Accumulation of Calcium, Phosphate, and Collagen in Bones and Accumulation of Creatine in Muscles of Mice with Acute Hepatic Intoxication during Shin Fracture Healing

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The intensity of calcium and phosphate accumulation in regenerating bone tissue decreased significantly during the posttraumatic period in 54 male CBA mice with acute hepatic intoxication. The formation of organic components of the bone decreased to a lesser degree. Creatine metabolism in the muscles of damaged segment virtually did not change.

Key Words: *trauma; acute hepatic intoxication; muscle; bone*

The significance of the liver for bone and muscle tissue metabolism is systemic. The liver regulates the distribution of energy substrates between the organs (and synthesizes creatine), is involved in calcium-phosphorus metabolism [4], produces growth factors which have anabolic effects on the skeletal muscles [9], on osteosynthesis and osteolysis processes [3,5]. Chronic hepatic diseases lead to growth inhibition in children and to osteopenia and osteoporosis in adults [6,8] and hence, to a higher incidence of limb bone fractures in this patient population [7]. This gave grounds to speak about the so-called hepatic osteodystrophy (or osteopathy) [2,11], its pathogenesis being attributed to reduced protein producing function of the liver [10]. However, it remains unclear to what degree the reduction of the synthetic function of the organ under conditions of acute hepatic insufficiency (AHI) is essential for tissue reparation processes after skeletal injury.

We studied accumulation of calcium, phosphate, and collagen in the bones and of creatine in the skeletal muscles after skeletal injury in mice with acute hepatic intoxication.

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MATERIALS AND METHODS

Experiments were carried out on 54 male CBA mice (25-30 g). The animals were distributed into 3 groups. In group 1 (n=18), closed fracture of the shin bone was modeled (the segment of the right hind limb was broken in ether-narcotized animals). In group 2 (n=18), AHI was induced by a single intraperitoneal injection of 20% carbon tetrachloride (CTC) in olive oil in a dose of 0.2 ml/100 g. Morphological studies carried out in mice of this group showed the development of focal hepatic necrosis on day 3 after CTC injection; the first signs of intracellular regeneration were detected on day 10. In group 3 (n=18), fracture of the shin bone was created 3 days after CTC injection. All animals were kept under standard vivarium conditions, with distilled water for drinking. The animals were sacrificed by decapitation: groups 1 and 3 on days 3, 7, 28 after the injury and group 2 on days 6, 10, and 31 after CTC injection. All manipulations on animals were carried out in accordance with the European Convention for Protection of Vertebrates Used in Experimental and Other Purposes and with the Regulations for Studies on Laboratory Animals (Supplement to the Order No. 755 of the Ministry of Health of the USSR of August 12, 1997). Experi-

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mental study was approved by Ethics Committee of G. A. Ilizarov Center.

After decapitation, the bones of the injured segment of the limb were cleansed from the connective and muscle tissues and lyophilized for 24 h. Lyophilized bone tissue was grinded in a ceramic mortar. Dry tissue was divided into 2 portions. One was used for measurements of calcium and phosphate. Lyophilized bone powder was calcified in a muffle furnace at 800°C for 4 h, after which the ashes were dissolved in 1 ml concentrated HCl, quantitatively transferred into graduated tubes, neutralized with alkaline solution, and the volume was brought to 10 ml. The concentration of phosphate was measured in the resultant solution by the reaction with ammonium molybdate, calcium content was determined by the reaction with *o*-cresolphthalein using Vital Diagnostics reagent kits. The other portion was used for measuring hydroxyproline; collagen concentration was calculated from its concentration by Zaides' formula. Hydroxyproline was measured after hydrolysis with 8 n HCl by reaction with Ehrlich's reagent. The content of creatine in the skeletal muscles of damaged segment of the limb was evaluated by the reaction with diacetyl, of creatine phosphate by the content of phosphorus in protein-free extract [1]. Activities of serum aminotransferases (ALT and AST) were measured using Vital Diagnostics reagent kits.

The results obtained in experimental animals were compared with the values in intact animals (n=16) using Wilcoxon W test for independent samplings.

