Ultrastructural Changes in Liver Sinusoid Endotheliocytes during Dextran Treatment of the Compression Syndrome

V. A. Shkurupii, E. S. Luk'yanova, and A. V. Efremov

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Electron microscopic and morphometric studies showed reduction in the intensity of plastic processes, impaired mitochondrial function, and increased proportion of secondary lysososomes in endothelial cells of liver sinusoids in rats with a moderate long-term compression syndrome. These processes were pronounced in dextran-treated rats, which may be due to impaired trophics of hepatocytes.

Key Words: *liver; endothelial cells of sinusoids; long-term compression syndrome*

Endotheliocytes of liver sinusoids (ELS) form a fenestrated lining of the sinusoids and have a high ability for endocytosis realized via pinocytosis and adsorptional pinocytosis [10]. Administration of high-molecular-weight preparations during stress leads to activation of endocytosis, labilization of lysosomal membranes, reduction of the fenestrae, and increase in the cytoplasm volume [8]. This may impair the conditions for the hepatocyte metabolism [8].

Correction of the long-term compression syndrome (LTCS) by infusions of plasma-replacing solutions creates conditions for the above-mentioned pathological changes which are aggravated by high blood and lymph levels of endogenous toxins from compressed tissues [1]. Bearing in mind the important role of the liver in the clearance, we investigated structural reorganizations occurring in ESL during "spontaneously" developing of LTCS and its correction by dextran infusions, basic therapy of LTCS [4,6].

MATERIALS AND METHODS

Male Wistar rats (n=75, body weight 180-200 g, age 6 months) were used. The animals were maintained on standard laboratory diet. The LTCS was modeled by compressing the left hind leg with a vice (compression area 4 cm^2) for 4 h; [3].

The rats were divided into three groups: group 1 rats (n=5) were not treated, group 2 rats (n=5) were treated by intraperitoneal infusions of 10% dextran (30-40 kD) in normal saline (10 ml/kg body weight) three times every other day according to the recommendations on the first aid to patients with LTCS [4]. Group (n=5) consisted of intact rats. Pieces of liver were excised from the central part of the left lobe 1, 3, and 7 days after decompression of the limb. This corresponded to the early, intermediate, and the beginning of the late period of LTCS development [5].

For electron microscopy the pieces were fixed in 1% OsO_4 in phosphate buffer (pH 7.2-7.4), dehydrated in ascending concentrations of ethanol, and embedded in Epon. Five blocks from each animal were cut into semithin and ultrathin sections. Semithin sections were stained with toluidine blue and ultrathin sections with uranyl acetate and lead citrate.

For morphometry [10], ELS (40-50 cells in each rat for each observation period) were photographed in a JEM-100S electron microscope at magnification 3500. The differences between the mean values were regarded as significant at $p<0.05$ according to Student's t test.

Department of Pathological Anatomy, Department of Pathological Physiology, Novosibirsk Medical Institute

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RESULTS

Under normal circumstances, unlike Kuppfer cells [12], ESL, which have a well-developed vacuolar apparatus, have a very low content of secondary lysosomes. This was confirmed by our findings (Table 1). In untreated rats, the total volume of primary lysosomes in ELS increased by 21%. It then decreased by 43% on the 7th day after decomperssion (Table 1). In dextran-treated rats these changes developed earlier and were more dynamic. A 24-50% decrease in the total volume of pinocytotic vesicles and a 29-58-fold increase in the total volume of secondary lysosomes in ELS of untreated (compared with the control) may be due to the entry of compounds with a very low molecular mass (presumably, myoglobin degradation products) into ELS. After dextran infusions, the intensity of pinocytosis increased at least 2-fold (Table 1), which was accompanied by a higher rate of increase in the total volume of secondary lysosomes on the first and third days of decompression. By the 7th day this parameter was similar in untreated and treated rats, which may be due to dextran hydrolysis (by the 7th day its liver content drops to 15% of the initial level [11]) and decreased energy supply to pinocytosis. This is confirmed by much earlier and severe mitochondrial damage in dextran-treated animals on the 2nd, 3rd and particularly on the 7th day of decompression. The surface area of internal membranes in the mitochondria decreased by 2-4 times (Table 1). The greater damage to the mitochondria may be associated with sorption of necrotoxins by dextran. At the same time, the results obtained imply that pinocytosis in not strongly influenced by functional state of the mitochondria. Indeed, on the first day a 2-fold decrease in the area of mitochondrial internal membranes in dextran-treated rats coincided with a sharp increase in the intensity of pinocytosis and in the amount of secondary lysosomes. A similar regularity was observed in subsequent periods. Another explanation is the fact that the major proportion of dextran and myoglobin degradation products uptaken at the early stage (up to 3 h) of

