EFFECT OF A SPLEEN-SPARING OPERATION ON FUNCTIONAL STATE OF LIVER MITOCHONDRIA OF RATS WITH EXPERIMENTAL CIRRHOSIS

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Interest in the effect of splenectomy (SE) on the intrahepatic hemodynamics, on the course of inflammatory changes in the liver, and on the immune reactions of the body has increased greatly in the last decade [1-4, 6, 12, 15]. Meanwhile, the indications for SE are still being interpreted widely, supported by arguments in favor of its decompressive effect, and the abolition of hypersplenism and autoimmune aggression [8, 10, 13]. This state of affairs makes the choice of a pathogenetically justified method of treatment of patients with cirrhosis of the liver more difficult and it necessitates a profound study of hepatolienal connections.

The aim of the investigation described below was to study the effects of a spleen-sparing operation combined with supplementary arterialization of the liver on the functional state of the mitochondria and on activity of the key mitochondrial enzymes of the liver in rats with experimental cirrhosis.

EXPERIMENTAL METHOD

Experiments were carried out on 102 male albino rats of a mixed population, with a body weight of 170-200 g. The experimental model of cirrhosis of the liver involved two intraperitoneal injections of the hepatotoxin heliotrine in a total dose of 8 mg/100 g body weight, and intraaortic injection of the vascular sclerosing preparation thrombovar, in a dose of 2 mg/100 g body weight. The interval between injections was 6-7 days. The animals were kept on the standard laboratory diet. The operations were performed in the 6th-7th week after first administration of the poison to the animal, when the initial signs of formation of cirrhosis of the liver became apparent morphologically. Animals of Group 1 underwent resection of 30-35% of the mass of the spleen at the lower pole together with ligation of the left gastric artery (RS); Group 2 consisted of rats undergoing SE; animals of the control group underwent laparotomy and inspection of the spleen (mock operation).

Liver mitochondria were isolated in a cold room at 0-2°C. Respiration and oxidative phosphorylation of the mitochondria were studied by the usual method, in a polarographic cell with open revolving platinum electrode [5]. The oxidation substrate used was sodium succinate: 5 mmoles per sample. The phosphate acceptor was ADP in a concentration of 200 μmoles per sample. Activity of succinate dehydrogenase (SDH) [5] and of magnesium-dependent ATPase (Mg^{2+}-ATPase) [11] and the mitochondrial protein content [9] were determined in all the animals. The results were analyzed by "Pravets-8A" computer.

EXPERIMENTAL RESULTS

Mortality among animals in the control group and Groups 1 and 2 was 46.6 (14 of 30 rats), 20% (six of 30), and 30% (nine of 30) respectively.

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TABLE 1. Changes in Respiration and Oxidative Phosphorylation in Liver Mitochondria of Rats with Experimental Cirrhosis of the Liver after SE and RS Combined with Ligation of the Left Gastric Artery

Group of animals	Time after operation, weeks	Respiration rate (ng·atoms O_2/min/mg protein)				ER	RC	ADP/O	Phosphorylation time ADP/t, sec
		V_1 (end)	V_2 (4-n)	V_3 (3 st)	V_4 (4-rest)				
Intact (n = 6)		11,6±1,47	14,23±1,29	42,15±4,60	11,91±1,94	2,962±0,27	3,846±0,53	1,776±0,12	49,7±6,22
Control (n = 4)	1	12,21±1,36	15,96±2,02	34,64±2,77	9,52±2,20	2,055±0,33*	2,885±0,61	1,342±0,06*	98,4±8,80*
1 (n = 8)	1	9,34±0,55	10,82±1,32	18,72±3,88*	10,48±1,72	1,804±0,41*	2,144±0,82	1,255±0,20*	146,0±22,4**
2 (n = 8)	1	8,21±1,21*	9,94±1,38*	19,90±2,75*	10,20±2,33	1,915±0,29*	2,255±0,37	1,120±0,13**	166,0±27,2**
Control (n = 6)	4	12,82±0,51	17,89±0,73*	33,25±3,15	16,41±0,81*	2,701±0,18	2,691±0,56	1,414±0,08*	58,0±5,20
1 (n = 8)	4	16,42±0,62	20,04±1,53*	30,86±2,53*	19,30±3,11**	1,965±0,27*	2,352±0,18	1,320±0,13*	125,0±17,3*
2 (n = 7)	4	17,21±0,98*	23,50±1,92*	25,00±5,14*	22,60±1,48**	1,924±0,33*	2,026±0,48*	1,237±0,19**	150,0±26,7**
Control (n = 6)	8	21,89±2,33*	24,69±2,03*	30,58±1,77**	21,75±1,50**	1,265±0,25**	1,414±0,066**	1,097±0,054**	211,0±12,3**
1 (n = 8)	8	11,66±0,75	16,93±1,36	48,79±5,18	14,97±1,28	2,463±0,35	2,991±0,56*	1,514±0,09*	81,0±17,7*
2 (n = 8)	8	16,98±0,81*	20,41±3,01*	32,84±2,83*	18,84±1,44*	1,988±0,38*	2,166±0,48*	1,208±0,14*	185,0±22,8**

Legend. Here and in Table 2: *$p < 0.05$, **$p < 0.01$.

