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EFFECTS OF TRANSPLANTATION OF MSCS AND PANCREATIC BETA CELLS ON METABOLIC PARAMETERS OF BLOOD PLASMA IN RATS WITH MYOCARDIAL INFARCTION WITH UNDERLYING DIABETES MELLITUS

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ABSTRACT

The purpose of this work was to study the role of transplantation of mesenchymal stem cells (MSCs) and pancreatic beta cells (BCs) in metabolism of rats with diabetes mellitus (DM) and myocardial infarction (MI). The animals formed 4 groups, 24 in each: control group - healthy Wistar-Kyoto animals, which served as the norm for the studied parameters; group 1 in which animals with diabetes mellitus had a myocardial infarction simulated by ligation of the left gastric artery after the first branch; group 2 – rats with the model of MI and DM which, on the day 2 after the modeling, had transplantation of rat mesenchymal stem cells taken from the bone marrow of tubular bones; group 3 – rats with DM+MI model which received transplantation of MSCs and rat pancreatic BCs on the day 2 after the modeling. We dynamically studied the activity of aspartate aminotransferase enzymes, creatine kinase-MB, blood plasma lactate dehydrogenase, and haptoglobin and ceruloplasmin proteins. The study revealed that cardiomyoplasty with MSCs caused a positive metabolic effect. MSC transplantation is accompanied by a decrease in the activity of ADA and LDH enzymes in the damaged myocardium, which indirectly indicates an increase in the energy balance of energy-forming substrates and a decrease in the degree of myocardial ischemia. A decrease in the accumulation of lipid peroxidation products and the tension of the non-enzymatic antioxidant protection is a good prognostic criterion for the restoration of the functional activity of the damaged myocardium. The transplantation of a mixed culture of MSCs and pancreatic BCs makes it possible to fully compensate for carbohydrate metabolism, to achieve the best performance in the studied groups in terms of lipid peroxidation regulation and activation of the antioxidant system.

Keywords: myocardial infarction, type 1 diabetes, stem cells, β-cells, mesenchymal stem cell (MSC) transplantation, oxidative stress markers

INTRODUCTION

Over 80% of mortality in type 1 diabetes is associated with the pathology of the cardiovascular system. Myocardial infarction in this structure occupies more than three quarters of cases. It has been established that more than 50% of patients with type I diabetes die from myocardial infarction during the first 5 years [1,2,3]. The modern method of treating myocardial infarction with underlying diabetes mellitus consists in placing stents in the coronary arteries. However, in the case of a multifocal peripheral lesion, or with previously performed coronary artery bypass grafting or stenting, this procedure is either not feasible or ineffective, which prompts the researchers to search for new correction methods of myocardial ischemia with underlying chronic hyperglycemia [4,5]. It has been found that patients with diabetes mellitus and myocardial infarction have an increase in the level of endogenous malondialdehyde (MDA) and diene conjugates in the blood serum. The results obtained indicate the presence of oxidative stress in diabetes mellitus and myocardial infarction [6,7,8].

Purpose: To study the role of transplantation of mesenchymal stem cells and pancreatic beta-cells in metabolism of rats with diabetes mellitus and myocardial infarction.

MATERIALS AND METHODS OF THE RESEARCH

During the experiment, we formed 4 groups of animals, 24 in each: control group - healthy Wistar-Kyoto rats, which served as the norm for the studied parameters; group 1 in which animals with diabetes mellitus had a myocardial infarction simulated by ligation of the left gastric artery after the first branch; group 2 – rats with the model of MI and DM which, on the day 2 after the modeling, had transplantation of rat mesenchymal stem cells taken from the bone marrow of tubular bones; group 3 – rats with DM+MI model which received transplantation of MSCs and rat pancreatic BCs on the day 2 after the modeling. The animals were maintained in compliance with current best practices and standards of care in laboratory animals.

