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Changes in Kupffer Cells after Reversible Ischemia in Rat Liver

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Occlusion of the portal vein results in the deposit of intestinal endotoxin (lipopolysaccharide) in the portal vein system. An increase of the lipopolysaccharide content in the blood may trigger the release of hepatic macrophage products from the cells, which may damage the liver parenchyma.

The aim of the present investigation was to study Kupffer cells (KC) after a 30-min reversible normothermal total ischemia in rat liver.

MATERIALS AND METHODS

For the study, 35 male Wistar rats weighing 200-220 g were used. The rats were kept fasting 12-14 h before the experiment, but water was given ad libitum. The operation was performed under nembutal anesthesia (40 mg/kg). Each experimental series consisted of 5 animals. In the operation the common bile duct was separated from the hepatoduodenal ligament and the latter was compressed for 30 min to produce ischemia of the liver. Rats were decapitated in the 30th min of normothermal ischemia before removal of the clamp and 2, 12, and 24 h and 3 and 7 days after recirculation. Sham-operated animals were the control. The tissue samples were processed for electron microscopy as described previously [2]. For ultrastructural study of KC, photographs were taken under an electron microscope with a power of 3,500. The morphometric indexes of organelles were measured at ×17,000 magnification using an open test grid. The data were processed statistically using an Elektronika DZ-28 computer. The differences between values were significant at $p<0.05$ (Student t test [1]).

RESULTS

After 30 min of ischemia (acute stage) the volume of secondary lysosomes in KC increased by 64% in relation to the control (Table 1). Active adhesion of monocytes to the endothelium was noted, and these monocytes accounted for 43% of the KC population (5% normally) (Fig. 1).

Recirculation during 2 h after 30-min ischemia resulted in a further increase of adhesion of KC bone marrow precursors to the endothelium. In this period the 3-fold increase of the volume of secondary lysosomes was predominantly due to erythrophagosomes (Figs. 2 and 3) and the latter were found in 64% of KC (normally in 2.5%) (Fig. 2). Massive erythrophagia caused atrophic

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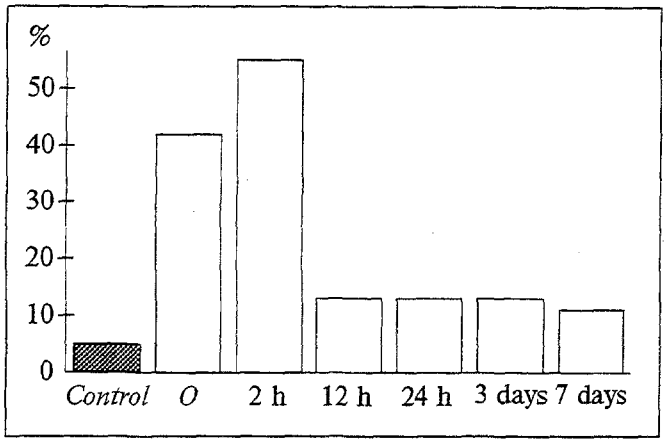

Fig. 1. Percentage of adhesive monocytes in relation to KC in hepatic sinusoids after 30-min ischemia in the liver (0) and at different times of recirculation.

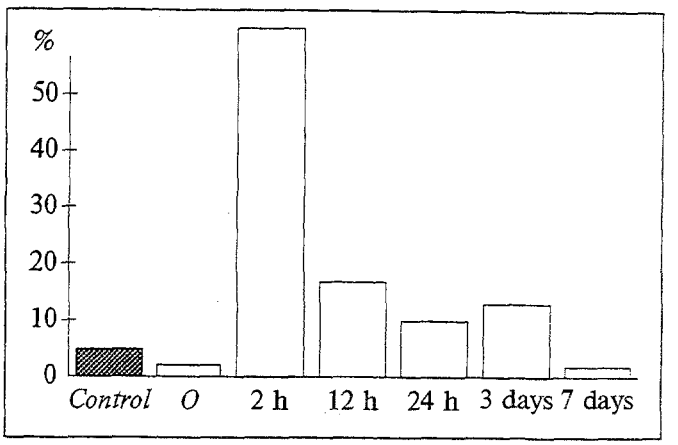

Fig. 2. Percentage of KC with erythrophagosomes after 30-min ischemia in the liver (0) and at different times of recirculation. C: control.

changes in KC such as a decrease of the density of ribosomes both in the granular reticulum and in the polysomes (free ribosomes) (Table 1).

The ongoing recirculation resulted in a fairly stable decrease of the density of ribosome content (Table 1), attesting to a drop of protein biosynthesis in KC. The lysosomal apparatus of KC remained active as long as the test period lasted, namely, the volume of secondary lysosomes was above the control level during 24 h recirculation and the volume of primary KC lysosomes also rose (Table 1). The increase of the relative volume of primary lysosomes may be due to the appearance of young macrophage forms in the KC population. When monocytes undergo differentiation to KC an increase in the volume and number of primary lysosomes typically occurs [9].

The percentage of adhesive monocytes to KC in the hepatic sinusoids is still above normal after 30-min ischemia of the liver (Fig. 1), indicating a marked influx of young forms to the KC population and consequent growth of it. It may be assumed that the increase in the density of sinusoidal cells in ischemia stemmed mainly from the growth of the hepatic macrophage population (Table 1).

