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CHANGES OF MACRO- AND MICROELEMENT COMPOSITION OF BLOOD SERUM AND BONE TISSUE IN THE DYNAMICS OF EXPERIMENTAL CRUSH-SYNDROME DEVELOPMENT

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Abstract

Crush syndrome (CS) is characterized by severe course, the development of systemic inflammatory response syndrome and disseminated intravascular coagulation, which lead to multiple organ failure The aim of this work was to study the dynamics of the macro- and microelement composition of blood serum and femur of rats in the model of endotoxicosis, which is formed in case of CS. The experimental studies were conducted on 40 nonlinear mature white male rats. The left hind limb of the rat was subjected to the mechanical pressure for 4 hours; the compressed area was 4 cm^2 with a compressive force of 4.25 kg/cm^2. It was found that the postcompression period of the experimental crush syndrome is characterized by an imbalance of macro- and microelements in both blood serum and femur. Thus, calcium content of the blood serum of rats decreases on the 1st day of observation (by 10.1%), but increases on the 14th day (by 23.0%); the magnesium content progressively decreases during all days of observation, and the content of inorganic phosphate increases by the 14th day of observation and by 36.6% ($p < 0.05$) prevails control data. The content of calcium, magnesium, zinc and copper in the femur significantly changes from the 3rd day of the postcompression period, in particular the content of bioelements relative to the control group progressively decreases by 30.6%, 42.7%, 43.9% and 30.1% respectively on the 14th day of observation.

Keywords: *crush syndrome, rats, bioelements, serum, bone tissue.*

Introduction

In recent decades, there has been a growing trend around the world of military conflicts, emergencies, natural and man-made disasters, accompanied by an increase of the number of victims (1–3). The significance of the study of this problem is reinforced by the events in eastern Ukraine, where an anti-terrorist operation has been taking place since the beginning of 2014. According to X. Bosch et al., crush syndrome (CS) develops in approximately 2–5% of all earthquake victims, more than 50% of traumatic rhabdomyolysis victims, and 10.5% of beaten victims (4). In addition, a type of CS is often observed – positional compression syndrome, in which the muscle mass is injured due to its compression by the mass of one's own body in case of a comatose state (1, 5).

Crush syndrome is characterized by severe course, the development of systemic inflammatory response syndrome and disseminated intravascular coagulation, which lead to multiple organ failure [6]. Despite the active introduction of the latest medical technologies, a significant reduction in mortality from CS, which in its severe forms reaches 85-90%, even in specialized hospitals has not yet been achieved (7). Conducting clinical trials of CS is difficult, due to the variety of injuries in victims and the difficulty of systematizing the obtained data (8, 9). In addition, providing medical care to a large number of victims in the face of a shortage of forces and resources also complicates research. All this determines the important role of the experiment in the study of CS (10).

The biochemical basis of CS is endogenous intoxication by the products of ischemia and tissue reperfusion (11). Metabolic disorders in compressed tissues, toxic products formed in the focus of compression, lead to the development of endotoxicosis with subsequent generalization of the process with damage to vital organs (12), especially the liver. Endogenous intoxication is accompanied by a complex of metabolic disorders, among which one of the markers is an imbalance of the activity of the antioxidant system and the level of free radical oxidation (13-17). Excess of toxic metabolites, formed during EI, can adversely affect the course of remodeling in bone tissue, which is a dynamic structure that is constantly updated and is controlled by many systemic and local factors (18, 19). Toxins, in addition to directly affecting bone tissue cells, can disrupt the mechanisms of regulation and metabolic support of this process. At the same time, metabolic processes in bone tissue and the balance of bone remodeling are largely determined by the content of macro- and microelements. It should be noted that for the normal metabolism is important not a single trace element, but a complex of bioelements and their balance (20, 21).

Therefore, the aim of our research was to study the dynamics of the macro- and microelement composition of blood serum and femur of rats in the model of endotoxicosis, which is formed in case of crush syndrome.

Methods

The experimental studies were conducted on 40 nonlinear mature white male rats weighing 180 - 200 g, which were distributed into the following five groups: the control group and four experimental groups (1, 3, 7 and 14 days of observation, respectively) with 8 animals for every group. The selected durations of the study were consistent with the generally accepted periods of crush syndrome development: early period (1 to 3 days), intermediate period (3 to 7 days) and late (restorative) period (7 to 21 days).

