180 ± 1.6 U / L).CPK activity increases again by 72 hours. In particular, in group I was198 ± 1.4 U / L, and in the second group - 190 ± 1.4 U / L. By the 7th day of the development of acute myocardial infarction in group I, its activity remained high, while in group II the average value of CPK activity decreased almost to the control value. On the 14th and 21st days of acute myocardial infarction, the indicators in both groups remained within the normal range.

A different picture was observed in the blood of animals of group III (Fig. 2c). High CPK activity in this group of animals persists for two weeks (24th hour - 377 1.64 U / L, 48th hour - 245 1.5 U / L, 72 hours - 265 1.2 U / L, 7th day - 214 1.62 U / L) after ligation of the left coronary artery. The positive effect of BRT sessions was best manifested in animals of group IV (Fig. 2c). In particular, the activity of CPK in the blood of white rats of this group increased by 24 hours to 290 2.5 U / L (control group - 183 1 U / L). However, the enzyme activity index is lower in comparison with groups I, II and III. By the 48th hour, after ligation of the left coronary artery, the CPK activity decreases, even below the control values, returning to normal by the 72nd hour- 179 1 U / L.

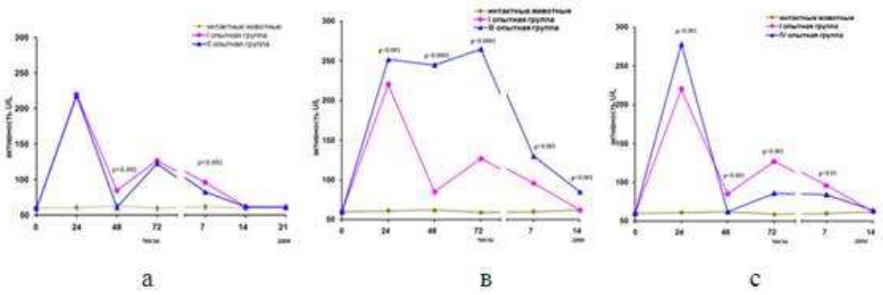
The most accurate way to assess the lesion of the heart muscle, in comparison with determining only the activity of creatine phosphokinase (CPK), is to determine its MV fraction (KFK-MV). The CPK-MB isozyme is an early indicator of cardiac muscle damage [14]. The research results are shown in Fig. 3.

In fig. 3 shows the dynamics of changes in the activity of CPK-MB in the blood of intact and experimental animals with experimental acute myocardial infarction.

In fig. 3a shows the results of changes in the activity of CPK-MB in the blood of intact animals and experimental animals of groups I and II. In fig. 3c - the results of studies of CPK-MB in the blood of intact and experimental animals of groups II and III are presented. In fig. 3c shows the results of studies of KFKMV in the blood of intact and experimental animals of groups II and IV.

As can be seen from Fig. 3a, the activity of the CF fraction of creatine phosphokinase (CPK-MB) in the blood of experimental animals, in groups I and II, sharply increases after 24 hours in comparison with intact animals (60 1.5 5 U / L) and is in group I - 220 2 U / L, in group II - 218 fifteen U / L.

By the 48th hour, in both groups there is a sharp decrease in the activity of CPK-MB. In the blood of animals of group I, with reproduced acute myocardial infarction, the activity of CPK-MB decreased to the level of 85 ± 1.5U / L, and in the second group of animals, which underwent endogenous BRT sessions against the background of acute myocardial infarction, the decrease in the activity of CPK-MB was still more pronounced- 61 ± 0.8 U / L, from which it follows that 48 hours after the creation of the AMI model, the activity of the CPKMV enzyme in the II experimental group, in contrast to the I experimental group (AMI model), normalized. In the next 24 hours, the activity of CPK-MB in groups I and II did not differ significantly (Fig. 3a).



Rice. 3.Changes in the activity of the creatine phosphokinase (CPK-MB) fraction in the blood of intact and experimental animals: a - Group II; in- Group III; with- IV group.

By the 72nd hour, after the development of acute myocardial infarction, the activity of CPK-MB increases again, but already with much lower values - 127 ± 2.4 U / L). On the 7th day after the formation of AMI, the activity of the CPK-MB enzyme remained elevated- 96 ± 1.4 U / L). On the 14th day after the formation of AMI, the CPK-MB indicator was within the normal range.

In group II, animals that received endogenous BRT for 30 minutes after 1 hour after the operation, creating a model of AMI, according to the algorithm described above, by 72 hours, the indicators of CPK-MBI and II groups practically did not differ.

By the 7th day of AMI formation, the CPK-MBI values of the group were slightly higher than the CPK-MBII values of the group, and by the 14th day of AMI formation, the CPK-MB index in both groups was similar to the control group (intact animals).

A different picture was observed in group III (Fig. 3c). As in the case of CPK, the high activity of its isoform, CPK-MB, in the blood of animals of group III, in comparison with group I, persists for two weeks (24 hours of CPK-MB of group III- 252 0.8 U / L; 48 hours- 245 1.5 U / L; 72 hours- 265 1,2 U / L; 7th day 130 1.5 U / L; 14th day- 85 1.5 U / L.

