Functional Activity of the Liver during Severe Compression Injury

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Severe compression injury in rats was accompanied by metabolic acidosis, cytolytic syndrome, disturbances in liver excretory function and detoxification, and change in biotransformation processes.

Key Words: *crush syndrome; compression injury; liver; metabolism*

Among a variety of emergency traumas (emergency medicine) and battle injuries, much attention is paid to extensive muscle damage due to long-term compression of soft tissues with the debris of defensive constructions and buildings, landslide, and rocks. The pathogenesis of damage to various organs and systems was extensively studied; renal failure is the most serious complication [1,2,6,8]. The similarity of ontogenetic and phylogenetic characteristics of the kidneys and liver determines the substitutive role of these organs in the development and course of various diseases. During severe compression injury, the course and outcome of crush syndrome depend on the degree of hepatorenal dysfunction [10]. Liver dysfunction under conditions of extensive muscle damage and myolysis is associated with disturbances in systemic hemodynamics and blood flow in organs and ischemia of the organ and damage to its structures caused by toxic products of autolysis in traumatized muscles. Clinical symptoms of liver dysfunction are detected 1 week after damage. They include icteric discolor of the sclera, soft palate, and skin, increase in bilirubin concentration in the blood, and suppression of detoxification in the liver [7]. It should be emphasized that liver dysfunction occurs on day 1 after injury. However, little is known about this process. A specific situation and poor condition of patients do not allow using the arsenal of methods for liver tests and functional diagnostics of liver dysfunction.

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Here we studied the effect of severe closed injury to skeletal muscles on functional activity of the liver during the early posttraumatic period.

MATERIALS AND METHODS

Experiments were performed on 80 awake male outbred rats (170-190 g). The study was approved by the Local Ethics Committee of the S. M. Kirov Military Medical Academy. Skeletal muscle injury was induced by 4-h compression of hindlimb soft tissues (area 5 cm^2) in a vice with a trough-shaped cut to prevent femoral bone fracture [8]. The control group consisted of immobilized animals. The samples were taken immediately or 6, 12, 24, and 72 h after trauma. Excretory function of the liver was estimated from the rate of bromsulphalein excretion from the circulation [3]. The process of detoxification was studied by measuring aminopyrine N-demethylase activity, contents of cytochromes P-450 and b_5, rate of antipyrine clearance from the circulation, and duration of hexenal-induced sleep (60 mg/kg intraperitoneally) [4]. Metabolic function of the liver was evaluated from transaminase activities and contents of lactate and pyruvate [5]. The results were analyzed by Student's *t* test.

RESULTS

Liver dysfunction occurs over the first hours after massive closed muscle injury. In intact rats, elimination of bromsulphalein (30 mg/kg intravenously) was

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TABLE 1. Bromsulphalein Concentration in the Blood of Rats (μmol/100 ml, n=10, $M\pm m$)

Period, min	Control	Time after injury, h		
		0	12	24
1	176±12	87±9*	0*	26±14*
2	186±8	190±14	45±19*	68±26*
4	157±7	169±9	108±11*	117±26*
8	95±5	139±11*	129±10*	115±10
12	57±7	106±8*	120±7*	100±12*
16	29±7	66±8*	95±19*	74±13*
32	5±2	20±10*	58±7*	29±16*

Note. *p<0.05 compared to the control.

described by a biphasic decay curve. The phase of rapid decrease in bromsulphalein concentration was followed by slow dye elimination. It is related to bromsulphalein accumulation in the liver and excretion with the bile. Severe injury was followed by deceleration of bromsulphalein excretion from the blood (Table 1). Analysis of the elimination curves showed that this state is accompanied by impairment of bromsulphalein accumulation in the liver and excretion with the bile. Bromsulphalein retention in the blood was most pronounced 12 h after injury. This conclusion was derived from measuring the retention coefficient of bromsulphalein. Our findings indicate that excretory dysfunction of the liver develops immediately after decompression, becomes most severe by the 12th hour, and persists for 1 day after injury.

