Protective Effect of the Lysosomotropic Preparation Rheopolyglucin in the Treatment of Iron Deficiency Anemia with Iron Saccharate

T. A. Ageeva and V. A. Shkurupii

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Levels, phagocytic capacity, and ultrastructure of Kupffer's cells and plastic processes in these cells were studied in Chinchilla rabbits with severe posthemorrhagic iron deficiency anemia (IDA) after their treatment with the iron-containing preparation Ferrum Lek (FL) alone or in combination with the dextran preparation Rheopolyglucin (RPG). In the rabbits treated with FL only, both the phagocytic capacity and number of Kupffer's cells were substantially reduced, whereas in those given FL after being injected with RPG the phagocytic capacity of Kupffer's cells approached that in the intact controls and their numbers exceeded the control level. The level of plastic processes in Kupffer's cells was significantly lowered in the untreated rabbits with IDA and even more so in those treated with FL alone. Preinjecting FL-treated rabbits with RPG led to an intensification of synthetic processes in Kupffer's cells and lowered FL levels in their secondary lysosomes.

Key Words: *Kupffer's cells; lysosomotropic substances; Rheopolyglucin; Ferrum Lek; iron deficiency anemia*

The concept of lysosomotropism formulated by de Duve *et al.* in 1974 [11] has given rise to a number of studies devoted to this phenomenon. Lysosomotropic properties are possessed by iron-containing drugs and dextrans [1]. Previously we reported unfavorable effects of the iron-containing drug FL on liver cells of intact animals following a single intravenous injection [9]. As this drug is used clinically to advantage in patients with severe IDA who receive it repeatedly during a treatment course, it appears appropriate to continue using it as before but to eliminate or minimize its negative effects.

With this aim in view, an attempt was made in this study on rabbits to utilize the low-molecular-weight dextran preparation RPG along with FL in the treatment of IDA. RPG, like FL, accumulates in the vacuolar apparatus of cells but is eliminated from it in 5 to 7 days [10] without adversely affecting lysosomal membranes [2].

MATERIALS AND METHODS

The study was carried out on Chinchilla rabbits in which a severe IDA was produced (hemoglobin levels ⩽ 70 g/liter) by repeated blood-letting (20 ml each time) from an auricular vein during a period of 38-43 days. There were three groups of test rabbits, 5 animals in each. Group 1 comprised untreated rabbits with severe IDA. Group 2 rabbits were injected intravenously with iron saccharate (Ferrum LEK, Yugoslavia) on alternate days for a total of 5 injections per course. The overall dos-

Department of Pathological Anatomy, Medical Institute, Novosibirsk. (Presented by Yu. I. Borodin, Member of the Russian Academy of Medical Sciences)

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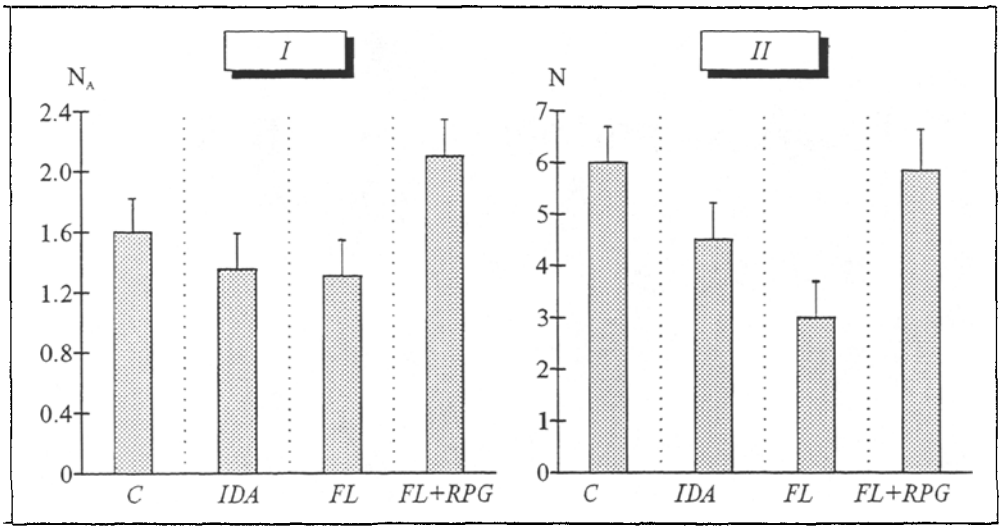

Fig. 1. Numbers and phagocytic activity of Kupffer's cells in treated and untreated rabbits. *I*) number of Kupffer's cells per 10,000 μ² (N_A); *II*) number of latex particles phagocytized by one Kupffer's cell (N); *C* = control; *A* = IDA; *FL* = IDA treated with FL; *RFL* = IDA treated with FL after preinjection with RPG.

age was calculated using the formula recommended by Miterev *et al.* [4] for treatment of patients with IDA. Group 3 rabbits were treated exactly like those of group 2 except that 24 h before the 1st and 3rd FL injections they were injected intravenously with RPG (a dextran of molecular weight 30,000-40,000 D) at a rate of 10 ml/kg body weight. Intact rabbits served as controls.