The nature of the curve shown in Fig. 3c, shows that the activity of the MB fraction of creatine phosphokinase (CPK-MB) in the blood of animals of group IV normalizes by 48 hours after ligation of the left coronary artery (24 hours of CPK-MBIV group- 278 3.2 U / L; 48 hours - 62 1 U / L; 72 hours - 86 1,3 U / L; 7th day84 1.7 U /L; 14th day- 64 1.1 U /L).

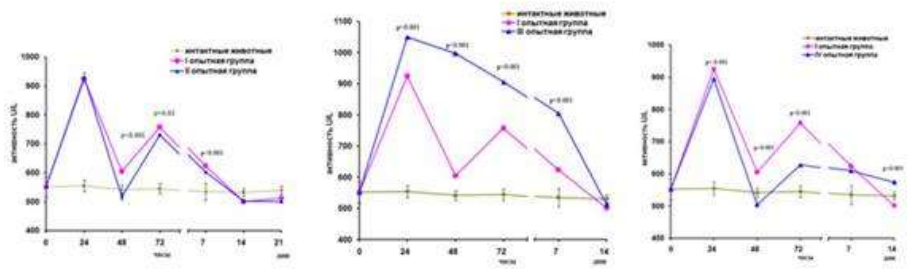
As for the activity of the enzyme LDH, according to the literature data, the activity of LDH in acute myocardial infarction increases more slowly than the activity of CPK and MV-CPK, and remains elevated for a longer time. Normalization of the activity of the enzyme LDH occurs within 2-3 weeks [11-fourteen].

In fig. 4 shows the dynamics of changes in the LDH activity in the blood of intact and experimental animals with experimental acute myocardial infarction.

In fig. 4a shows the results of changes in the LDH activity in the blood of intact animals and experimental animals of groups I and II. In fig. 4c shows the results of LDH studies in the blood of intact and experimental animals of groups II and III. Figure 4c shows the results of LDH studies in the blood of intact and experimental animals of groups II and IV.

The results of our research, presented in Fig. 4a, on the study of the effect of endogenous BRT on the activityLDH in the blood of animals showed that the activity of the enzyme increases by 24 hours in both groups I and II (24 hours Control - 552 17.9 U / L; Group I - 925 3.6 U / L; II group - 930 1.5 U / L)

(Fig.4a). 48 hours after ligation of the left coronary artery in group II, the enzyme activity decreases to the control level (Control- 552 17.9 U / L; I group - 605 2.2 U / L; II group - 518 11.2 U / L).



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Rice. 4.Influence of endogenous bioresonance therapy on the activity of lactate dehydrogenase (LDH) in the blood of intact and experimental animals in dynamics during experimental acute myocardial infarction: a - Group II; in- Group III; with- IV group.

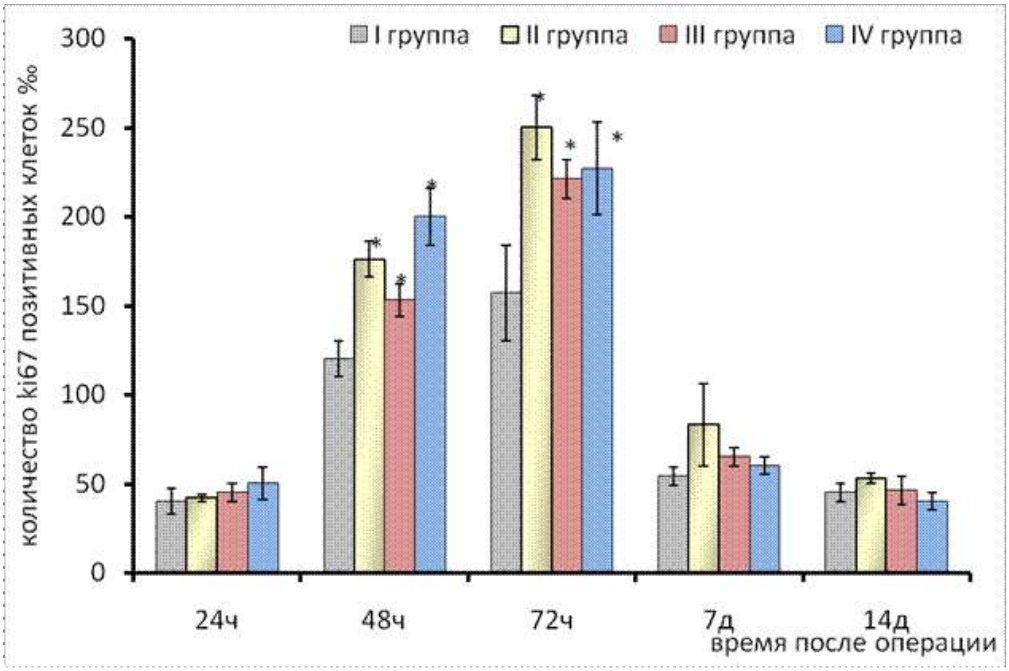
In the following days, the activity of the LDH enzyme increases in both experimental groups within the same limits. As for the results of group III studies, the analysis of the curves shown in Fig. 3c showed that, unlike CPK and CPK-MB, the positive effect of endogenous BRT sessions on the normalization of the LDH enzyme content is not pronounced. Normalization of the LDH enzyme content in the blood of animals occurs only after two weeks. BRT sessions do not cause positive changes in the activity of the LDH enzyme in the groupIII (rice. 4c). As in group I, normalization of LDH activity is achieved after two weeks (14th day), control- 552 17.9 U / L; I group - 501 1.8 U / L; III group - 513 1.5 U / L.

