Effects of Dextran on the Ultrastructure of Kupffer Cells in Crush Syndrome

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Ultrastructural stereometric analysis showed that the ratio of secondary lysosomes increased, the intensity of plastic processes decreased, and the protein-synthesizing apparatus did not change in rats subjected to crush syndrome and treated or untreated with dextran. Energy production in mitochondria considerably decreased. These structural changes were most pronounced in animals treated with dextran on the 7th day after recirculation in the hind limb.

Key Words: *crush syndrome; Kupffer cell; dextran; ultrastructure; stereometry*

Crush syndrome (CS) is a severe disease characterized by high mortality rate induced by polymorphic pathology (especially, renal diseases). The liver plays an important role in clearing functions in the organism. On the other hand, recirculation in crushed tissues is accompanied by high levels of toxic substances in the blood. These data suggest that damage to the liver during CS determines the development of pathological changes in the body.

Dextran solutions (rheopolyglucin and polyvinylpyrrolidone) display a considerable lysosomotropism. These substances are intensely entrapped in Kupffer cells and can modify damage and reparation processes [1,9,10].

Therefore, the study of changes observed in Kupffer cells, the largest compartment of the mononuclear phagocyte system, are important for the understanding the mechanisms of CS and for adequate infusion therapy with plasma substitutes that is necessary in the treatment of patients with CS [3].

MATERIALS AND METHODS

Experiments were performed on 6-month-old male Wistar rats (n=75) weighing 180-200 g kept under standard laboratory conditions. Moderate CS was modeled as described previously [2] by applying 5-cm^2 metal disks on the left hind limb parallel to the inguinal ligament for 4 h. Liver samples were taken from the central part of the left lobe. Control rats were subjected to CS without treatment. Experimental rats were subjected to CS and received intraperitoneal infusion of 10% dextran (molecular weigh 30-40 kDa) and isotonic NaCl in a dose of 10 ml/kg body weight (3 times with one-day intervals) in accordance with recommendations on emergency care for patients with CS [3]. The periods of liver examinations corresponded to the early (1-3 days) and intermediate (3-7 days) stages and the beginning of CS (the 7th day) [5].

For electron microscopy, liver samples were fixed in 1% OsO_4 in phosphate buffer (pH 7.2-7.4), dehydrated in increasing ethanol concentrations, and embedded into Epon. Five blocks were prepared from liver samples of each rat. Semithin Epon slices were stained with toluidine blue. Ultrathin slices were stained with uranyl acetate and lead citrate. Morphometry was performed using a JEM-100S electron microscope (×3500). Kupffer cells (n=30-40) corresponding to different observation periods were photographed. Stereometric assays were performed as described elsewhere [8]. The results were analyzed using Student's t test.

RESULTS

Taking into account the specialization of Kupffer cells, the state of the vacuolar apparatus was analyzed. On

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the 1st and 3rd days of recirculation in the limb, vacuolar apparatus of treated and untreated animals increased slightly compared with the control and were similar (Table 1). This increase was obviously associated with increased content of secondary lysosomes. In dextran-treated rats, this increase was more pronounced than in untreated animals. In untreated rats, low-molecular-weight molecules (products of myoglobin degradation) entered Kupffer cells after the onset of blood recirculation in the limb. This did not affect pinocytosis (Fig. 1, *a*). In untreated animals with CS, the reproduction of primary lysosomes was maintained at relatively high level and their total volume did not decrease (Fig. 1, *b*). The decrease in the content of primary lysosomes in dextran-treated animals was due to their decreased reproduction and pinosome-lysosome fusion. This was confirmed by a higher volume density of these structures in treated rats (in comparison with untreated animals) and a decrease in the surface area of the vacuolar apparatus. On the 7th day of recirculation in the limb, the levels of pinocytosis in animals of both groups sharply decreased (to zero in treated rats; Fig. 1, *a*). The contents of primary and secondary lysosomes in Kupffer cells of dextran-treated rats were 2.5-fold lower and 2-fold higher, respectively, compared with those levels in intact animals. However, in untreated rats these indexes returned to normal (Fig. 1, *c*). On the 7th day of recirculation, the intensity of plastic processes (total concentration of membranes of cytoplasmic organelles) [8,10] in animals subjected to CS in both groups was 2 times lower than in intact rats (Table 1). We observed no considerable changes in the content of polysomes that could contribute to a decrease in the intensity of plastic processes.

On the 7th day of recirculation in the limb, the surface area of the inner mitochondrial membrane sharply decreased in dextran-treated and untreated animals with CS (in all periods of observations, Table 1).