The experiments were carried out under deep anesthesia using intraperitoneal administration of ketamine hydrochloride (at 100 mg/kg of body weight). The left hind limb of the rat was subjected to the mechanical pressure for 4 hours, using an apparatus designed for this purpose at the Department of Functional and Laboratory Diagnostics of the I. Horbachevsky Ternopil National Medical University. The compressed area was 4 cm^2 with a compressive force of 4.25 kg/cm^2 (22). The integrity of large vessels and bony structures of the lower extremity was preserved. Thus, the crush syndrome of moderate degree was modeled in animals.

All manipulations with experimental animals were carried out in accordance with the rules of the European Convention for the Protection of

Vertebrate Animals used for Experimental and other Scientific Purposes (23).

Determination of total calcium, magnesium and inorganic phosphate contents in blood serum was performed on a semi-automatic biochemical analyzer Humalyzer 2000 (Human, Germany) using standard reagent kits. The results were expressed in mmol/l. The content of calcium, magnesium, copper and zinc in the femur was determined by atomic absorption spectrometry on a Selmi C-115 M spectrophotometer (24). Analytical parameters were chosen according to the literature data (25): pressure 0.4 kg/cm^3 and 20 mm of water column; flame temperature 2250 ° C, wavelength: Ca – 495.5 nm, Mg – 285.2 nm, Zn – 213.9 nm, Cu – 324,7 nm. After determining the content of the element in the solution, the mass of the sample was introduced and the content of the element in 1 gram of study tissue was obtained. The results were expressed in mg/g of ash.

Statistical processing of digital data was performed using Excel software (Microsoft, USA) and STATISTICA 6.0 (Statsoft, USA). The analysis of the study results was carried out using non-parametric statistical methods, the choice of which was based on the correctness of the distribution of values. Quantitative characteristics of the features were presented in the form of medians and quartiles (lower and upper) – Me (Lq; Uq). Comparison of the two quantitative characteristics was performed using the Mann-Whitney U test. Comparison of three or more groups on a quantitative basis was carried out using the Kruskal-Wallis test.

Results

The results of our studies showed changes of all three macroelements – calcium, magnesium and inorganic phosphate in the blood serum of animals with an experimental crush syndrome (Table 1). Thus, the calcium content on the 1st day of the postcompression period significantly decreased by 10.1%, on the 3rd and 7th days of the postcompression period practically did not differ from control values and on the 14th day of observation was significantly higher by 23.0 % in relation to controls. At the same time, the magnesium content practically did not change in the early postcompression period (during the first three days), while after 7 and 14 days it significantly decreased, respectively, by 15.8% and 25.4% against the control values. The level of inorganic phosphate in the blood serum of rats increased on the 1st day of observation by 7.8%, continued to increase on the 3rd (by 11.6%) and 7th day (by 36.6%) of the postcompression period and remained significantly high by the 14th day in relation to controls.

The calcium content in the femur of rats with an experimental crush syndrome on the 1st day of the postcompression period did not change significantly (Table 2). Subsequently, this index decreased on the 3rd day of observation by 8.6%, continued to decrease to 7th day (by 26.7%) and remained significantly low until 14th day (was lower by 30.6% compared to controls). The same trend was observed for the dynamics of changes of the magnesium content in the femur of rats with experimental crush syndrome, in particular, this index progressively decreased: on the 3rd day of the postcompression period by 16.4%, on the 7th day – by 32.7% and on the 14th day – by 42.7% in relation to the control group. The content of zinc in the femur of rats with experimental crush syndrome gradually decreased from the 3rd day (by 17.3%) to the 7th day (by 33.3%) and remained significantly low on the 14th day of observation (by 43.9% was lower relative to controls). The same trend was observed for the dynamics of changes of the content of copper in the femur of rats with CS, in particular, this index significantly decreased on the 3rd (by 20.3%) and 7th (by 28.7%) days of observation and remained decreased on 14th day (by 30.1% against control values).