Diabetes in rats was modeled by administering an aqueous solution of alloxan subcutaneously at a dose of 200 mg/kg. The alloxan solution was prepared by dissolving the crystalline substrate Alloxan Tetrahydrate from Fluka-Sigma (Germany) in sterile distilled water. After dissolution of the crystals of the substance, the sterility of the solution was achieved by passing it through a Millex-GV membrane with a 0.22 μm filter from MILLIPORE (France) after which it was placed into sterile rolled vials. To reduce mortality from hypoglycemic coma, the animals were given water with sugar 2–4 hours later (after blood glucose control).

To obtain MSC culture we used bone marrow of healthy animals. To prevent bacterial contamination, they were washed with saline containing antibiotics. The bone marrow of tubular bones of rats was processed mechanically and enzymatically. Then it was placed in a thermostat at 37°C for 10-15 minutes. After 10-15 minutes, the inhibited cell suspension was centrifuged and the supernatant was discarded. A growth medium containing 10% fetal bovine serum (Biolot, St. Petersburg) was added to the cell pellet and it was placed in a flask. The cell pellet was resuspended in Igla growth medium (Biolot, St. Petersburg) containing 10% FBS (Biolot, St. Petersburg). Cells, in the amount of 1,5-2,0×106 cells/ml, were placed in a culture flask and cultivated in a CO_2 incubator at 37 °C with 5% CO_2 content and 95% humidity. The medium was changed every three days in all cultures. Before transplantation, the confluent cell culture was washed with a buffer solution and transferred into suspension using a standard trypsin solution (2.5 g) on Hanks without Mg^{2+} and Ca^{2+} (Sigma, USA). The cell suspension was inhibited by the addition of serum, then centrifuged. The supernatant was removed and the cell suspension in saline was given for transplantation. The cell culture was injected into the femoral vein of rats at the rate of 1,000,000 per 1 animal.

Determination of the activity of the aspartate aminotransferase (AST) enzymes, creatine kinase MB-fraction (CK-MB), lactate dehydrogenase (LDH) of blood plasma and plasma proteins of haptoglobin and ceruloplasmin was done on a Cobas Integra 400+ device using standard original kits from Roche-Diagnostics (Switzerland).

The Shapiro-Wilk test (W) was used to check the data distribution for normality, which made it possible to use it even with a small sample ($n<30$). To identify significant differences between the mean values of different populations of comparable groups, methods of variation statistics were applied using Student's t-test with Bonferroni correction for multiple comparisons with a type I error probability $p=0.05$. Data were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Ischemic myocardium is characterized by increased anaerobic metabolism and hypoxic type of metabolism. However, even the most enhanced metabolism is not able to protect the already damaged myocardium for a long time. Hypoxia naturally causes activation of lipid peroxidation (LPO) processes.

So, when modeling myocardial infarction, the accumulation of TBA-active products (TBA-test) occurred from 1.09±0.1 to 2.81±0.8 μmol/l (at t=2.13; p<0.05). An interesting fact is that in the group with MSC transplantation, this indicator was 1.74±0.5 μmol/l, which did not differ significantly from that in healthy animals (at t=1.11; p>0.05). In terms of absolute increase, it was 1.2 μmol/l lower than in MI, but nevertheless higher than the norm, which indicated the minimization of the damaging factor by MSC transplantation and a decrease in the intensity of LPO. When studying the content of haptoglobin, we found its increase in MI from 37±8 to 58±6 mg/dl (at t=2.2; p<0.05), no difference between animals with MI and MI with MSC transplantation (at t=0.63; p>0.05) and between norm and MI + MSC transplantation (at t=0.95; p>0.05). A different picture was present when studying the content of ceruloplasmin: there was no difference between the norm of 13.2±1.1 and animals with MI 15.5±4.1 (at t=0.54; p>0.05); there was also no difference between MI and MI + MSC transplantation 21.5±3.1 (at t=1.2; p>0.05). However, there was a significant increase in ceruloplasmin when comparing the norm and the group with MI + MSC transplantation (at t=2.5; p<0.05). In the group of rats with MI+MSC+BC, there was a slight increase in haptoglobin from 37±8 to 42±4 mg/dl (at t=1.3; p<0.05), while ceruloplasmin was significantly higher than the norm and in groups 1 and 2, being 25.3 ± 2.8 mg/dl, which indicated that the stabilization of the carbohydrate profile by pancreatic beta-cells led to the normalization of LPO and increased antioxidant protection of cells, which in turn led to the preservation of a larger number of cells (tab. 1).