Kupffer cells act as a filter between the portal vein and systemic circulation. Systemic endotoxemia of intestinal genesis is thus a sign of damage to KC or a decrease of their phagocytic activity [5,6]

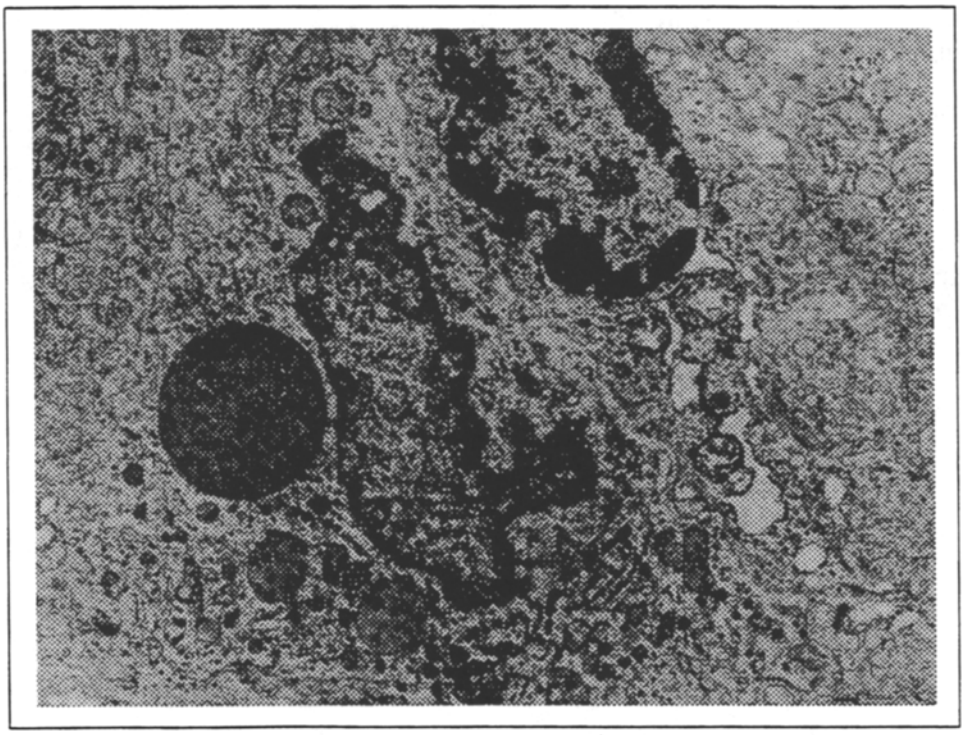

Fig. 3. Erythrophagosomes in KC after circulatory restoration for 2 h after 30-min ishemia of the liver. ×7,700

TABLE 1. Number of Sinusoidal Cells and Changes of Quantitative Indexes of Ribosomes and Lysosomes in KC after 30-min Ischemia in Rat Liver ($M \pm m$)

Parameter	Control	30-min ischemia	Time of recirculation				
			2 h	12 h	24 h	3 days	7 days
Sinusoidal cells	3.30±0.20	4.94±0.27*	3.98±0.17*	4.37±0.27*	3.84±0.19*	4.95±0.36*	3.88±0.27
Primary lysosomes	0.07±0.057	0.19±0.099	0.05±0.044	0.17±0.090	0.66±0.250*	1.41±0.360*	0.62±0.230*
Secondary lysosomes	7.5±0.98	12.3±1.40*	22.3±2.30*	11.7±1.34*	13.2±1.28*	10.4±1.19	9.6±0.95
Free ribosomes	20.1±2.2	15.9±1.6	13.3±1.2*	21.2±1.7	12.5±0.9*	17.5±1.5	13.4±1.0*
Attached ribosomes	3.4±0.5	3.3±0.4	1.7±0.2*	2.2±0.4	1.4±0.2*	1.5±0.2*	1.4±0.2*

Note. Asterisk signifies reliable difference from control.

due to excessive endotoxin. Under our experimental conditions the portal vein accumulated a marked concentration of intestinal endotoxin due to 30-min portal stasis when the hepatoduodenal ligament was clamped. Endotoxin is found in KC, endothelium, and hepatocytes with the maximum labeling in KC [8]. In one study intravenous administration of high doses of endotoxin resulted in a significant increase of phagocytic activity of hepatic phagocytes [7]. Captured material comprised erythrocytes and their parts, platelets, and damaged neutrophils. The authors postulate that endotoxin destroys erythrocytes, and damaged ones are captured subsequently by hepatic macrophages.

Kupffer cells cleared the portal blood of a great number of erythrocytes after the 30-min ischemia (Figs. 2 and 3). The erythrophagocytosis stemmed both form the alterative effect of the endotoxin overdose and from the end of the life cycle for a certain proportion of the erythrocytes during the course of 30-min blood stasis.

Liver damage with endotoxin in a sublethal dose reportedly results in KC proliferation and monocyte transformation to KC [3,4]. We noted the above transformation after a 30-min ischemia of the liver.

Our study demonstrated an inhibition of protein synthesis due to a decrease of the density of free and attached ribosomes in KC. These destructive features resulted from the overdoses of intestinal endotoxin affecting the macrophages and from excessive KC clearance for erythrocytes.

The presence of a large marginal pool of monocytes [10] in the liver (56% of blood monocytes from the bone marrow are converted to KC) promoted the beginning of monocyte differentiation into KC before the clamp was removed from the hepatoduodenal ligament. This phenomenon provided for clearance of a large dose of intestinal endotoxin and of postischemically altered erythrocytes. Before the restoration of circulation in the liver the volume of secondary lysosomes increased in KC. These findings point to an activation of hepatic phagocytes, evidently causing the adhesion of monocytes to the endothelium and their further transformation into KC. Boosted function of the KC population due to increased numbers of these cells may play a key role in protecting the liver from ischemia and recirculatory damage.

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