A change in the activity of lactate dehydrogenase (LDH) was detected in the blood of animals of group IV (Fig. 4c). It follows from the figure that in this group the LDH activity decreases by the 48th hour. Three days later, a second peak of activity appears, but significantly lower than the corresponding indicator of group I (72nd hour) (Control- 552 17.9 U / L; I group - 758 1.8 U / L; Group IV - 628 1.8 U / L).

To assess the proliferative activity of cells in the myocardium, carried out immunohistochemical analysis. The results of the studies have shown that cell proliferation is observed in the damaged area within 24 hours after ligation of the left coronary artery. However, active proliferation is found in the period from the second to the seventh day. The maximum number of Ki-67-positive cells can be seen on the third day. According to the literature, cardiomyocytes actively proliferate only in the peri-infarction area, and not in the necrosis focus, while the proliferation of scar-forming fibroblasts is noted precisely in the necrosis zone [15]. To assess mitotic activity, in addition to the classical morphological analysis, the authors used the immunofluorescent determination of the Ki-67 protein in the nuclei of cardiomyocytes. When examining the human heart soon after ligation of the coronary artery at a later date, it has also been shown that

In order to identify morphological types of cells stained for the Ki-67 protein in our experiments, we used antibodies to vimentin (a marker protein of fibroblasts). Staining was performed on parallel sections. The presence of positive cells in the area of the scar was revealed, they were mainly fibroblasts. However, in the same zone, we detected Ki-67 positive cells that were not stained for vimentin. From which it follows that not only fibroblasts, but also myocytes or myocyte precursors proliferate in the scar zone. Such, though single cells in the scar zone, and not only in the peri-infarction area, as described in the literature [15], we have identified in groups I, II and IV. In order to establish their true number, a series of experiments should be carried out using immunofluorescent antibodies,

As can be seen from the data below (Fig. 5, Table 1), the immunohistochemical assessment of the proliferation process by the marker protein - Ki-67 between groups II, III, IV did not reveal a significant difference in indicators (* p> 0.05), while at 48 and 72 hours, after the model of acute myocardial infarction, an increase in the number of Ki-67-positive cells in the myocardium of white rats after ligation of the left coronary artery in dynamics compared with group I (p <0.005).



Rice. five.Change in the number of Ki-67-positive cells in the myocardium of white rats after ligation of the left coronary artery in dynamics

Table 1

Change in the number of Ki67-positive cells in the myocardium of white rats after ligation of the left coronary artery in dynamics (-ppm)

Group no.	24th hour	48th hour	72nd hour	7th day	14th day
I	40 ± 7	120 ± 10	157 ± 27	54 ± 5	45 ± 5
II	42 ± 2	176 ± 10	250 ± 18	83 ± 23	53 ± 3
III	45 ± 5	153 ± 9	221 ± 11	65 ± 5	46 ± 8
IV	50 ± 9	200 ± 16	227 ± 26	60 ± 5	40 ± 5

Based on the results obtained, as well as the stimulating effect of BRT sessions on the proliferation of bone marrow cells described in earlier studies, it can be assumed that the positive effects of BRT sessions established by us on the model of myocardial infarction may be mediated by bone marrow activation. At the same time, the results obtained by us give the right to believe that they have not only theoretical, but also practical significance, the possibility of their use, in order to enhance the proliferative activity of the ischemically damaged myocardium.

conclusions

On the model of experimental myocardial infarction, reproduced by ligation of the anterior descending branch of the left coronary artery in white rats, it was found that:

1 BRT sessions conducted in groups II and IV have a positive effect on the recovery processes in the damaged heart tissue.

2 Conducting sessions of bioresonance therapy (endogenous BRT and the fourth group BRT algorithm) in acute myocardial infarction in the experiment, accelerates the process of normalization of serum enzymes at the initial stage of recovery growth.

3 The normalizing effect of BRT sessions is most pronounced in the case of the CF fraction of creatine phosphokinase (CF-CPK).

4 BRT sessions (endogenous BRT and BRT algorithm of the fourth group) in acute myocardial infarction in the experiment, accelerate morphological transformations at the initial stage (24 hours) of recovery growth.

five Conducting sessions of bioresonance therapy in animal groups II and IV, stimulates cell proliferation in the area of the scar.

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