Discussion

The pathogenesis CS combines various mechanisms, including ischemia, endotoxicosis and inflammation, which cause multiorgan changes. In our opinion, the imbalance of macro- and microelements is an equally important trigger for the development of pathobiochemical reactions that occur in case of CS. In the present study we found that in the blood serum of rats with experimental CS calcium content decreases on the 1st day of the postcompression period, but increases on the 14th day; the magnesium content

progressively decreases during all days of observation, and the content of inorganic phosphate gradually increases, and on the 14th day of the postcompression period by 36.6% ($p<0.05$) exceeds the control data. The content of calcium, magnesium, zinc and copper in the femur significantly changes from the 3rd day of the postcompression period, in particular the content of bioelements relative to the control group progressively decreases by 30.6%, 42.7%, 43.9% and 30.1% respectively on the 14th day of observation.

Under physiological conditions, the skeleton contains 99% of calcium, 87% – phosphorus, 58% – magnesium of the total content in the body, while the mineral part of the bones is in constant contact with the surrounding tissue fluid (26, 27). The release of various organic acids (eg, lactic, uric acid) from destroyed muscle cells in case of CS leads to metabolic acidosis, which further exacerbates hyperkalemia and causes redistribution of bioelements in blood and body tissues, including bone tissue (28).

We found multidirectional changes of calcium and magnesium contents in blood serum – hypercalcemia and hypomagnesemia. It is known that magnesium deficiency is accompanied by an increase of oxidative stress with a simultaneous weakening of antioxidant protection (29), which is confirmed by the results of our previous study (30). One of the probable mechanisms of such a multidirectional dynamic of changes of these two trace elements is the development of inflammation, which develops during CS.

As for inorganic phosphate, a significant increase of its content in blood serum was found in rats with experimental CS. During the destruction of muscle cells, inorganic and organic phosphorus components are dissolved, and a large amount of inorganic phosphorus is released into the blood plasma, which leads to hyperphosphatemia (31). Hyperphosphatemia causes the deposition of calcium phosphate on damaged muscle cells and other tissues (32). In addition, suspending the renal 1α-hydroxylase enzyme, which is responsible for producing the active form of vitamin D, leads to early hypocalcemia, which is usually asymptomatic. After complete cell necrosis, calcium, first entering the cytoplasm of muscle cells, is released back into the plasma. This, in combination with secondary hyperparathyroidism, which develops as a result of early hypocalcemia and high levels of vitamin D (produced in large numbers by glomerular cells), leads to late hypercalcemia (31).

For zinc and copper, the content of these trace elements in the femur gradually decreased during all days of observation and on the 14th day of the postcompression period was 56.1% and 69.9% of the control data. Zinc plays an important role in bone metabolism, which makes it an important component of the calcified matrix. Excessively low concentrations of zinc in bone tissue are the cause of gradual loss of bone mass (33, 34). Cu refers to bioelements, the deficiency of which leads to significant violations of metabolic processes, in particular in bones and connective tissue. The highest content of Cu is found in young osteons, which promotes the synthesis of collagen and elastin and mineralization of bone tissue. Cu provides bone tissue strength by influencing collagen metabolism through prolyl- and lysyl hydroxidase (35).

Previous studies of the content of zinc and copper in the liver of rats with an experimental crush syndrome also found pronounced violations of the content of these trace elements (36). The content of zinc in the liver gradually increased from the 1st day of the postcompression period (by 12.8%) to the 14th day (by 34.0%) of observation in relation to controls. The same trend was observed for the dynamics of changes of copper, but its content significantly increased only from the 3rd day of the postcompression period.

Such changes of zinc and copper may be related to the expression of Zn-, Cu-containing superoxide dismutases and metallothioneins. Excessive intake of copper increases the concentration of free metal forms in body tissues, which leads to the activation of the formation of reactive hydroxyl radicals, which are toxic to cell membranes, leading to their destruction (37). Copper leads to the formation of structural changes in internal organs, including the liver, by activating tissue processes of lipid peroxidation (38).

Thus, the established bioelement imbalance in the studied tissues of rats with experimental CS disrupts

cellular metabolism, deepens oxidative stress, thereby activating other systemic processes, leading to progression of CS and deepening of multiorgan damages (39, 40).

Conclusions

The postcompression period of the experimental crush syndrome is characterized by an imbalance of macro- and microelements in both blood serum and femur, which is important for the regulation of metabolic processes. Thus, calcium content of the blood serum of rats decreases on the 1st day of observation (by 10.1%), but increases on the 14th day (by 23.0%); the magnesium content progressively decreases during all days of observation, and the content of inorganic phosphate increases by the 14th day of observation and by 36.6% ($p < 0.05$) prevails control data. The content of calcium, magnesium, zinc and copper in the femur significantly changes from the 3rd day of the postcompression period, in particular the content of bioelements relative to the control group progressively decreases by 30.6%, 42.7%, 43.9% and 30.1% respectively on the 14th day of observation. The obtained results complement the existing data on the mechanisms of the course of the experimental crush syndrome depending on the postcompression period term and are the basis for finding effective means of correcting dysmacro- and dysmicroelementosis that occur in case of this pathology.