To label Kupffer's cells, a suspension of latex particles (Dow latex) 1.1 μ in diameter was injected into the rabbits intravenously (0.1 ml/kg) 1.5 h before their sacrifice. Liver specimens for electron microscopy were prepared in the conventional way [6]. Kupffer's cells were photographed in a JEM-100S electron microscope, obtaining 30-40 negatives for each group. Cytoplasmic structures of these cells were subjected to morphometry. FL levels in the vacuolar apparatus were determined morphometrically by counting the number of dots in the test system in steps of 1 cm at a 59 500-fold final magnification of the cytoplasmic negatives; the number of test system dots projected to all structures of the vacuolar apparatus was taken as 100%. The number of phagocytic Kupffer's cells as well as the number of latex particles phagocytized by one Kupffer's cell were counted on toluidine blue-stained semithin Epon sections as described previously [8]. Differences between the mean values being compared were considered significant at $p<0.05$ by Student's t test.

RESULTS

In the untreated rabbits with severe IDA, the phagocytic capacity of Kupffer's cells was reduced, as evidenced by the decreased numbers of these cells and of latex particles phagocytized by one cell (Fig. 1), which was probably a consequence of the prolonged hemic hypoxia. In the rabbits treated with FL alone, the number of phagocytized latex

TABLE 1. Results of Morphometry of Kupffer's Cells

Ultrastructure	Control	IDA	FL	RPG+FL
Ribosomes:				
attached (N_A)	23±3	14±2*	8±1**	13±1***
free (N_A)	46±5	24±3*	14±1**	19±2***
Mitochondria:				
outer membrane (S_V)	0.7±0.10	0.5±0.09	0.3±0.05**	0.5±0.06***
inner membrane (S_V)	2.0±0.27	1.3±0.22*	0.6±0.11**	1.0±0.12***
Rough endoplasmic reticulum:				
(S_V)	2.0±0.19	1.2±0.14*	1.2±0.11*	1.6±0.15***
(V_V)	9.6±1.60	5.9±0.90*	5.7±0.74*	6.5±0.80*
Golgi apparatus:				
(S_V)	2.3±0.30	1.8±0.20	0.7±0.11**	1.3±0.20***
$\Sigma(S_V)$	7.0	4.8	2.8	4.3

Note. The asterisks denote significant differences: one asterisk from the control; two, from the group with IDA; three, from the FL–treated group. S_V = surface density of membranes (μ²/μ³); N_A = numerical density (number per 10,000 μ² of cytoplasmic section area; V_V = volume density (% of cytoplasmic volume).

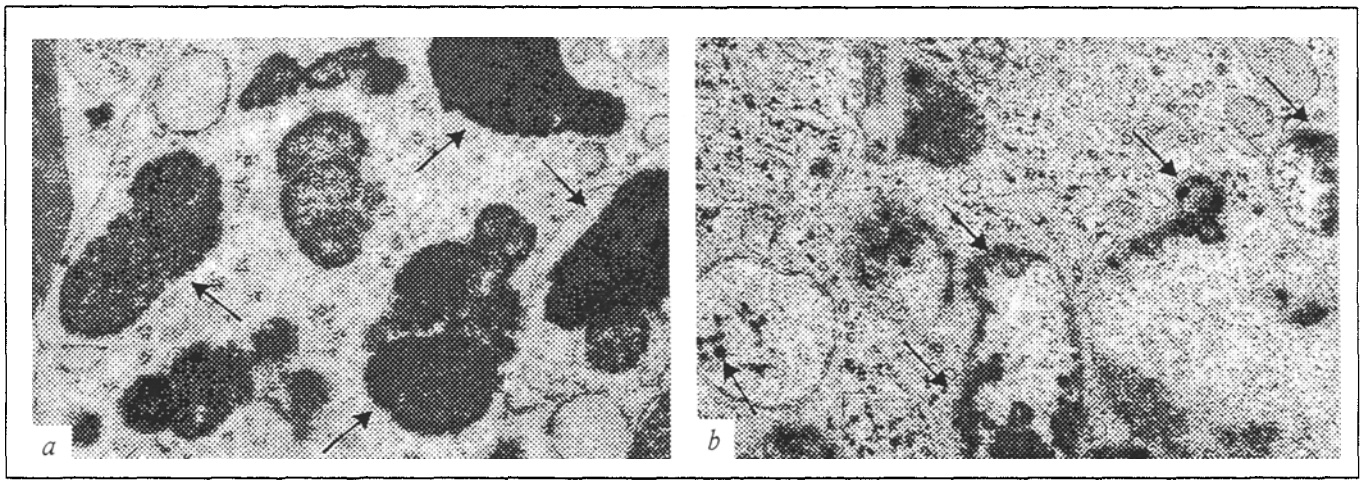

Fig. 2. Ultrastructure of Kupffer's cells from rabbits with severe IDA treated with FL and RPG. *a*) pinolysosomes with electron-dense FL grains (arrows); *b*) pinolysosomes containing preinjected RPC, which appears as flakes of low electron density; electron-dense FL grains are located peripherally (arrows). ×21,000.

particles as well as that of latex-phagocytizing cells were even lower than in the untreated anemic rabbits. In contrast, as shown in Fig. 1, both the phagocytic capacity of Kupffer's cells and their numbers returned to normal in the FL-treated animals that had also received RPG.

