Ultrastructure of Hepatocytes and Sinusoidal Endothelial Cells for the Use of Competition between Lysosomotropic Preparations Applied in the Treatment of Iron-Deficiency Anemia

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It is shown that the level of repair processes in endothelial cells progressively declines in posthemorrhage iron-deficiency anemia treated with Ferrum Lek. Administration of Ferrum Lek inhibits the repair processes in hepatocytes, leading to the development of dystrophy and necroses in them. Administration of rheopolyglucin prior to Ferrum Lek reduces the Ferrum Lek content in endothelial cells lysosomes and activates the repair processes in hepatocytes and endothelial cells. This is thought to be due to competition of rheopolyglucin for cell vacuoles and the ability of this preparation to stimulate the repair processes.

Key Words: *hepatocytes; sinusoidal endothelial cells; lysosomatotropic substances; iron-deficiency anemia*

As a biological phenomenon, lysosomotropism is probably attended by blocking of endocytosis to varying degrees [2]. The use of drugs with lysosomal properties can thus have negative results, since these properties are usually not taken into consideration. At the same time, the development of new generation of drugs with targeted delivery calls for research into the biological and medical aspects of lysosomotropism.

We studied the morphological relationship between the reticuloendothelial cells and liver parenchyma cells during treatment of severe iron-deficiency anemia (IDA) with the lisosomotropic preparations Ferrum Lek (FL) and rheopolyglucin (RPG). We also attempted to mitigate the negative effect of FL on the liver [7] - an organ with a pronounced clearing function - which results in a substantial accumulation of lysosomotropic preparations and, consequently, may have a strong impact on the liver and the organism as a whole.

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MATERIALS AND METHODS

Experiments were performed on Chinchilla rabbits. Posthemorrhagic severe IDA (a hemoglobin content of 70 g/liter or less) was attained by daily 20-ml bleedings from the ear vein during a 38-43-day period. The animals were divided into 3 equal groups (n=5). Group I consisted of rabbits with severe IDA; group II rabbits were given intravenous injections of FL (Ferrum Lek, Fe saccharide, Yugoslavia) for the correction of IDA (5 injections per course, the total dose being calculated from the formula recommended for IDA patients [3]); group III rabbits were treated with FL using the same total dose and scheme of administration, but 24 h before the first and third injections they were given intraperitoneal injections of RPG (a dextran with

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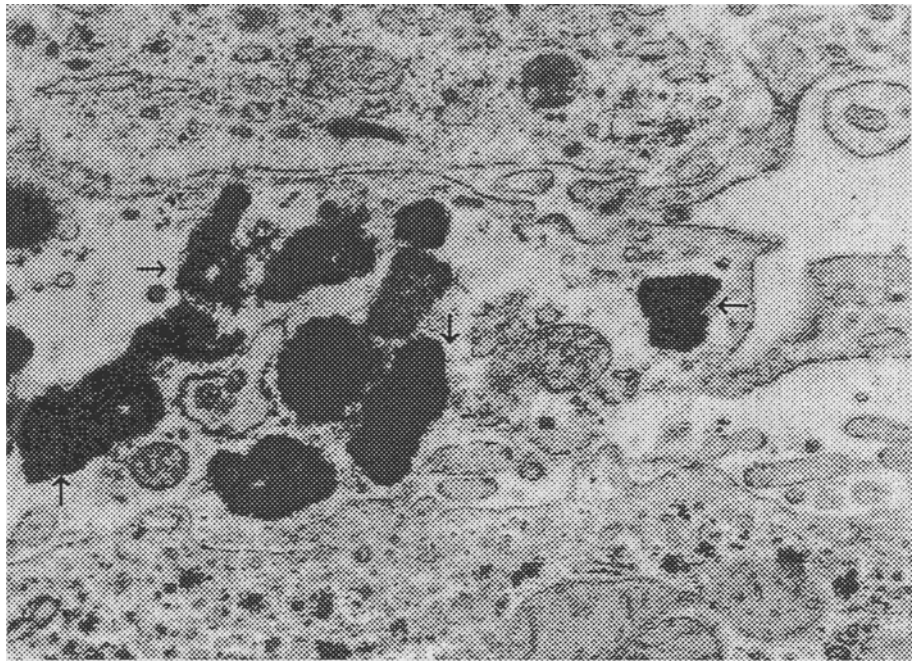

Fig. 1. Ultrastructure of sinusoidal endothelial cells for treatment of severe IDA with FL, ×21,000. Electron-dense particles of Fe saccharide in pinolysosomes are indicated with arrows.

a molecular weight of 30,000-40,000 D) in a dose of 10 ml/kg. Intact animals served as the control. Liver specimens for electron microscopy were processed using conventional methods [5] and viewed in JEM 100S electron microscope. Hepatocytes (100 negatives in each group) and sinusoidal endothelial cells (50 negatives in each group) were photographed, and their cytoplasmic structures were studied morphometrically [6]. The FL content in endothelial cells vacuoles was determined morphometrically by counting the number of test system points with 1-cm intervals at a final magnification of the cytoplasm negative of 59,500. The differences between the mean values were considered to be significant at $p<0.05$ (Student's t test).

