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CHANGES IN LIPID PEROXIDATION IN EXPERIMENTAL TRAUMATIC MUSCLE INJURY AND THEIR CORRECTION WITH MESENCHYMAL STEM CELLS

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Abstract

The aim. To compare the dynamics of lipid peroxidation (malonic dialdehyde and diene conjugates) during experimental traumatic muscle injury and mesenchymal stem cell correction.

Materials and methods. The experimental study was performed on 92 nonlinear white rats, which were divided into three groups - control and experimental: I - thigh muscle injury, II - thigh muscle injury with correction of mesenchymal stem cells. Animals in the experimental groups underwent thigh muscle injury under thiopental-sodium anesthesia using a developed percussion mechanism. According to the developed method, blood was taken for biochemical parameters and production and administration of mesenchymal stem cells were performed. Changes in lipid peroxidation in experimental white rats were assessed by plasma levels of primary lipid peroxidation products such as diene conjugates and malonic dialdehyde.

Results. Our studies found that in the early post-traumatic period after traumatic muscle injury, hyperactivation of free radical lipid oxidation is detected on the first day of the experiment and increases until the seventh day of the post-traumatic period compared with the intact group. Analyzing the indicators of peroxide oxidation in the late post-traumatic period, we note that malonic dialdihyde decreases on the fourteenth day compared with the seventh day. After analyzing the data, we found that the correction of mesenchymal stem cells has a positive effect on the dynamics of thiobarbituric acid products from the first day of observation. The concentration of malonic dialdihydride in the corrected animals also decreased, but slightly less than the level of diene conjugates. Under the conditions of experimental correction with the use of mesenchymal stem cells, the activity of free radical processes is inhibited, which leads to a decrease in the level of products of lipid peroxidation.

Conclusions. After analyzing the results of our experiments, we found that during traumatic injury of the thigh muscles, hyperactivation of free radical lipid oxidation occurs from the first day and reaches a maximum on the fourteenth day of the post-traumatic period compared to the control group. Under the influence of mesenchymal stem cells in the blood there is a decrease in the activity of lipid peroxidation, which leads to a decrease in the signs of inflammation in the injured muscle.

Keywords: *Mesenchymal stem cells, traumatic muscle injury, malonic dialdihydride.*

Introduction

The treatment of soft tissue injuries, such as muscle tears, ligaments and tendons, is a complex issue in the 21st century, as it involves physical, chemical and cellular processes [1]. Mesenchymal stem cells are increasingly used in wound healing processes, and the study of its effect on biochemical parameters remains relevant [2, 3].

One of the most important oxidative processes in the body is lipid peroxidation (LPO), which is the main cause of cell membrane damage. LPO is also one of the main causes of cell damage and death due to the action of reactive oxygen species [4, 5, 6].

This process is regulated by the lipid composition of biomembranes, and also participates in the synthesis of leukotrienes, prostaglandins, catecholamine metabolism, and affects the ability of membranes to penetrate and transport substances through them [7, 8]. One of the most common aldehydes is malonic dialdehyde (MDA) with the formula $CH_2(CHO)_2$, which is formed during the secondary oxidation of lipids, and is also most often used as a marker of oxidation. Malonic dialdehyde is formed during the free radical oxidation of membrane lipids and accounts for 70% of the total amount of similar lipids [9, 10]. Increased concentration of malonic dialdehyde indicates an acceleration of lipid peroxidation, with a violation of important membrane functions, such as enzymatic and receptor activity, ion transport and others [11, 12].

Also, the products of lipid peroxidation include diene conjugates, which are their primary products. Diene conjugates (DC) are toxic metabolites that damage proteins, enzymes and nucleic acids [13].

The aim of study. To compare the dynamics of lipid peroxidation (malonic dialdehyde and diene conjugates) during experimental traumatic muscle injury and mesenchymal stem cell correction.

Methods

The experimental study was performed on 92 nonlinear white rats weighing 180–210 g, which were divided into three groups - control (12 rats) and experimental: I - thigh muscle injury (observation days 1st, 7th, 14th, 21st (40 animals)), II - injury of the thigh muscle with correction of mesenchymal stem cells (days of observation 1st, 7th, 14th, 21st (40 animals)). Animals in the experimental group under thiopental-sodium anesthesia (40 mg / kg body weight of the rat intraperitoneally) were simulated using a percussion mechanism to injure the thigh muscle. Femoral muscle injury was simulated by applying a single dosed blow to a specially designed device on the left thigh with a cylindrical surface, which caused injury to the thigh muscle without causing skin damage and fracture of the femur.

During the experimental work with animals, we followed the rules of treatment of experimental animals according to the EU Council Directive 2010/63 / EU on compliance with regulations, laws, administrative regulations of the EU on animal protection, which are used for scientific purposes [14, 15]. Blood sampling for biochemical parameters and production and administration of mesenchymal stem cells were performed according to the developed method [16].

Changes in lipid peroxidation in experimental white rats were assessed by plasma levels of primary lipid peroxidation products such as diene conjugates and malonic dialdehyde. Determination of diene conjugates according to the method of I.D. Stalnaya (1977) [17]. Malonic dialdehyde was determined by the method of I.D. Stalnaya, M.G. Garishvili (1987) [18].

Statistical processing of the material was performed using a personal computer and an application program for working with Microsoft Excel spreadsheets using the package "STATISTICA-10 for Windows®-6, 0". Graphs were designed using "Microsoft Excel 7.0".

Results

To assess the impact of traumatic muscle injury and corrective therapy, we compared the blood parameters of experimental animals, in the post-traumatic period without correction and after correction with mesenchymal stem cells. To assess disorders of muscle injury, lipid peroxidation parameters were determined: malonic dialdehyde, diene conjugates. The analysis revealed deviations of the studied parameters in the group of animals with simulated traumatic muscle injury from similar indicators of intact animals.