The electron microscopic study revealed Kupffer's cells of increased size in all test groups, mainly because of the hypertrophy and hyperplasia of the lysosomal apparatus. Secondary lysosomes predominated.

FL appeared in the lysosomes as minute grains of high electron density that formed large aggregates (Fig. 2, *a*). Kupffer's cells rendered obviously inviable through overloading of their lysosomes with FL were encountered, and in such cells destroyed membrane structures of cytoplasmic organelles were seen. Lysosomes suffered greater damage than did other organelles, so that their hydrolytic enzymes were released into the cell [5]. The observed intracellular lesions were due to the hypoxia which occurs in IDA and to the prooxidant properties of iron ions in the FL [12]. In the group treated with both lysosomotropic drugs, secondary lysosomes contained floccular material of low electron density (RPG) and also, in small numbers and not in all cells, electron-dense FL grains (Fig. 2, *b*).

In the group of untreated anemic rabbits, the number of Kupffer's cells that had phagocytized erythrocytes was much (by 31%) higher than in the control group, which resulted in a 87% increase in the volume of the lysosomal apparatus (Fig. 3). In the FL-treated group, the volume of secondary lysosomes was much higher than in the untreated group with IDA, exceeding fourfold the control level, and a similar increase in the volume of such lysosomes occurred in the group given both FL and RPG. In the latter group, however, only 11.5% of the secondary lysosome volume was filled with iron saccharate as compared to 50.2% in the group treated with FL alone (Fig. 3).

Levels of plastic processes in Kupffer's cells were then evaluated by estimating numerical densities of attached and free ribosomes and the total membrane concentration (i.e., surface densities of membranes in cytoplasmic structures, namely outer and inner mitochondrial membranes, membranes of the rough endoplasmic reticulum, and the Golgi apparatus per μ^2 of cytoplasm). The results are

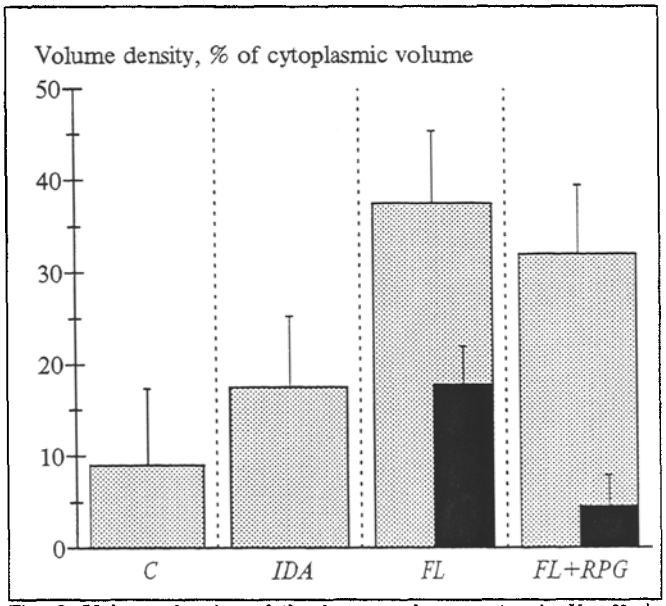

Fig. 3. Volume density of the lysosomal apparatus in Kupffer's cells in treated and untreated rabbits. Black bars indicate the percentage content of the iron-containing drug (FL) in the lysosomal apparatus. *C* = control; *A* = IDA; *FL* = IDA treated with FL; *RFL* = IDA treated with FL after preinjection with RPG.

presented in Table 1. It can be seen that the level of plastic processes in the untreated anemic rabbits was significantly lower than in the control group and that it was still lower in the group treated with FL only. Preinjecting the animals with RPG led to an intensification of synthetic processes in the cell. Thus, the surface density of rough endoplasmic reticulum membranes was close to that in the control group, while the values of all other parameters were similar to those in the untreated anemic rabbits and significantly higher than in the FL-treated group (Table 1). It should be noted that, as shown by latex tests, the phagocytic function of hepatic macrophages was restored in the group that had received both drugs. The tendency toward the restoration of plastic processes would probably be more pronounced if the observation period were prolonged, as is suggested by the results obtained in clinical trials of this combination treatment (FL+RPG) in patients with severe IDA [3].

Presumably, the protective effect of RPG is brought about through its competition with FL for plasmalemma receptors, partial blockage of macrophage endocytic capacity by RPG, and intensification of plastic processes in liver cells [7].

The results of this study provide an example of how the principle of competition between lysosomotropic preparations may be applied taking into account both the negative and positive aspects of lysosomotropism.

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