TABLE 2. Changes in Enzyme Activity of Liver Mitochondria of Rats with Experimental Cirrhosis of the Liver After SE and RS Combined with Ligation of Left Gastric Artery

Group of animals	Time after operation, weeks	Activity of		Mitochondrial protein content, mg/g
		Mg^{2+}-ATPase, mmoles/mg/30 min	SDH, μg/mg/min	
Intact (n = 6)		0,650±0,040	0,245±0,0008	26,01±2,04
Control (n = 4)	1	0,707±0,063	0,0372±0,0016**	21,87±63
1 (n = 8)	1	0,403±0,017**	0,0117±0,0016**	18,15±0,88*
2 (n = 8)	1	0,457±0,037**	0,0121±0,0019**	16,22±1,86**
Control (n = 6)	4	0,782±0,089	0,0200±0,28	17,53±1,84*
1 (n = 8)	4	0,869±0,047*	0,0187±0,004*	18,85±1,68*
2 (n = 7)	4	0,912±0,055*	0,0129±0,0017**	15,29±2,17**
Control (n = 6)	8	1,222±0,09**	0,0170±0,001*	16,12±2,31**
1 (n = 7)	8	0,777±0,084	0,0214±0,003	19,92±2,06*
2 (n = 6)	8	0,985±0,119*	0,0123±0,004**	17,31±1,34**

The investigations showed that 1 week after the operation, a low-energy shift in the functional state of the liver mitochondria was developing in animals of the control group. This was manifested as a decrease in the respiration rate of the mitochondria in activated and regulated states (V_3 and V_4), a tendency for the enhancement ratio (ER) and the respiratory control (RC) to fall, and for the phosphorylation time to increase (Table 1). An increase in activity of SDH and Mg^{2+}-ATPase was observed (Table 2). The operations greatly worsened the functional state of the liver mitochondria and led to the development of a more marked shift of the energy state. In both Group 1 and Group 2 the respiration rate in metabolic states 1, 2, and 3 (V_1, V_2, and V_3) was considerably reduced, whereas in state V_4 it remained at the control level, which was accompanied by a decrease in the values of the coefficients ER, RC, and ADP/O. The phosphorylation time increased by 48-69% ($p < 0.01$). Changes in SDH and Mg^{2+}-ATPase activity were expressed as a significant decrease in the values of the parameters in both groups of experiments and lowering of the mitochondrial protein level.

In the 4th week after the operation, the changes observed in Groups 1 and 2 continued in the same direction. However, processes of respiration and oxidative phosphorylation were different in character. For instance, the respiration rate of the mitochondria in states V_1, V_2, and V_4 increased, whereas in state V_3 it remained below the control values. The coefficient RC, and ADP/O, while showing a tendency to increase, were nevertheless below the control level. The phosphorylation time was increased by 115.5-158.6% ($p < 0.001$). Activity of Mg^{2+}-ATPase was twice that observed in Stage 1 of the investigation, but a significant increase in SDH activity was observed only in Group 1.

Toward the end of the experiments (8 weeks after the operation) worsening of the developing low-energy shift was observed in the liver mitochondria of animals undergoing the mock operation, and also in the splenectomized rats. Coupling of oxidation and phosphorylation was greatly weakened compared with that both in intact rats and in animals undergoing RS combined with ligation of the left gastric artery. Values of the coefficients ER, RC, and ADP/O remained low. The phosphorylation time was more than quadrupled. The marked increase of ATPase activity was accompanied by a low level of SDH activity and by hypoproteinemia. These changes reflect profound disturbances of the functional state of the hepatocyte organelles during progressive experimental cirrhosis of the liver, and the impossibility of preventing them by removal of the spleen.

In the group of animals undergoing RS with supplementary arterialization of the liver, changes indicative of the favorable action of resection with arterialization on the state of the hepatocyte mitochondria were observed in the late period after the operation. In this group, 2 months after the operation an increase was observed in the rate of oxygen consumption in the active state, with an increase in values of the coefficients ER, RC, and ADP/O and reduction of the phosphorylation time compared both with data for the previous period of the investigation and the values obtained on splenectomized animals. Activity of the mitochondrial enzymes moved close to this level in intact rats and the protein concentration rose.

Thus the development of cirrhosis of the liver in rats, produced by combined injection of the hepatotoxic agent heliotrine and the vascular sclerosing agent thrombovar, gives rise to considerable disturbances of the functional state of the liver mitochondria. In the early period after SE and resection of 30-35% of the mass of the spleen (1-4 weeks) the developing low-energy shift in the liver organelles becomes intensified. At later stages of observation, in animals undergoing RS with supplementary arterialization of the liver, the coupling of oxidation with phosphorylation was more marked whereas after SE, activation of oxidative processes was observed but the coefficient of phosphorylation remained low.

Disturbance of energy-determined regulation of respiration and reduction of the efficiency of phosphorylation in animals with experimental cirrhosis of the liver, and undergoing SE, were probably due to deficiency of splenic humoral factor [4, 14], leading to disturbance of the functions of the most important organelles of the hepatocytes, responsible for detoxication processes and energy production in the liver cells. Preservation of part of the spleen, combined with supplementary arterialization of the liver, significantly improved the functional state of the hepatocyte mitochondria and the effects of RS were superior to those of SE.

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