MORPHOLOGY AND PATHOMORPHOLOGY

Morphological Changes in the Liver during Experimental Modeling of Acute Ischemia and Reperfusion of the Limb

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The development of 4-h ischemia of the limb in dogs was paralleled by disorders in liver microcirculation manifesting in increased vascular permeability, capillary plethora, hemorrhages, and degenerative changes in hepatocytes, which progressed during the reperfusion period.

Key Words: *ischemia; reperfusion; limb; liver*

Ischemia—reperfusion injuries are serious problems of surgery, because of increasing incidence of cardiovascular diseases all over the world, wider practice of interventions requiring temporary circulation arrest in organs, and introduction of transplantation methods into clinical practice [3]. Ischemia-reperfusion injuries develop in syndromes of long-term compression and position compression, tourniquet application on the limbs, bloodflow recovery after embolectomy or thrombectomy, replantation of the limbs [1].

The ischemia—reperfusion syndrome can lead to severe polyorgan failure. The morphological substrate of polyorgan failure in this condition is progressive degenerative changes in vital organs [4].

We studied morphological changes in the liver of animals with ischemia—reperfusion syndrome of the limb.

MATERIALS AND METHODS

The study was carried out on 44 adult male mongrel dogs (12-15 kg). The animals were divided into 3 groups: group 1 animals (n=14) were fixed and narcotized; group 2 comprized animals with 4-h ischemia (n=15), and group 3 included dogs with 4-h ischemia followed by 72-h reperfusion (n=15).

Acute ischemia—reperfusion syndrome was induced by tourniquet application on isolated vascular bundle of the experimental limb proximally from the origin of the deep femoral artery until cessation of peripheral pulsation on the limb distally from the site of tourniquet application verified by palpation [2]. Reperfusion of the experimental limb was started after 4 h (the tourniquet was removed).

Liver biopsy specimens were fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin. Morphological changes were studied on semithin sections (0.5-1.0 μ) stained with hematoxylin and eosin. The histological picture of the organs was evaluated under an Olympus light trinocular microscope.

Morphometric analysis was carried out using an ocular insert grid. The karyocytoplasmic index (KCI) served as the marker of the severity of degenerative processes; the regeneration processes were evaluated by the percent of degenerative hepatocytes (DH) in visual fields; the intensity of morphological substratum of free radical damage was evaluated by percent (per visual field) of elements of leukocytic infiltration of the liver parenchyma (LILP).

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RESULTS

The histological picture of the liver tissues in group 2 animals was characterized by disordered blood circulation, which was confirmed by the appearance of centrolobular hyperemia, sinus capillary plethora, and hemorrhages in the center of the hepatic lobules (Fig. 1).

In some cases, destruction of vascular walls became the cause of increased vascular permeability. However, in 33% cases perivascular inflammatory infiltration was also observed in the presence of apparently intact endothelium. Presumably, some other mechanisms were responsible for increased vascular permeability for infiltration elements in these cases.

The development of degenerative processes in hepatocytes in the first microcirculatory zones of hepatic acinuses was paralleled by the appearance of numerous foci of cholestasis, diffuse edema between the cords with foci of proinflammatory cellular infiltration consisting of lymphocytes, plasmacytes, and neutrophils.

Signs of cord discomplectation were observed after 4 hours of acute ischemia of the limbs (Fig. 2).

Hyperchromatic nuclei, many of them in a state of karyopyknosis, were detected in hydropic hepatocytes. The number of binucleated hepatocytes increased in degeneration foci.

In group 3 the picture of the liver tissues seemed to indicate improvement of blood supply to the liver. Despite less pronounced centrolobular hyperemia, diffuse edema between the cords and hydropic changes in the hepatocyte cytoplasm in the presence of augmenting arteriolar spasm in the portal tracts persisted in the majority of observations. The hepatocytes looked as large vacuoles squeezing the cell nuclei to the periphery. Focal lipofuchsinosis of the cytoplasm in hepatocytes adjacent to the central venules was observed in 40% animals in group 3. Many of these hepatocytes had no nuclei.