Table 1. Indicators of lipid peroxidation and antioxidant status of cardiomyocytes in experimental myocardial infarction and after MSC transplantation

Experimental conditions	Content of plasma proteins		TBA-active products, μmol/l
	Haptoglobin, mg/dl	Ceruloplasmin, mg/dl	
Control n= 24	37,3±4,2	13,2±1,1	1,09±0,1
MI n=24 (group 1)	58,2±6,1*	15,5±4,1	2,81±0,8*
MI+MSCs n=24 (group 2)	50,3±4,3*	21,5±3,1*	1,74±0,5*
MI+MSCs+BCs n=24 (group 3)	42,1±3,1	25,3±2,8*	1,18±0,4*

** - the difference between the norm and the study group is significant (p<0,05)*

The accumulation of TBA-active products and a compensatory increase in the content of ceruloplasmin and haptoglobin indicate a breakdown of compensatory mechanisms. Accordingly, with the formation of an extensive myocardial infarction, cardiomyocyte membranes are destroyed and LPO is activated, which is reflected in an increase in TBA-active products. An interesting fact is that MSC transplantation causes a significant decrease in the concentration of TBA-active products. When studying the level of plasma proteins with antioxidant properties, it was found that the level of haptoglobin significantly increased with MI, although in the group with MSC transplantation, the increase was less pronounced, possibly due to a decrease in myocardial alteration caused by ischemia. The data obtained also indicate the possible activation of peroxide hemolysis of erythrocytes caused by hypoxia. A significant, almost 2-fold, increase in ceruloplasmin in the MI + MSC transplantation group indicates stimulation of the factors of the natural antioxidant system by the transplanted MSCs. Ceruloplasmin protects the myocardium from the prooxidant action of ferrous iron and, thus, inhibits LPO processes. TBA products in plasma of group 3 animals were significantly lower than those of groups 1 and 2 and equal to 1.18±0.4 μmol/l (at t=1.82; p<0.05 and t=1.67; p<0, 05 respectively).

Thus, transplantation of MSCs significantly limits the rate of LPO and reduces the expression of antioxidant proteins in blood plasma. In combination, transplantation of MSCs and pancreatic BCs makes

it possible to achieve greater success in stabilizing LPO and activating the antioxidant system, which is associated not only with angiogenesis and cytokine effects of MSCs on the body as a whole and the pathological process, but also with the normalization of carbohydrate metabolism by pancreatic beta-cells.

The degree of myocardial damage in MI was assessed by the activity of cardiac enzymes of creatine kinase (CK-MB) and AST. So, when modeling MI, a significant increase in CK-MB from 5125±123 to 7700±140 U/l (at t=13.8; p<0.001) occurred already in a day, which indicated large-focal MI in rats. There was a difference in the data of this indicator between the groups of MI and MI + MSC transplantation. In the group with MSC transplantation, the level of CK-MB was 5855.5±129 U/l, which was significantly lower than in MI in untreated animals (at t=9 .7; p<0.001) and did not differ from the indicators of healthy animals (at t=0.62; p>0.05). AST turned out to be less specific for MI volume. So, in the MI group it increased from 150±22 to 273±15 U/l (at t=4.6; p<0.001). In the group with MSC transplantation, this indicator was 262±28 U/l, which did not differ from the group with MI (at t=0.35; p>0.05) and was significantly higher than normal (at t=3.1 p<0.01). In group 3, as well as 1 and 2, there was an increase in CK-MB and AST. However, it should be noted that the studied indicators were much lower, which indicated the protective effect of mixed cell transplantation (table 2).