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Table 1. Changes of the macroelement composition of the blood serum of rats in the dynamics of the postcompression period of the crush syndrome (Me [Q25-Q75])

Index	Group of animals				
	Control, n=8	1st day, n=8	3rd day, n=8	7th day, n=8	14th day, n=8
Calcium, mmol/l	2.38 [2.32; 2.41]	2.14 [2.08; 2.19] $p_1<0.05$	2.27 [2.23; 2.30] $p_1>0.05$ $p_2>0.05$	2.40 [2.36; 2.46] $p_1>0.05$ $p_3<0.05$	2.63 [2.61; 2.65] $p_1<0.05$ $p_4>0.05$
Magnesium, mmol/l	0.73 [0.69; 0.75]	0.71 [0.69; 0.73] $p_1>0.05$	0.70 [0.69; 0.72] $p_1>0.05$ $p_2>0.05$	0.62 [0.60; 0.64] $p_1<0.05$ $p_3<0.05$	0.53 [0.51; 0.55] $p_1<0.05$ $p_4<0.05$
Inorganic phosphate, mmol/l	1.34 [1.29;1.37]	1.45 [1.41;1.47] $p_1<0.05$	1.50 [1.47;1.54] $p_1<0.05$ $p_2>0.05$	1.83 [1.60;1.95] $p_1<0.05$ $p_3<0.05$	1.83 [1.79;1.85] $p_1<0.05$ $p_4>0.05$

Note. p_1 – changes are significant in relation to the indices of control animals; p_2 – significance of changes between the group on the first day of observation and rats on the third day of observation; p_3 – significance of changes between the group on the third day of observation and rats on the seventh day of observation; p_4 – significance of changes between the group on the seventh day of observation and rats on the fourteenth day of observation.

Table 2. Changes of the macro- and microelement composition of the femur of rats in the dynamics of the postcompression period of the crush syndrome (Me [Q25 – Q75])

Index	Group of animals				
	Control, n=8	1st day, n=8	3rd day, n=8	7th day, n=8	14th day, n=8
1	2	3	4	5	6
Calcium, mg/g of ash	289.03 [283.59; 300.60]	282.71 [277.05; 293.43] $p_1>0.05$	264.18 [259.54; 266.96] $p_1<0.05$ $p_2<0.05$	211.93 [209.23; 217.57] $p_1<0.05$ $p_3<0.05$	200.58 [199.41; 211.54] $p_1<0.05$ $p_4>0.05$
Magnesium, mg/g of ash	35.60 [33.60; 36.90]	32.42 [31.60; 33.01] $p_1>0.05$	29.77 [29.05; 30.24] $p_1<0.05$ $p_2>0.05$	23.98 [23.08; 24.73] $p_1<0.05$ $p_3<0.05$	18.59 [17.51; 19.11] $p_1<0.05$ $p_4<0.05$
Zinc, mg/g of ash	0.41 [0.39;0.45]	0.40 [0.37;0.42] $p_1>0.05$	0.34 [0.32;0.35] $p_1<0.05$ $p_2<0.05$	0.27 [0.24;0.29] $p_1<0.05$ $p_3<0.05$	0.23 [0.21;0.27] $p_1<0.05$ $p_4>0.05$
Copper, mg/g of ash	17.65 [17.08; 18.20]	17.13 [16.82; 17.24] $p_1>0.05$	14.08 [13.50; 14.25] $p_1<0.05$ $p_2<0.05$	12.59 [12.03; 13.59] $p_1<0.05$ $p_3<0.05$	12.33 [12.13; 12.68] $p_1<0.05$ $p_4>0.05$

Note. p_1 – changes are significant in relation to the indices of control animals; p_2 – significance of changes between the group on the first day of observation and rats on the third day of observation; p_3 – significance of changes between the group on the third day of observation and rats on the seventh day of observation; p_4 – significance of changes between the group on the seventh day of observation and rats on the fourteenth day of observation.