These data suggest that recirculation is accompanied by a release of low-molecular-weight substances in crushed tissues. These substances decrease the energy production in mitochondria, which is associated with structural changes (destruction of organelles). On the 7th day of recirculation, the volume density of mitochondria decreased by 20% in untreated rats and 5-fold in dextran-treated ani-

TABLE 1. Morphometry of Kupffer Cell Ultrastructure during CS and Its Treatment with Dextran (*M±m*)

Parameters		Intact rats	Time after decompression, days							
			1		3		7			
			control	experiment	control	experiment	control	experiment		
Secondary/primary lysosome ratio	Vv	1.2	2.0	9.5	2.6	10.5	2.2	7.0		
Vacuolar apparatus	Vv	13.6±3.46	22.3±4.6*	21.4±6.14	19±2.87	22.9±4.78	15.4±2.8	16.3±4.72		
	Sv	2.1±0.32	2±0.18	0.7±0.09*	1.9±0.2	0.8±0.08*	0.8±0.08*	0.8±0.09*		
Mitochondria	Vv	5.1±1.3	4.5±0.96	3.6±0.82	6±0.67	5±0.97	4.2±0.75	1.1±0.4*		
inner membranes	Sv	1.6±0.3	1.6±0.31	0.6±0.01*	2.3±0.29	0.8±0.1*	0.7±0.1*	0.2±0.05*		
outer membranes	Sv	0.7±0.1	0.5±0.1	0.2±0.03*	0.8±0.09	0.8±0.05	0.3±0.04*	0.1±0.02*		
Granular endoplasmic reticulum and Golgi complex	Vv	4.5±0.45	4.2±0.42	3.7±0.44	5.4±0.53	3.8±0.42	4.5±0.08	4.2±0.44		
	Sv	3.6±0.37	3.0±0.38	0.9±0.12	4.2±0.48	1.6±0.18*	1.5±0.17*	1.0±0.13*		
Rough endoplasmic reticulum	Vv	5.2±0.42	6.9±0.45*	7.0±0.95*	6.0±0.57	7.1±0.47*	6.4±0.42*	7.1±0.47*		
	Sv	3.9±0.25	4.1±0.38	1.7±0.19*	4.9±0.34*	2.7±0.15*	2.2±0.13*	2.1±0.27*		
Number of membranes of cytoplasmic organoids	Sv	11.9±1.34	11.2±1.35	4.1±0.44*	14.1±1.4	6.7±0.56*	5.5±0.52*	4.2±0.56*		
Attached ribosomes		26.6±2.95	29.1±2.87	17.7±3.5	26.2±3.26	34.4±3.55	30.9±1.76	31.0±4.41		
Free ribosomes		30.1±3.63	29.2±3.91	37.7±4.52	37.6±3.36	38.4±3.13	45.1±3.8*	36.9±5.2*		

Note: *$p<0.05$ compared with intact rats.

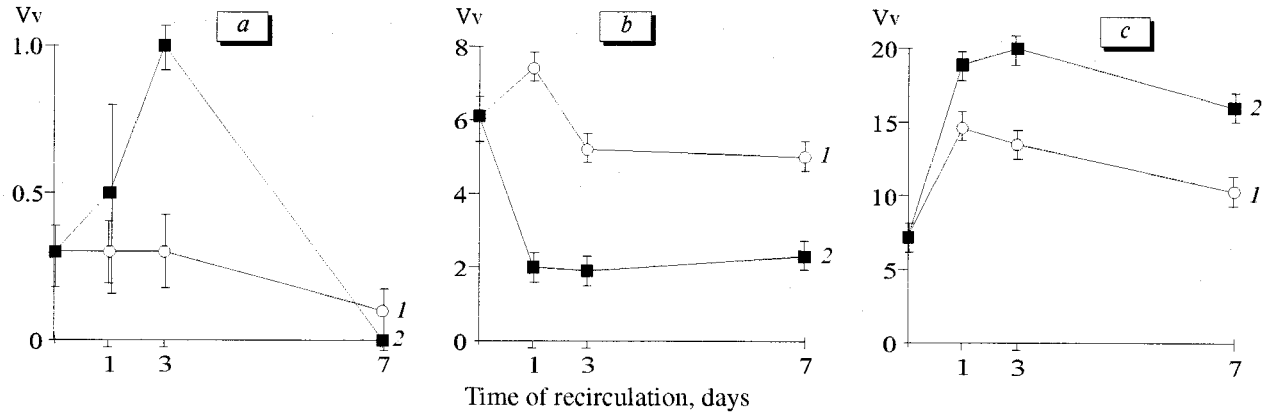

Fig. 1. Volume density of pinosomes (*a*) and primary (*b*) and secondary lysosomes (*c*) in untreated (*1*) and dextran-treated animals (*2*).

mals (Table 1). This was one of the main factors responsible for the blockade of synthesis of structural and, probably, lysosomal proteins. This affected the total concentration of membranes of cytoplasmic organelles and the volume density of primary lysosomes (Table 1, Fig. 1, *b*).

Our findings show a lower energy dependence of pinocytosis in comparison with the synthesis of structural proteins and enzymes (Table 1).

Dextran has lysosomotropic activity, changes rheological characteristics of the blood, and adsorbs toxic substances. This contributes to accumulation of toxic substances in the blood and aggravates pathological changes in Kupffer cells observed after administration of dextran.

During moderate CS, the 7th day of recirculation is a critical period not only for the hepatic compartment of the mononuclear phagocyte system. In view of our previous studies of the elimination rate for dextran of a specified molecular weight [11], these data suggest that dextran infusion for compensation of plasma losses and antishock therapy induces a partial blockade of mononuclear phagocytes (the 7th-8th day) and labilizes lysosomal membranes [4]. Thus, dextran can be considered as a risk factor for the development of septic and necrobiotic complications of infected wounds in patients with CS. Similar complications can be expected during the use of polyvinylpyrrolidone that also displays considerable lysosomotropism. Moreover, its retention time in the vacuolar apparatus of Kupffer cells, sinusoidal endotheliocytes, and hepatocytes exceeds 1.5 months [1].

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