RESULTS

Summary content of creatine and creatine phosphate in the skeletal muscles of damaged segment in mice after shin bone fracture virtually did not differ from the normal levels throughout the experiment (Table 1). A statistically significant increase in these substrate levels on days 7 and 28 was noted in mice with AHI. A significant decrease of creatine and creatine phosphate levels was observed in mice with shin fractures and AHI on day 3 after the injury, but later the tissue levels of these substances were within the normal range. The detected elevation of creatine and creatine phosphate levels in the muscles of mice in group 2 and their slight

TABLE 1. Summary Content of Creatine and Creatine Phosphate (μmol/g tissue) in Skeletal Muscles of Injured Limb in Experimental Mice (Median, Interquartile Range)

Group	Intact mice	Day after injury		
		3	7	28
1	81.3 (78.4-84.3)	85.4 (82.5-86.3)	79.2 (77.5-83.1)	78.3 (76.9-81.6)
2		82.5 (80.6-84.2)	86.3* (83.8-87.8)	89.9* (86.0-94.70)
3		75.4* (73.2-78.7)	82.3 (79.6-85.0)	84.5 (81.3-89.4)

Note. Here and in Tables 2 and 3: statistically significant differences *$p \leq 0.05$ compared to normal.

TABLE 2. Serum Transaminase Activities (μmol/sec×liter) in Experimental Mice (Median)

Transaminase	Intact mice	Day after injury		
		3	7	28
AST				
group 1	0.207	0.241*	0.236*	0.213
group 2	0.207	0.302*	0.330*	0.198
group 3	0.207	0.312*	0.240*	0.226
ALT				
group 1	0.267	0.385*	0.276	0.246
group 2	0.267	0.481*	0.348*	0.203
group 3	0.267	0.370*	0.307	0.204

TABLE 3. Calcium, Phosphate, and Collagen Content in the Fractured Shin Bone in Experimental Mice (Median, Interquartile Range)

Group	(Ca+P), g/100 g dry residue				Collagen g/100 g dry residue			
	normal value	day 3	day 7	day 28	normal value	day 3	day 7	day 28
1		21 (20-23)	27 (24-31)	30* (28-33)		4.9* (4.5-6.0)	9.2 (7.9-11.8)	12.5* (11.1-13.5)
2	23 (21-25)	15* (12-17)	24 (23-25)	28* (27-30)	10.4 (8.7-10.6)	5.7* (5.4-6.1)	7.8* (7.3-7.9)	7.5* (7.1-8.2)
3		11* (7-18)	17* (16-19)	25 (24-26)		5.8* (5.5-6.5)	8.1* (7.4-8.4)	11.1 (9.5-13.9)

loss in group 3 during the early posttraumatic period were presumably caused by compensatory activation of the synthetic processes in hepatocytes. Later attenuation of cell necrosis contributed to this trend, which was shown by the dynamics of serum aminotransferase activities (Table 2). The detected significant increase in activities of these enzymes in group 1 was caused by skeletal muscle injury in the fracture zone.

The study revealed a significant reduction of the intensity of accumulation of mineral and organic substances in the shin bones of mice with AHI after fracture. Tissue collagen level decreased in animals with shin fracture (group 1) on day 3 after the injury; no appreciable changes in calcium and phosphate levels were detected; in addition, on day 28, excessive accumulation of these elements was detected in tissue (Table 3). Shin fracture in the presence of AHI (Group 3) was associated with reduction of calcium, phosphate, and collagen levels in the shin bones of injured limb on days 3 and 7 after the injury. Importantly, the greatest reduction was observed for the mineral constituent. The (Ca+P)/collagen ratio in this group was 1.90 and 2.10 on days 3 and 7 after the injury, respectively (*vs.* normal value of 2.21). However, by the end of the experiment the levels of calcium, phosphate, and collagen returned to normal, similarly as the (Ca+P)/collagen ratio, reaching 2.24. A significant decrease in collagen concentration in the shin bones was observed in mice with AHI (group 2) throughout the experiment; calcium and phosphate levels in tissues decreased during the early period after CTC injection.

Hence, posttraumatic osteoreparation in mice with AHI was paralleled by disorders in bone mineralization and in the synthesis of its organic component. Importantly that AHI was virtually inessential for creatine metabolism in the damaged limb muscles during the posttraumatic period.

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