RESULTS

Hemic hypoxia in IDA led to the inhibition of repair processes in endothelial cells: the surface area and the volume of the rough endoplasmic reticulum (RER) decreased 34% with a simulataneous decrease in the ribosome number (Table 1). The sinusoidal lining was thickened and edematous, with occasional exfoliations, the number of fenestrae was decreased, and the space of Disse was widened. Impaired transport via the sinusoidal lining was manifested in a diminished surface are of the inner mitochondrial membrane, in a decrease in the number of free and, in particular, of bound ribosomes, and in depletion of the glycogen re-

TABLE 1. Morphometry of the Ultrastructure of Liver Sinusoidal Endothelial Cells ($M \pm m$)

Ultrastructure	Control	IDA	FL	RPG + FL
Mitochondria				
outer membrane (S_v)	0.6±0.07	0.6±0.07	0.4±0.05*	0.4±0.05*
inner membrane (S_v)	1.2±0.16	1.0±0.13	0.5±0.07**	0.6±0.09**
RER (S_v)	2.3±0.15	1.5±0.13*	1.1±0.08**	1.3±0.07*
(V_v)	10.4±1.30	6.8±0.90*	5.5±0.57*	6.4±0.66*
Golgi apparatus (S_v)	3.0±0.33	2.5±0.28	1.0±0.12**	1.3±0.16**
Ribosomes				
bound (N_A)	23±2	13±2*	7±1**	10±1**
free (N_A)	43±5	29±4*	15±1**	17±2**
Macropynocytotic vesicles (V_v)	4.1±0.86	3.3±0.70	3.4±0.73	2.8±0.67
$\Sigma(S_v)$	7.1	5.6	3.0	3.5

Note. Here and in Table 2: S_v is the surface density of the membranes (μ^2/μ^3); N_A is the numerical density (the number in 10,000 μ^2 of cytoplasm section area); V_v is the volume density (% of cytoplasm volume). One asterisk indicates values significantly different from the control, two asterisks from IDA rabbits, and three asterisks from FL-treated rabbits.

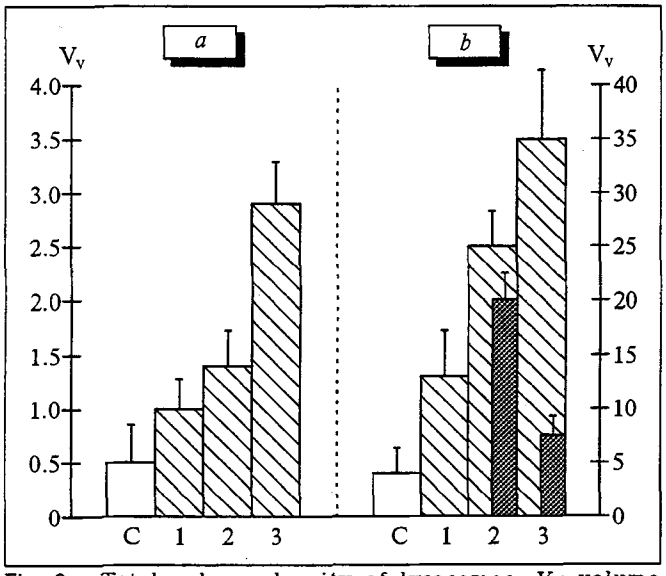

Fig. 2. Total volume density of lysosomes. V_v: volume density (% of cytoplasm volume); *a*) hepatocytes; *b*) sinusoidal endothelial cells; *C*: control; *1*) severe IDA; *2*) IDA treated with FL; *3*) IDA treated with FL + RPG. Dark bars: content (%) of Fe saccharide of entire lysosomal apparatus in the given group.

serves (Table 2). Autophagocytolysis in hepatocytes was activated.

Treatment with FL aggravated the impairments of synthetic processes in both cell types. In the endothelial cells the surface area of the inner mitochondrial membrane, RER, and Golgi apparatus, and the number of free ribosomes decreased even more than in IDA (Table 1). In the endothelial cells lysosomes accumulated FL was seen as electron-dense particles. Cell volume increased markedly (almost 6-fold compared with the control and 2-fold compared with that in IDA, Fig. 2) due to both edema and enlargement of secondary lysosomes (Fig. 1). Endothelial cells contained lysosomes overloaded with FL, the membranes being destroyed in some of them; completely destroyed endothelial cells and free aggregates of Fe saccharide were seen in sinusoids. The surface area of the inner mitochondrial membrane decrease 41% compared with the control, and not only the volume but also the surface are of RER diminished. There were fewer free ribosomes than in the control (Table 2). The volume of secondary lysosomes increased 40% compared with that in IDA (Fig. 2). The increase in the volume of secondary lysosomes in IDA and during treatment with FL was accompanied by an increase in the diffusion of lysosomal hydrolases in the vascular bed [4], which evidently results from hypoxia in IDA and from the prooxidant properties of the Fe ions contained in FL [10].