Analyzing the data in table 1, in the group where no correction was performed, in the early post-traumatic period, lipid peroxidation increased, so the level of MDA on the first day was 7.02 ± 0.02 (µmol /L), on the seventh day - 7.64 ± 0.01 (µmol /L), which compared with intact increased 2.3 times on the first day and 2.6 times on the 7th day, the level of DC - 3.12 ± 0.02 (conv.un), on the first day, on the 7th day - 4.32 ± 0.02 (conv.un), which compared with intact increased by 3.3 times on the 1st day and 5.1 on the 7th day ($p < 0.05$).

In the experimental group, which was corrected by mesenchymal stem cells, in the early post-traumatic period the following changes were detected, namely the level of MDA on the first day was 6.83 ± 0.02 (µmol /L), on the seventh day - 5.26 ± 0 , 02 (µmol /L), which was 2.1 times higher than intact on the first day and 1.6 times on the 7th day, the level of DC was - 3.1 ± 0.01 (conv. un.). On on the first day, on the 7th day - 3.81 ± 0.01 (conv.un.), which compared with intact increased by 3.1 times on the 1st day and 3.9 times on the 7th day ($p < 0.05$).

Analyzing the indicators of peroxide oxidation in the late post-traumatic period in the first experimental group, we note that on the 14th day MDA decreases compared to the seventh day and is 5.63 ± 0.04 (µmol /L), and on the 21st day this figure is 5.14 ± 0, 04 (µmol /L), and compared with intact higher 1.7 times on day 14 and 1.6 times on day 21, respectively. The level of DC on the 14th day was 4.01 ± 0.02 (conv.un.), Compared with the intact group of rats, this figure was 4.01 times higher. On the 21st day of the experiment, the index was 3.21 ± 0.02 (µmol /L), and therefore exceeded the index of the intact group by 3.3 times.

In the second experimental group where mesenchymal stem cell correction was performed in the late post-traumatic period, it was found that index MDA on the 14th day was 4.34 ± 0.03 (µmol /L), and on the 21st day 4.06 ± 0.01(µmol /L), which is 1.38 times and 1.26 times higher than intact. The level of DC at day 14 was at the level of 3.15 ± 0.02 (conv.un.), and compared with animals of the intact group, it was 3.2 times higher. And on the 21st day of the experiment it was 1.68 ± 0.01 (conv.un.) and exceeded the intact index by 1.7 times.

Reactive forms of oxygen are formed in skeletal muscle during traumatic muscle damage and reduce cell viability at the site of injury. Our studies found that in the early post-traumatic period after traumatic muscle injury hyperactivation of free radical oxidation of lipids is detected on the 1st day and increases to the 7th day of the post-traumatic period compared with intact which can be seen in table 1. Analyzing the peroxide oxide in the late post-traumatic period, we note that index MDA decreases by 26.3% on the 14th day compared to the seventh day and on the 21st day did not reach intact, but these indicators are significantly higher than control (see Table 1). Analyzing the data in table 5.1, we can assume that the correction of MSCs has a positive effect on the dynamics of thiobarbituric acid products from the 1st day of observation, as the DC on the 1st day of observation did not differ significantly from that of animals not corrected, but on the 7th day amounted to 88.0% of the animals without correction, on the 14th - 78%, and on the 21st - 52%, significantly not exceeding the level of animals without simulated pathology. The concentration of MDA in animals that underwent correction was also reduced, but slightly less than the level of DC: On the 1st day, this decrease was not significant, but on the 7th indicator was 68.8%, on the 14th - 77%, on the 21st - 78% of the level of animals that were not corrected., (Table 1). Under the conditions of experimental correction with the use of MSK there is a suppression of the activity of free radical processes, which leads to a decrease in the level of LPO products.

Conclusions

According to the analyzed results of our studies, it was found that traumatic injury of the thigh muscles causes hyperactivation of free radical oxidation of lipids from the first day and reaches a maximum by the 14th day of the post-traumatic period compared with the control group. The use of mesenchymal blood stem cells reduces the activity of lipid peroxidation, which reduces the signs of inflammation in the injured muscle.

After analyzing the results of our experiments, we found that during traumatic injury of the thigh muscles, hyperactivation of free radical lipid oxidation occurs from the first day and reaches a maximum on the fourteenth day of the post-traumatic period compared to the control group. Under the influence of mesenchymal stem cells in

the blood there is a decrease in the activity of lipid peroxidation, which leads to a decrease in the signs of inflammation in the injured muscle.

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Table 1. Comparative levels of malonic dialdehyde and diene conjugates in animals with traumatic muscle injury without correction and with mesenchymal stem cell correction injury (M ± m)

Group		Indicator	
		Diene conjugates (conv. un.)	Malonic dialdehyde (µmol / l)
Intact (n=12)		0,97 ± 0,01	3,14 ± 0,01
First group (n=40)	1st day (n = 9)	3,12 ± 0,02*	7,02 ± 0,02*
	7th day. (n = 8)	4,32 ± 0,02*	7,64 ± 0,01*
	14th day. (n = 8)	4,01 ± 0,02*	5,63 ± 0,04*
	21st day (n = 7)	3,21 ± 0,02*	5,14 ± 0,04*
Second group (n=40)	1st day (n = 9)	3,1 ± 0,01*	6,83 ± 0,02*
	7th day (n = 9)	3,81 ± 0,01*	5,26 ± 0,02*
	14th day (n = 8)	3,15 ± 0,02*	4,34 ± 0,03*
	21st day (n = 8)	1,68 ± 0,01*	4,06 ± 0,01*
Note. * - values that are statistically significant from similar indicators in the control group of animals (p <0,05).			