SELECTIVE ACCUMULATION OF RHEOPOLYGLUCIN AND LATEX IN LIVER CELLS AND RESPONSE OF LIVER PARENCHYMA TO ACUTE CCl_4 POISONING

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Lysosomotropic substances (LS) [8] can modify the physicochemical properties of lysosomes taking part in the realization of various functions of the organs and tissues. In this connection research has been undertaken with the aim of studying the possibility of corrective action to influence the development of pathological processes [2, 8]. In the investigation described below the effect of rheopolyglucin and a latex suspension, substances which accumulate in lysosomes of various liver cells, on the response of the liver after administration of a hepatotropic poison, was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male BALB mice aged 2 months and weighing 19-21 g, divided into groups (4 mice in each group). Animals of group 1 served as the control. Mice of group 2, under superficial ether anesthesia, received an injection of a 10% solution of rheopolyglucin (partially hydrolyzed dextran with mol. wt. of 30,000-40,000 daltons) in a dose of 1 ml/100 g body weight into the caudal vein, mice of group 3 were injected with a suspension of latex microspheres (Dow Latex) 1.1 μ in diameter (a marker of Kupffer cells) [9] in a dose of 0.5 ml/100 g body weight. Like the animals of group 2, they were decapitated 1 h after the experiment. Animals of groups 4, 5, 6, and 7 received CCl_4 by inhalation. Its concentration in the chamber was 0.025 ml in 1 liter of air, and the duration of exposure in the chamber was 10 min. These mice were decapitated 1, 2, 3, and 4 days later respectively. Animals of groups 8, 9, 10, and 11 received rheopolyglucin in the same dose and by the same method as mentioned above. Mice of groups 12, 13, 14, and 15 were given an injection of latex suspension similarly. They all received CCl_4 1 h later, as described above, and they were decapitated 1, 2, 3, and 4 days later respectively. Samples of liver for electron microscopy were fixed in 1% OsO_4 solution and embedded in Epon. Sections 1 μ thick were cut from these blocks, stained with toluidine blue, and used for morphometry. Ultrathin sections of the liver specimens from mice of the control group (1) and groups 6, 10, and 14 were studied in the IEM-100S electron microscope. From each animal 25 areas of cytoplasm of hepatocytes were photographed under a magnification of 7,000, and the negatives were studied stereometrically [12]. In parallel tests, samples of mouse liver from all groups were fixed in 10% formalin solution and embedded in paraffin wax. Sections 6-7 μ thick were stained with hematoxylin and eosin and used to determine volumes of necrosis by stereometry. During the stereometric investigation, closed test systems of squares were used. Differences between the mean values compared were considered significant at the $P < 0.05$ level.

EXPERIMENTAL RESULTS

One hour after the injection of rheopolyglucin and latex (animals of groups 2 and 3) the former was found in pinosomes and lysosomes of endothelial and Kupffer cells and of hepatocytes (Figs. 1 and 2). Latex was found only in heterophagosomes and heterophagolysosomes of Kupffer cells. The same tendency of the distribution of both LS also was maintained at subsequent times of observation. A weak positive effect from administration of

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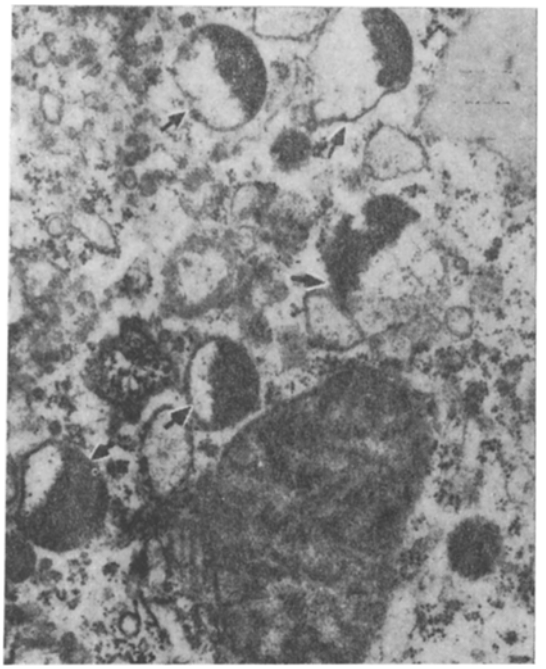

Fig. 1

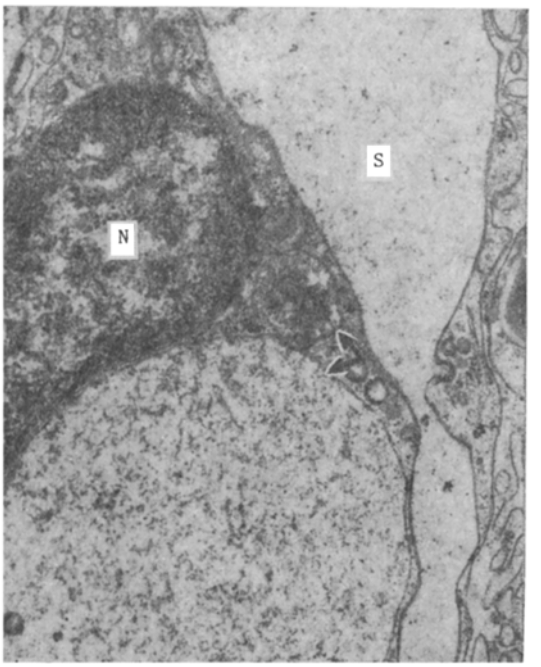

Fig. 2

Fig. 1. Part of cytoplasm of a mouse hepatocyte 1 h after injection of rheopolyglucin. Arrows indicate heterophagolysosomes containing rheopolyglucin. 30,000 ×.