TABLE 1. Morphometry of Organelles of Liver Sinusoid Cells in Long-Term Compression Syndrome and with and Without Dextran Treatment (*M±m*)

Parameter	Control	Days after decompression					
		1		3		7	
		no treatment	dextran treatment	no treatment	dextran treatment	no treatment	dextran treatment
Lysosomes (Vv)							
primary	2.8±0.63	3.4±0.69*	0.2±0.14*	2.9±1.00	1.4±0.50	1.6±0.51	0*
secondary	0.1±0.01	2.9±0.95*	4.6±1.00*	3.5±1.20*	4.9±0.82*	5.8±1.30*	5.8±1.50*
Membranes (Sv)							
Pinosomes (Vv)	7.3±1.90	3.7±1.00*	14.1±2.40*	4.8±0.89	18.1±3.60*	5.6±1.20	10.3±3.60
Mitochondria (Vv)	8.2±1.50	7.0±1.00	7.2±1.60	6.8±0.90	4.7±085*	6.5±1.40	4.5±1.50*
internal	1.2±0.19	1.5±0.23	0.6±0.13*	2.1±0.38*	0.6±0.11*	0.6±0.15	0.3±0.09*
external	0.5±0.08	0.6±0.08	0.2±0.04*	0.8±0.14	0.2±0.03*	0.3±0.06*	0.1±0.04*
Golgi complex							
Vv	6.4±0.77	5.2±0.57	5.5±0.66	7.04±0.68	4.9±0.66	7.4±0.64	0.3±0.66
Sv	2.4±0.25	2.6±0.40	1.2±0.12*	6.0±0.08*	1.5±0.20*	1.9±0.25	1.1±0.09*
Rough endoplasmic reticulum							
Vv	9.1±2.28	6.0±0.59	7.0±0.52	6.6±0.57	6.8±0.33	6.5±0.42	9.1±0.19
Sv	3.3±0.27	3.6±0.30	1.7±0.14*	5.2±0.38*	2.1±0.30*	1.7±0.16*	1.5±0.24*
Membranes of cytoplasmic organelles (Sv)	8.2±0.85	9.2±1.12	4.3±0.50	15.2±1.13	5.2±0.57	5.0±0.69	7.9±0.53
Ribosomes (Nv)							
attached	35.4±3.69	57.0±5.81*	28.9±2.87	32.3±3.56	35.1±2.98	28.0±3.01	37.6±5.79
free	31.1±4.92	37.6±4.39	35.1±3.89	48.9±3.63*	36.5±3.81	38.0±3.26	28.0±4.81

Note. Volume (Vv), surface (Sv), and numerical (Nv) density of structures; *$p<0.05$ compared with the control.

recirculation. Further changes in the total volume of pinocytotic vacuoles depend on their fusion with lysosomes and on membrane stability.

A 47% decrease in the volume density of mitochondria and a 4-fold decrease in the area of their membrane occurring in dextran-treated rats on the 7th day of decompression, which markedly surpassed these parameters in untreated animals (Table 1), point to impaired mitochondrial function and destruction of some mitochondria. Changes in the Golgi apparatus, which is functionally associated with lysosomes, were more pronounced than in untreated rats.

In dextran-treated rats, the decrease in the area of the rough endoplasmic reticulum was observed earlier than in untreated rats. This was due to more than a 2-fold decrease in the content of cytoplasmic membranes 1-3 days after decompression [7] (Table 1).

Thus, lower intensity of plastic processes in ELS during LTCS is strongly associated with energy metabolism. An increase in the area of internal mitochondrial membranes on the 3rd day of decompression in untreated rats was associated with an almost 2-fold increase in the content of cytoplasmic membranes, while a 4-fold decrease in the area of internal mitochondrial membranes in dextran-treated rats on the 7th day coincided with more than a 2-fold decrease in the total membrane content (Table 1).

It is noteworthy that overload of ELS vacuoles by dextran, a lysosomotropic compound, was not accompanied by an increase in the volume density of the sinusoid endothelial lining.

We observed a similar phenomenon after administration of dextran and hemodez under other experimental conditions and explained it by overload of the Kuppfer cell vacuoles with a lysosomotropic compound and lower intensity of cytoplasmic processes in these cells [9]. Widening of sinusoids in dextran-treated rats by more than 3 times is comparison with untreated rats may be associated with shock. More pronounced manifestations of shock in dextran-treated rats may result from increased blood content of necrotoxins due to the ability of dextran to improve blood rheology and microcirculation after decompression. In dextran-treated animals, ELS were edemic and the fenestrae were reduced. Thus, low intensity of plastic processes, cytoplasmic edema, increased proportion of secondary lysosomes with labile membrane [2], and decreased energy production by mitochondria occur in rat ELS during a moderate LTCS. These changes are aggravated by dextran, particularly 7 days after decompression, which markedly impairs hepatocyte trophics.

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