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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

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***Vyacheslav Mykhaylichenko*[1], *Andrey Pilipchuk*[1], *Dmitry Parshin*[2]✉, *Yuri Kostyamin*[1]**

[1] *S.I. Georgievsky Medical Academy, Simferopol*
[2] *Astrakhan State Medical University, Astrakhan, Russia*

✉ *parshin.doc@gmail.com*

ABSTRACT — Experimental modeling of myocardial infarction in rats was carried out by ligation of the anterior intergastric artery after the first division. There were 3 groups of 20 animals each: control group I — to verify normal parameters, group II — a model of myocardial infarction, and group III — animals which, after modeling myocardial infarction, underwent transplantation of mesenchymal stem cells. The level of lipid peroxidation products — diene conjugates and malondialdehyde — was studied by spectrophotometry. Comparison of the content and their ratio in the cytoplasm and mitochondria of myocardiocytes was carried out. It turned out that transplantation of mesenchymal stem cells significantly levels the activation of lipid peroxidation processes in subcellular fractions of cardiomyocytes, which is accompanied by a decrease in the primary and secondary products of oxidative stress. The ratio of malondialdehyde to diene conjugates both in the cytoplasm and in the mitochondria of cardiomyocytes after transplantation returned to control values. This indicates the normalization of physiological processes with underlying ischemic heart damage. The results indicate the cytoprotective effect of mesenchymal stem cell transplantation and the preservation of a larger number of cell pools, compared with the control group of animals that did not receive any treatment.

KEYWORDS — myocardial infarction, stem cell transplantation, lipid peroxidation, mesenchymal stem cells, subcellular fractions of cardiomyocytes, diene conjugates, malondialdehyde.

INTRODUCTION

Despite significant research devoted to myocardial infarction (MI) and its treatment, a number of pathophysiological questions remain open and require further study [1]. Existing therapies reduce early mortality, prevent additional damage to the heart muscle, and reduce the risk of further heart attacks. However, there is a need for a treatment to improve the infarction area by replacing damaged cells after MI. [2, 3]. The irreversible loss of cardiomyocytes and the formation of scar tissue after myocardial infarction leads to progressive impairment of cardiac function. Thus, cardiac repair (replacement, repair and regeneration) is essential for cardiac function after myocardial infarction. It is a well-known factor that the earlier the treatment of myocardial infarction is started, the more effective the therapy is. However, for a number of reasons, it is not always possible to make a diagnosis on time and, accordingly, begin treatment [4, 5]. Recently, in addition to standard methods of treatment, transplantation of various cell fractions and cytokine cocktails for myocardial infarction has been increasingly used. Mesenchymal stem cells (MSCs) are obtained from a wide variety of sources and are easy to isolate and culture. MSCs are capable of amplification and self-renewal in vitro, have low immunogenicity and immunomodulatory properties, and under certain conditions MSCs can differentiate into various cells. In the cardiovascular system, MSCs can protect the myocardium by reducing inflammation, promoting myocardial cell differentiation around infarction areas and angiogenesis, increasing resistance to apoptosis and suppressing fibrosis, which is ideal for cardiovascular recovery.

Therefore, stem cell regeneration of heart tissue may be an effective treatment option. Recently, they are increasingly inclined towards myocardial regeneration using stem cells [6, 7]. The potential benefits and the possibility of improving cardiac function with stem cell therapy require further research, which was the reason for this study.

The aim of the study is to study the dynamics of lipid peroxidation (LPO) products in rats with myocardial infarction and the effect of transplantation of MSCs on them.

MATERIALS AND RESEARCH METHODS

An experimental study was carried out on Wistar-Kayoto rats weighing 200–220 g. Research at all stages was carried out in a certified laboratory. The experiment was carried out in compliance with the "European Convention for the protection of vertebrate animals used for experiments or other scientific purposes" [Directive 2010/63 / EU]. We formed 3 groups of 20 animals each: control group I — to verify normal parameters, group II — a model of myocardial infarction, and group III — animals that received MSC transplantation after modeling myocardial infarction. Thoracotomy and ligation of the anterior intergastric artery after the first division were performed under intra-abdominal anesthesia with calypsol + xylazine, thus forming a model of myocardial infarction. In normal animals, after MI modeling, hearts and myocardiocytes were removed after 60 minutes and 1 day under anesthesia.