Fig. 2. Part of cytoplasm of endothelial cell of mouise liver 1 h after injection of rheopolyglucin. Arrow indicates endocytic vacuole containing rheopolyglucin. N) nucleus of sinusoidal endothelial cell; S) sinusoid. 8250 ×.

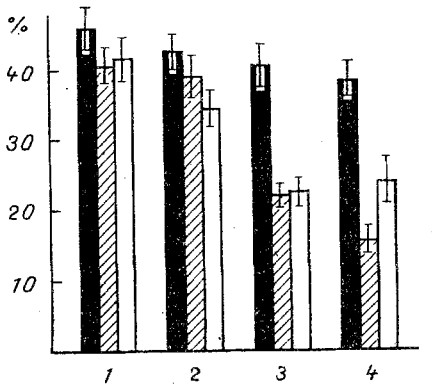

Fig. 3. Results of morphometry of necrotic zones and parenchyma of mouse liver after inhalation of CCl_4. Abscissa, time (in days) after inhalation of CCl_4; ordinate, volume of necrotic foci (in percent). Black columns — animals receiving CCl_4 only, obliquely shaded — animals receiving latex before CCl_4 poisoning, unshaded columns — animals receiving rheopolyglucin before CCl_4 poisoning.

both LS was observed 24 h after inhalation of CCl_4, as shown by a decrease in the volume of necrotic foci (by 12-14%) in the liver parenchyma (Fig. 3). It can be tentatively suggested that this character of manifestation of the protective properties of such widely different preparations was due not to their direct action, but to the state of stress arising in the animals in connection with the procedure of injection of LS. A state of stress is accompanied by a fall in the concentration of microsomal cytochrome P-450 [1, 7], possibly in connection with increased production of glucocorticoid hormones, which in high concentrations may lead to inhibition of the mono-oxygenase system, and to a reduction in the content of cytochrome P-450, which plays an important role in metabolism of xenobiotics, in the microsomes of hepatocytes [3]. After two days the differences in the volumes of necrosis between the three groups of animals were small (Fig. 3).

TABLE 1. Results of Morphometry of Semithin Sections through the Liver (M ± m)

Parameter studied	Control	Experiment	Time after end of CCl_4 inhalation, days			
			1-	2-	3-	4-
Volume of hepatocytes ($\bar{v}$), μ^3	3213,2±364,1	I	4807,7±496,2*	10332,3±1136,4*	8663,1±1009,8*	7624,0±821,2*
		II	4454,0±490,9*	8881,0±900,2*	4232,4±389,7	4820,6±458,0
		III	6137,5±746,5*	10168,9±1152,5*	7929,1±936,5*	6107,8±518,0*
Numerical density of Kupffer cells (N_A), number of cells per 10^4 μ^3 of liver tissue section	5,0±0,30	II	3,6±0,39*	3,0±0,39*	2,5±0,30*	3,0±0,38*

Legend. I) CCl_4, II) latex + CCl_4, III) rheopolyglucin + CCl_4. *) difference from control significant.

TABLE 2. Results of Morphometry of Ultrastructures of Hepatocytes in Animals 3 Days after Administration of CCl_4 and LS (M ± m)

Parameter studied	Control	Experiment		
		CCl_4	latex + CCl_4	rheopolyglucin + CCl_4
Mitochondria:				
Outer membrane (S_V)	1,32±0,050	0,84±0,042[a]	0,98±0,049[a,б]	1,00±0,040[a,б]
Inner membrane (S_V)	4,73±0,205	3,66±0,273[a]	3,90±0,248[a]	3,91±0,299[a]
Rough endoplasmic reticulum (S_V)	3,35±0,220	2,72±0,154	3,14±0,164	3,59±0,168
Total concentraiton of membrane (S_V)	9,40±0,304	7,22±0,314[a]	8,02±0,301[a]	8,50±0,287[a,б]
Lysosomes (N_V)	0,22±0,038	0,26±0,037	0,20±0,040	0,28±0,039
Ribosomes:				
Free (N_A)	24±2	16±1[a]	23±2[б]	21±1[б]
Fixed (N_A)	15±1	11±1[a]	16±1[б]	17±1[б]
Glycogen (V_V)	3,9±0,55	0,5±0,21[a]	2,1±0,78[б]	1,6±0,51[б]
Lipid inclusions (V_V)	2,4±0,50	29,3±2,32[a]	11,9±1,64[a,б]	6,5±1,23[a,б]

Legend. S_V) surface density of membranes of organoids (in μ^2/μ^3 of cytoplasm); V_V) numerical density of structures (number pf μ^3 of cytoplasm); N_A) numerical density of structures (number per μ^2 area of section of cytoplasm); V_V) bulk density of structures (in percent of colume of cytoplasm). Total concentration of membrane — includes inner and outer mitochondrial membranes, rough endoplasmic reticulum (in μ^2/μ^3 of cytoplasm). a) Difference from control is significant, b) difference from animals receiving CCl_4 only is significant.