The treatment of IDA with preliminary administration of the second lysosomotropic drug RPG probably led to competition of these drugs for the plasmalemma, vacuoles, and, presumably, the receptor system. In endothelial cells, secondary lysosomes occupied 40% of the cytoplasm (a 2-fold increase compared with animals treated with FL alone). However, in just FL-treated rabbits lysosomes were 73.7% filled with Fe saccharide, while in RPG-pretreated animals this value was 17.7%, indicating an almost 2,5-fold decrease in the FL content per cell (Figs. 2, 3). Although the volume of endothelial cells increased considerably, sinusoid contained no necrotized endotheliocytes. The parameters of the ultrastructure reflecting the intensity of repair process did not differ appreciably from those in animals treated only with FL, but there was some improvement (Table 1). In hepatocytes of the rabbits treated with both drugs the repair processes were more intensive, as was manifested in an increase in the surface areas of the inner and outer mitochondrial membranes and

TABLE 2. Morphometry of Hepatocyte Ultrastructure ($M \pm m$)

Ultrastructure	Control	IDA	FL	RPG + FL
Mitochondria				
outer membrane (S_v)	0.9±0.04	0.8±0.04	0.8±0.04	1.0±0.04***
inner membrane (S_v)	2.7±0.18	1.9±0.16*	1.6±0.12*	2.6±0.20***
Peroxysomes (V_v)	1.3±0.20	0.9±0.10	1.0±0.13	0.9±0.10
RER (S_v)	1.6±0.16	1.3±0.13	1.1±0.11*	2.0±0.15***
(V_v)	7.3±0.91	3.6±0.40*	3.9±0.48*	7.3±0.56***
Ribosomes				
bound (N_v)	301±36	206±25*	203±33*	234±18
free (N_v)	308±36	230±34	150±18**	331±30***
Autophagosomes, autophagolysosomes (V_v)	0.2±0.07	0.4±0.12	0.5±0.12*	0.4±0.09
Glycogen (V_v)	34±2	25±1*	19±1**	25±2*
$\Sigma(S_v)$	5.2	4.0	3.5	5.6

Note. N_v is the numerical density (the number in 1.0 μ^3 of cytoplasm section area).

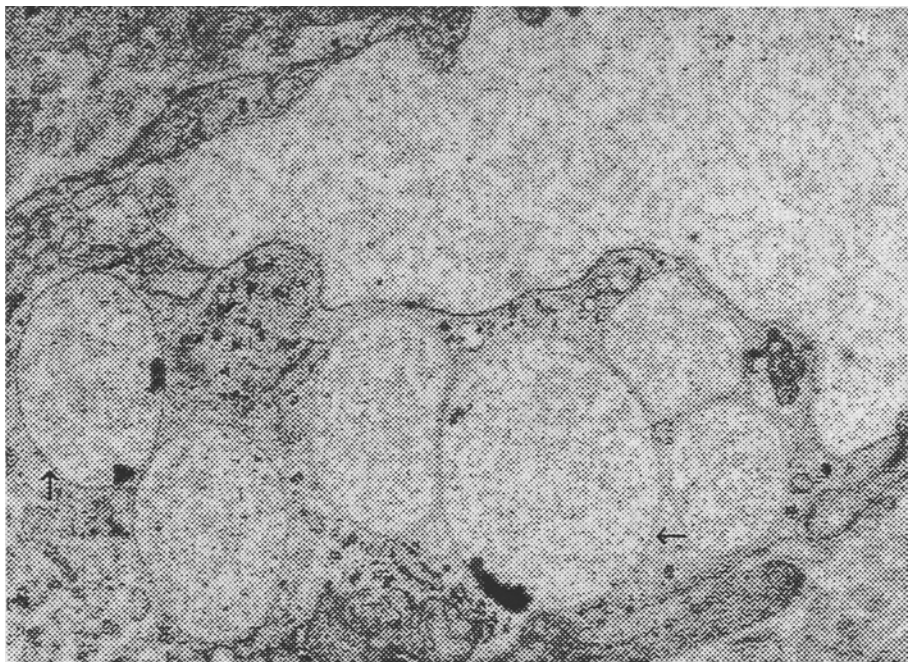

Fig. 3. Ultrastructure of sinusoidal endothelial cells for treatment of severe IDA with FL and RPG, ×21,000. Arrows indicate pinolysosomes containing RPG as low-electron-density flakes of Fe saccharide, which are located at the periphery.

RER, in the RER volume, and in the number of ribosomes. These parameters reached the control values (Table 2). The total volume of lysosomes in hepatocytes increased 2-fold due to the accumulation of RPG (Fig. 2), but visually the lysosomes contained no Fe saccharide. These alterations in endothelial cells and hepatocytes may be associated with a partial blocking of endocytosis, which prevents FL entry in hepatocytes and evidently accounts for the improvement of the repair processes in hepatocytes. On the other hand, as was demonstrated previously [8], RPG can stimulate repair processes in cells. Blockage of endothelial cells by RPG can be regarded as temporary, taking into account that the half-life of RPG is 5-7 days [9], in contrast to polyvinylpyrrolidone, a plasma-expanding preparation that blocks the reticuloendothelial system and system of mononuclear phagocyte for a long time [1]. Our results show a possible way of overcoming the negative consequences of lysosomotropism.

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