The method for determining the content of diene conjugates (DC) is based on the intense absorption of conjugated diene structures of lipid hydroperoxides in the 232 nm. Determination of the content of malonic dialdehyde (MDA) in lipid systems was studied by its interaction with 2-thiobarbituric acid leading to the formation of a chromogen with a maximum absorption in the red region of the visible spectrum at a wavelength of 532 nm. The measurements were carried out on an SF-56 spectrophotometer (Russia). Comparison of the content and their ratio in the cytoplasm and mitochondria of myocardiocytes was carried out.

Statistical data processing was carried out using Statistica 8.0 software packages. The statistical significance of the differences was determined using the Mann–Whitney rank test. The critical level of significance when testing statistical hypotheses was taken equal to $p < 0.05$.

RESULTS AND THEIR DISCUSSION

After studying the effect of MSC transplantation on the total level of peroxidation in the blood plasma after MI, it seemed important to assess the intensity of LPO directly in cardiomyocytes at the level of subcellular fractions. In experimental animals, the content of primary and secondary lipid peroxidation products in subcellular fractions of rat cardiomyocytes was studied 1 hour and a day after MI. The content of MDA and DC was determined in the cytoplasmic and mitochondrial fractions of cardiomyocytes.

Myocardial ischemia naturally caused an increase in the content of MDA and DC (LPO products). 1 hour after MI, the most significant increase in the content of primary DC products was 5.8 and 3.3 times in the cytoplasmic and mitochondrial fractions, respectively. The concentration of MDA after an hour of ischemia also increased: in the cytoplasmic fraction of cardiomyocytes, an increase of 4.6 times was noted, and in the mitochondral fraction — 2.7 times compared with the control (Table 1).

From the results obtained, it follows that that under conditions of ischemia in the subcellular fractions of cardiomyocytes, the processes of peroxidation after MI are more pronounced than the total indices of blood plasma. Both DC and MDA accumulate, which indicates the most significant peroxide modification of cardiomyocytes. A decrease in the ratio of MDA and DC is characteristic, reflecting the depth of LPO development in myocardial tissue in normal conditions and in MI.

When studying the parameters of primary and secondary lipid peroxidation in subcellular fractions of rat cardiomyocytes 1 day after MI, a slight decrease in the level of DC in the cytoplasm and in mitochondria was found. The total indicator — the MDA / DC ratio in the cytoplasm decreased by only 8% compared to the level of peroxidation 60 minutes after MI (Table 2).

Thus, in the group with MI, the concentration of DC in the cytoplasm is 7.13 ± 0.24 mol/g of wet weight -10'5, and in the control one — 1.39 ± 0.07 mol/g of wet weight — 10^5 at $p < 0.05$. In the group of animals with MSC transplantation, a decrease in the level of DC in the cytoplasm was established to 2.42 ± 0.08 mol/g of wet weight — 10^5, which is significantly lower than in the MI group, but nevertheless higher than in the control one.

A similar picture is observed when studying the MDA level in the cytoplasm of cardiomyocytes. So, in the control group, the MDA level is 0.146 ± 0.006 mol/g of wet weight — 10^5, and with MI this indicator decreased compared to the group of animals 60 minutes after the MI, but, nevertheless, remained much higher than the control one — 0.533 ± 0.027 mol/g of wet weight — 10^5 at $p < 0.05$. The same tendency was observed in the MI + MSC transplantation group: the MDA level was slightly higher than in the control one — 0.188 ± 0.014 mol/g of wet weight — 10^5 at $p < 0.05$, but much lower than in the group with MI.It is especially significant that the ratio coefficient (MDA / DC), which significantly decreased with MI to 0.072 ± 0.004 mol/g of wet weight — 10^5 at $p < 0.05$, did not differ from the control one in the group MI + MSC transplantation at $p > 0.05$ and amounted to 0.095 ± 0.005 mol/g of wet weight —10^5.