Table 2. Biochemical markers of metabolic activity of cardiomyocytes in experimental myocardial infarction and after cardiomyoplasty with MSCs

Experimental conditions	Enzyme activity			
	CK-MB, U/l	AST, U/l	ADA, nmol/min•l	LDH, U/l
Control n=24	5125±123	150±22	102±32	780±101
MI n=24	7700±140*	273±15*	170±48*	1071±215*
MI+MSCs n=24	5855,5±129€	262±28*	83±31*	671±150€
MI+MSCs+BCs n=24	5486,3±132	196±18	78,9±24*	650±124*

**- the difference between the norm and the compared group is significant (p<0,05)*
€- The difference between the groups of MI and MI with MSC transplantation is significant (p<0,05)

Thus, it can be concluded that with extensive MI, there is an increase in CK-MB and AST, but in terms of sensitivity for the size of necrosis, CK-MB is more prognostic than AST, which only confirms the presence of MI.

The level of oxygen supply to cardiomyocytes was judged by the activity of erythrocyte ADA enzymes and LDH in blood plasma. The protective effects of adenosine in ischemia have been shown in numerous works. It has been established that ADA plays an important role in the hypoxic type of metabolism. There is much less information about changes in ADA activity in erythrocytes. Thus, in MI, no significant differences in ADA activity were found between groups of healthy animals and rats with MI, 102±32 and 170±48 nmol/min*l, respectively, at t=1.17; p>0.05. Also, there was no difference in the groups of MI and MI + MSC transplantation - 83±31 nmol/min*l at t=1.5; p>0.05.

The LDH values in the group with MI were significantly higher than in the group of healthy animals: 1071±215 and 780±101 U/l, respectively (at t=1.97; p<0.05). In the group with MSC transplantation, it was 671±150 U/l, which did not differ from normal values (at t=0.6; p>0.05) and was much lower than in the group of animals with MI (at t=2 .1; p<0.05). In group 3, the levels of LDH and ADA were also significantly lower compared to groups 1 and 2.

A decrease in LDH activity indirectly indicates an increase in myocardial oxygenation and activation of aerobic pathways for the oxidation of energy-producing substrates.

The ADA enzyme involved in the catabolism of purine nucleosides, is localized in the cytoplasm of cells of

all tissues and catalyzes the deamination of adenosine and its transformation into inosine, and of deoxyadenosine – into deoxyadenosine. An increase in the rate of deamination of adenosine prevents the adenosine kinase reaction, which exacerbates energy deficiency. The accumulation of hypoxanthine, into which adenosine is transformed after deamination, enhances free radical oxidation reactions with the participation of xanthine oxidase.

Adenosine stimulation of postsynaptic A1-adenosine receptors localized in purinergic synapses located on the cell membranes of contractile cardiomyocytes of the atria and ventricles of the heart causes a decrease in their cAMP content and, consequently, a decrease in the contractility of the heart muscle, i.e. the negative inotropic effect of adenosine is realized. In addition to the specific effect on A1-adenosine receptors, adenosine reduces the activating effect of catecholamines on the heart. The source of erythrocyte adenosine is AMP catabolism. It can be assumed that the inhibition of uncompensated ATP decomposition in erythrocytes reduces the level of the substrate for ADA. A decrease in the enzyme activity after MSC transplantation preserves the adenosine pool and is a good prognostic sign [9, 10, 11].

Thus, cardiomyoplasty with MSCs causes a positive metabolic effect. MSC transplantation is accompanied by a decrease in the activity of ADA and LDH enzymes in the damaged myocardium, which indirectly indicates an increase in the energy balance of energy-producing substrates and a decrease in the degree of myocardial ischemia. A decrease in the accumulation of LPO products and the tension of the non-enzymatic antioxidant protection is a good prognostic criterion for the restoration of the functional activity of the damaged myocardium.

Based on the analysis of the obtained experimental data, two main directions of metabolic therapy with MSCs in myocardial infarction can be assumed:

- optimization of energy production and consumption processes;
- normalization of the balance between the intensity of free-radical oxidation and antioxidant protection.