Revascularization of the injured limbs after long-term ischemia was followed by the appearance of bioactive metabolites with membranotoxic properties in the blood and lymph. Endotoxemia was accompanied by impairment of detoxification function of the liver (neutralization of endo- and exotoxins by hydroxylating monooxygenases localized in the endoplasmic reticulum of liver cells). These reactions are of considerable importance after injury. Monooxygenase reactions are closely related not only to biotransformation of endotoxins and xenobiotics in the liver, but also to activity of the immune system. *In vivo* evaluation of functional activity of microsomal monooxygenases in animals receiving hexenal showed that the duration of sleep significantly increases at various periods of the study. These changes reflect inhibition of biotrans-

TABLE 2. Concentrations of Lactate and Pyruvate during Severe Compression Injury (n=10, $M\pm m$)

Treatment	Time after treatment, h	Lactic acid, μmol/g	Pyruvic acid, μmol/g
Intact	–	1.73±0.09	0.054±0.10
Immobilization	0	1.77±0.06	0.038±0.002
Injury		2.21±0.18*	0.054±0.009*
Immobilization	6	2.52±0.08	0.028±0.004
Injury		4.14±0.05*	0.035±0.002*
Immobilization	12	1.99±0.05	0.044±0.003
Injury		3.69±0.04*	0.052±0.002*
Immobilization	24	1.85±0.11	0.055±0.007
Injury		1.84±0.12	0.063±0.009*
Immobilization	72	1.71±0.02	0.051±0.001
Injury		1.71±0.03	0.057±0.001

Note. *p<0.05 compared to immobilized animals.

formation in the liver. For example, the duration of hexenal-induced sleep in treated rats 1 and 72 h after injury surpassed the control value by more than 3 and 2 times, respectively (Fig. 1). Similar results were obtained for antipyrine clearance from the circulation.

Since biotransformation of barbiturates (*e.g.*, hexenal) occurs mainly in liver microsomes, the observed potentiation of hypnotic activity of hexenal is most likely associated with dysfunction of liver microsomal monooxygenases. The content of cytochromes P-450 and b_5 in rat liver was shown to decrease immediately after injury from 0.870±0.023 to 0.371±0.027 nmol/mg protein ($p<0.001$) and from 0.552±0.044 to 0.434±0.028 nmol/mg protein ($p<0.05$), respectively. During decompression, the content of cytochrome P-450 changed more significantly than the concentration of cytochrome b_5. The lowest content of cytochrome P-450 (0.331±0.049 nmol/mg protein) and cytochrome b_5 (0.336±0.026 nmol/mg protein) were found on days 1 and 2 after injury, respectively. By the 3rd day after injury, cytochrome content in the liver remained below the control level. A correlation was found between the content of microsomal monooxygenases and activity of aminopyrine N-demethylase. Demethylation activity of the liver decreased immediately after injury (from 2.48±0.12 to 1.44±0.15 nmol HCOH/mg protein/min, $p<0.001$). Then, activity of aminopyrine N-demethylase significantly decreased and was minimum on day 1 after injury (0.67±0.04 nmol HCOH/mg protein/min). Demethylation activity of the liver tended to increase on days 2 and 3 after injury, but remained below the control level.

Thus, severe compression injury is accompanied by impairment of biotransformation in the liver and changes in detoxifying activity. Dysfunction of liver microsomal enzymes during injury is mediated by a variety of pathochemical mechanisms. Severe mechanical damage is followed by pathological changes due to pain syndrome, circulatory disturbances, activation of catabolism, and toxemia (release of metabolic transformation products from the traumatized muscle to the blood). Endotoxins are accumulated and neutralized mainly in the liver. Therefore, these changes play an important role in inhibition of detoxification during mechanical damage. Changes in hepatic circulation after injury were followed by inhibition of NADPH-specific flavoprotein (initial component of the hydroxylation system in the endoplasmic reticulum). Hypoxia is a cause of functional changes in the hepatic monooxygenase system during severe injury. It is accompanied by energy deficiency, activation of free radical reactions, and dysfunction of membrane-bound enzymes in hepatocyte microsomes. Activation of phospholipase A_2 leads to hydrolysis of phospholipids that play a role in various stages of microsomal hydroxylation. The conformation and catalytic activity of cytochrome P-450 are modified in the follow-up period.