In the study of the content of primary and secondary LPO products in the mitochondrial fraction of cardiomyocytes, a similar picture was

Table 1. *Content of primary and secondary LPO products in subcellular fractions of rat cardiomyocytes in normal conditions and 60 minutes after MI*

Fraction of myocardium	Conditions of the experiment	DC, mol / g of wet weight -10^5	MDA, mol / g of wet weight -10^5	MDA / DC ratio
	Control	1,39 ±0,07	0,146 ±0,006	0,102 ±0,003
Cytoplasm	MI 60 min.	8,26 ±0,34*	0,652 ± 0,031*	0,081 ± 0,005*
	Control	0,43 ± 0,03	0,047 ± 0,002	0,106 ±0,004
Mitochondria	MI 60 min.	1,48 ±0,04*	0,14 ±0,003*	0,089 ±0,004*

Note: *the difference between the norm and the group with MI is significant (p <0.05) — *.*

Table 2. *Content of primary and secondary lipid peroxidation products in subcellular fractions of rat cardiomyocytes 1 day after MI and in the MI + MSC group*

Fraction of myocardium	Group	DC, mol / g of wet weight -10'5	MDA, mol / g of wet weight -10'5	MDA / DC ratio
	Control n= 20	1,39 ±0,07	0,146 ±0,006	0,102 ±0,003
Cytoplasm	MI n=20	7,13 ±0,24*	0,533 ± 0,027*	0,072 ± 0,004*
	MI+MSC n=20	2,42±0,08**,***	0,188±0,014**,***	0,095±0,005**,***
	Control n=20	0,43 ± 0,03	0,047 ± 0,002	0,106 ±0,004
Mitochondria	MI n=20	1,21 ±0,03*	0,12 ±0,011*	0,089 ±0,004*
	MI+MSC n=20	0,61±0,04**,***	0,057±0,003**,***	0,094±0,005**,***

Note: *the difference between the norm and the group with MI is significant (p <0.05) — *;*
*the difference between the groups of MI and MI with MSC transplantation is significant (p <0.05) — **;*
*the difference between the normal and MI + MSC groups is significant (p <0.05) — ***.*

observed. Thus, 1 day after modeling MI, the level of DC in comparison with the norm decreased slightly (1.21 ± 0.03 mol/g of wet weight — 10^5 at p <0.05). In the group MI + MSC transplantation, it was 0.61 ± 0.04 mol/g, which was significantly higher than in healthy animals, but lower than in the group of rats with MI.

The content of MDA in mitochondria of cardiomyocytes in the group of animals with MI remained at a high level of 0.12 ± 0.011 mol/g of wet weight — 10^5 at p <0.05 compared to the control one; and in the group of MI + transplantation MSC it was 0.057 ± 0.003 mol/g of wet weight — 10^5, which is lower than in the group with MI, but higher than in healthy animals at p <0.05. An interesting fact is that the integral MDA/DC index decreased in the group with MI to 0.089 ± 0.004 at p <0.05, and in the MI + MSC transplantation group it did not significantly differ from the norm and amounted to 0.094 ± 0.005 at p <0.05.

CONCLUSION

Thus, MSC transplantation significantly reduces the activation of LPO processes in subcellular fractions of cardiomyocytes, which is accompanied by a decrease in the primary and secondary LPO products. It should be emphasized that the MDA/DC index both in the cytoplasm and in the mitochondria of the cardiomyocyte after MSC transplantation returned to the control values, which indicates the normalization of LPO processes during MSC transplantation with underlying ischemic heart damage. The obtained results indicate the cytoprotective effect of MSCs due to various mechanisms that lead to the preservation of a larger number of cell pools, compared with the group of animals that did not receive any treatment.

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