CONCLUSION

The use of transplantation of a mixed culture of MSCs and pancreatic BCs makes it possible to fully compensate for carbohydrate metabolism, and to achieve the best performance in the studied groups in terms of LPO regulation and activation of the antioxidant system.

REFERENCES

1. Cui J., Liu Y., Li Y., Xu F., Liu Y. Type 2 Diabetes and Myocardial Infarction: Recent Clinical Evidence and Perspective. Front Cardiovasc Med. 2021;8:644189. DOI: 10.3389/fcvm.2021.644189
2. Arnold S.V., Bhatt D.L., Barsness G.W., Beatty A.L., Deedwania P.C., Inzucchi S.E., Kosiborod M., Leiter L.A., Lipska K.J., Newman J.D., Welty F.K. American Heart Association Council on Lifestyle and Cardiometabolic Health and Council on Clinical Cardiology. Clinical Management of Stable Coronary Artery Disease in Patients With Type 2 Diabetes Mellitus: A Scientific Statement From the American Heart Association. Circulation. 2020;141(19):e779-e806. DOI: 10.1161/CIR.0000000000000766
3. Mykhaylichenko V.Y., Kubyshkin A.V., Samarin S.A., Fomochkina I.I., Anisimova L.V. Experimental induction of reparative morphogenesis and adaptive reserves in the ischemic myocardium using multipotent mesenchymal bone marrow-derived stem cells. Pathophysiology. 2016;23(2):95-104. DOI: 10.1016/j.pathophys.2016.04.002
4. Ozdemirler G., Mehmetçik G., Oztezcan S., Toker G., Sivas A., Uysal M. Peroxidation potential and antioxidant activity of serum in patients with diabetes mellitus and myocard infarction. Horm Metab Res. 1995;27(4):194-6. DOI: 10.1055/s-2007-979938
5. Wan X-X., Zhang D-Y., Khan M.A., Zheng S-Y., Hu X-M., Zhang Q., Yang R-H., Xiong K. Stem Cell Transplantation in the Treatment of Type I Diabetes Mellitus: From Insulin Replacement to Beta-Cell Replacement. Front. Endocrinol. 2022;13:859638. DOI: 10.3389/fendo.2022.859638
6. Madani S., Amanzadi M., Aghayan H.R. et al. Investigating the safety and efficacy of hematopoietic and mesenchymal stem cell transplantation for treatment of T1DM: a systematic review and meta-analysis. Syst Rev. 2022;11:82. DOI: 10.1186/s13643-022-01950-3
7. Karimova M.V., Gvazava I.G., Vorotelyak E.A. Overcoming the Limitations of Stem Cell-Derived Beta Cells. Biomolecules 2022;12:810. DOI: 10.3390/biom12060810

8. Koehler N., Buhler L., Egger B., Gonelle-Gispert C. Multipotent Mesenchymal Stromal Cells Interact and Support Islet of Langerhans Viability and Function. Front Endocrinol (Lausanne). 2022;13:822191. DOI: 10.3389/fendo.2022.822191
9. Tokuda K., Ikemoto T., Yamashita S. et al. Syngeneically transplanted insulin producing cells differentiated from adipose derived stem cells undergo delayed damage by autoimmune responses in NOD mice. Sci Rep. 2022; 12:5852. DOI: 10.1038/s41598-022-09838-x
10. Kawada-Horitani E., Kita S., Okita T. et al. Human adipose-derived mesenchymal stem cells prevent type 1 diabetes induced by immune checkpoint blockade. Diabetologia. 2022;65;1185–1197. DOI: 10.1007/s00125-022-05708-3
11. Mykhaylichenko V., Pilipchuk A., Parshin D., Kostyamin Y. Content of primary and secondary lipid peroxidation products in subcellular fractions of cardiomyocytes during myocardial infarction in rats in an experiment and their correction by transplantation of mesenchymal stem cells. Archiv EuroMedica. 2021;11(4):89-92 DOI: 10.35630/2199-885x/2021/11/4.exsu

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