On the 3rd and 4th days after poisoning of the mice with CCl_4 the rates of resorption of the necrotic foci in the liver parenchyma were greatly increased in the animals receiving LS (Fig. 3). These differences were evidently determined by the increased rate of degradation of residues of necrotic hepatocytes. A leading role in this process is played by blood macrophages and organ-specific macrophages.

Intensification of endocytosis by macrophages is linked with what has been called a metabolic burst [4]. According to data in the literature [4], phagocytosis of latex microspheres by macrophages led to an increase in their lysosome population, and in the Kupffer cells 1 h after injection of latex into mice their cathepsin D activity was increased by 50% [9]. There is reason to suppose that Kupffer cells migrate into zones of necrosis [6, 7] and secrete lysosomal enzymes, thus facilitating resorption of the remnants of necrotically changed hepatocytes [11]. Indirect evidence of migration of Kuppfer cells from undamaged zones of hepatic lobules is given by the results of counting Kupffer cells labeled with latex (Table 1). It can be tentatively suggested that the more dynamic character of resorption of necrotic foci in the liver parenchyma of mice receiving LS before inhaling CCl_4 is due both to an increase in functional activity of the Kupffer cells (stimulation of synthesis of lysosomal hydrolases and of their secretion), and to the larger scale of migration of the Kupffer cells into the zones of necrosis. The possibility cannot be ruled out that overloading of Kupffer cells with LS may facilitate their detachment from the endothelium of the sinusoids [5, 13].

The effect of rheopolyglucin on the physicochemical properties of lysosomes has received little study, although in this case some similarity can be expected with the changes arising when latex or polyvinylpyrrolidone particles are injected into animals. The latter possessed more marked protective properties against the harmful action of CCl_4 on the liver parenchyma [5].

From the first day after inhalation of CCl_4 animals of all groups showed hypertropy of the hepatocytes, which was least marked in the mice receiving latex (Table 1).

Injection of both LS led to more rapid normalization of the subcellular organization of the hepatocytes on the 3rd day after inhalation of CCl_4 (Table 2). However, this process developed optimally in the hepatocytes of mice which received rheopolyglucin beforehand (Group 10). This was shown by the mild degree of fatty degeneration, restoration of the value of the surface density of membranes of the rough endoplasmic reticulum, the number of ribosomes, and the highest concentration of cytoplasmic membranes compared with that in hepatocytes of animals of all other groups (6 and 14) receiving CCl_4 (Table 2).

It can be tentatively suggested that the stronger positive effect of rheopolyglucin than of preliminary injection of latex is connected both with changes in the functional state of lysosomes of hepatocytes and Kupffer cells and with its well-known detoxicating properties.

The results point to possible ways of correcting pathological processes at different stages of their development with the aid of LS.

LITERATURE CITED

1. L. M. Deev, M. Ya. Akhalaya, and Yu. B. Kudryashov, Byull. Éksp. Biol. Med., No. 8, 28 (1981).
2. T. A. Korolenko, Usp. Sovrem. Biol., 97, No. 1, 101 (1984).
3. I. N. Marokko, T. G. Khlopushina, E. M. Lysenkova, et al., Byull. Éksp. Biol. Med., No. 12, 713 (1984).
4. I. S. Freidlin, The System of Mononuclear Phagocytes [in Russian], Moscow (1984).
5. V. A. Shkurupii, V. N. Gavrilin, T. A. Korolenko, et al., in: Pathogenesis and Morphogenesis of Chronic Disease [in Russian], Riga (1984), p. 185.
6. V. A. Shkurupii, Byull. Éksp. Biol. Med., No. 6, 754 (1985).
7. Chung Ho and D. R. Brown, Toxicol. Appl. Pharmacol., 37, 2 (1976).
8. C. De Duve, T. De Barsy, B. Pool, et al., Biochem. Pharmacol., 23, 2595 91974).
9. A. M. De Leeuw, E. C. Sleyster, M. T. Quist, and D. L. Knook, Kupffer Cell Bull., 4, 4 (1982).
10. E. W. Perry, J. Comp. Path., 88, 481 (1978).
11. E. W. Perry, J. Comp. Path., 89, 205 (1978).
12. E. R. Weibel, W. Stabli, H. R. Ghagi, and F. A. Hess, J. Cell Biol., 42, 68 (1969).
13. E. Wisse, Med. Chir. Dig., 6 , 409 (1977).