Hence, metabolic changes caused by microsomal oxidation disturbances are associated with the interaction between individual factors of the multicomponent oxygenase system, phasic state of membrane lipids, and hydration environment of the membrane. A vicious cycle is created: disturbances in hepatic circulation and endotoxemia are followed by ultrastructural changes in the liver, which impairs the energy-producing function and activates free radical oxidation. The membrane-toxic effect of endotoxins becomes more significant under these conditions. These factors probably serve as the major cause of dysfunction in liver microsomal monooxygenases during severe injury.

Severe compression injury is accompanied by labilization of cell membranes due to changes in regional blood flow and endotoxemia. Transaminase activity in the blood and liver is a reliable criterion for cytolytic syndrome in the liver. We showed that extensive muscle damage is accompanied by a significant increase in alanine transaminase (ALT) activity in blood plasma 6, 12, and 24 h after injury. ALT activity was maximum 6 h after injury (4.04±0.41 *vs*. 1.58±0.36 μmol/ml/h in the control). By the 3rd day, enzyme activity in traumatized animals did not differ from the control. Aspartate transaminase (AST) activity in the plasma increased insignificantly under these conditions.

Other results were obtained in studying the liver of traumatized rats. Six hours after injury, AST activity decreased from 14.72±2.99 to 6.98±0.68 μmol/g/h ($p<0.05$). ALT activity remained unchanged during this period. Twelve hours after injury, we revealed a significant decrease in activities of ALT (from 88.72±10.67 to 53.07±1.55 μmol/g/h) and AST (from 9.34±2.44 to 2.93±0.82 μmol/g/h). By the 3rd day of the post-

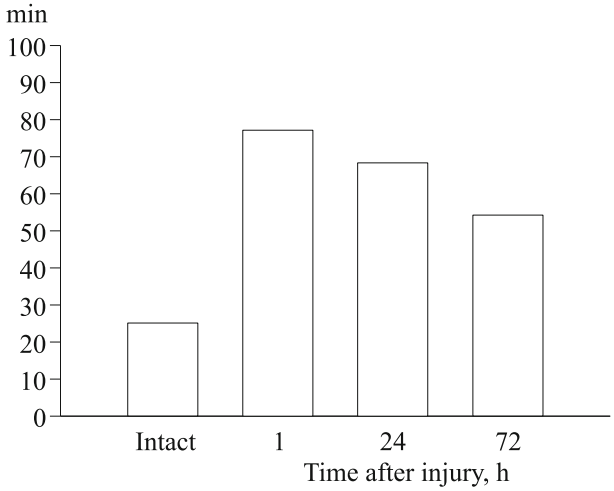

Fig. 1. Duration of hexenal-induced sleep in rats with severe compression injury.

trauma period, transferase activities in the liver of animals returned to the baseline level.

Taking into account the process of transaminase compartmentalization in the cell, it can be hypothesized that the observed changes are associated with damage to mitochondrial membranes. The release of intracellular enzymes serves as the criterion for damage to the plasma membrane, which is considered as an early and constant sign of nonspecific adaptive syndrome [9]. Labilization of lysosomal membranes and destruction of intracellular structures by lysosomal enzymes play an important role in the pathogenesis of hyperenzymemia. Hyperenzymemia after extensive muscle damage can be related to disturbances in transferase elimination from the blood, which involves the reticuloendothelial system of the liver. However, the development of cytolytic syndrome attests to ultrastructural changes and metabolic dysfunction of the liver.

The concentrations of lactic acid and pyruvic acid in the liver of animals significantly increased after injury, which attests to changes in aerobic and anaerobic processes in the organism. The concentrations of pyruvate and lactate increased immediately after injury (from 0.038±0.002 to 0.54±0.01 μmol/g; and from 1.78±0.06 to 2.510±0.012 μmol/g, respectively). The content of pyruvate and lactate in the liver of animals decreased progressively in the follow-up period and was lowest 6 and 12 h after injury (Table 2). One day after injury, the content of pyruvate and lactate did not differ from the control level.

We conclude that functional and metabolic properties of the liver are impaired in the early period after severe closed injury to skeletal muscles.

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