TABLE 1. Dynamics of Degenerative Changes in Liver Parenchyma in Dogs Exposed to 4-h Ischemia and 72-h Reperfusion ($M \pm m$)

Parameter	Group		
	1	2	3
Liver KCl	0.92±0.01	0.31±0.01*	0.26±0.01*+
DH, %	3.6±0.1	26.0±0.8*	32.7±0.3*+
LILP, %	0	26.7±0.8*	32.5±0.2*+

Note. $p<0.001$ compared to: *group 1, +group 2.

On the whole, changes in the liver tissues were characterized by cell polymorphism: many dark and clear hepatocytes were seen in the visual fields. The morphological picture of some sites of the parenchyma resembled a "honeycomb" and it seemed that some hepatocytes without nuclei formed accumulations against the background of cells with karyopyknotic changes.

The number and size of necrotic foci in group 3 animals increased from focal cellular after 4-h ischemia to focal segmentary and more massive ones.

Accumulations of acidophilic necrotic mass with blood cells were detected in foci of colliquation necrosis of hepatocytes. Leukocytic infiltration was seen at the periphery (Fig. 3).

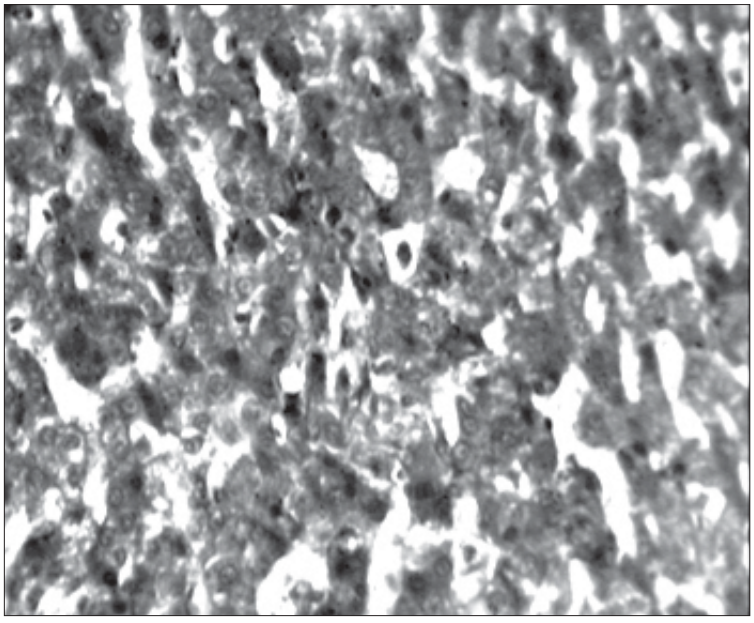

Fig. 1. Centrolobular hemorrhages in animals after 4-h acute ischemia of the limbs. Here and in Figs. 2, 3: hematoxylin and eosin staining, ×40.

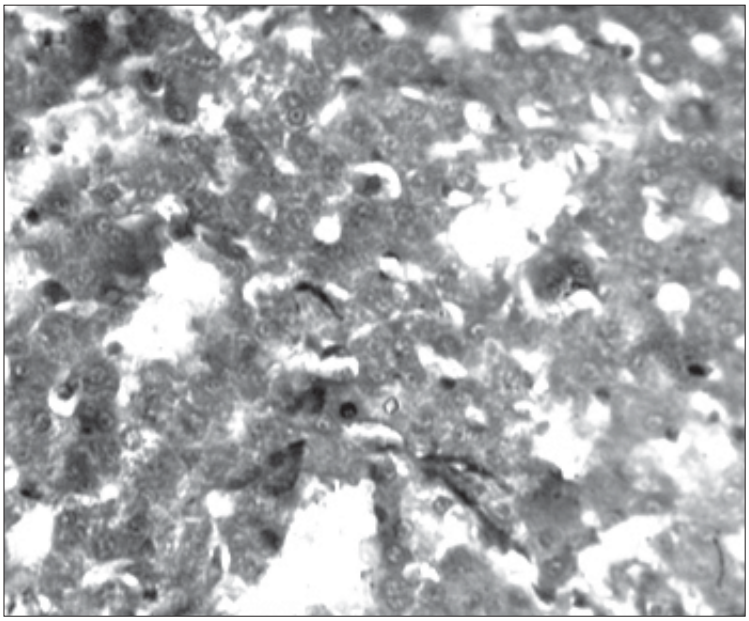

Fig. 2. Discomplectation of hepatocyte cords after 4-h acute ischemia of the limbs (in the center of the picture).

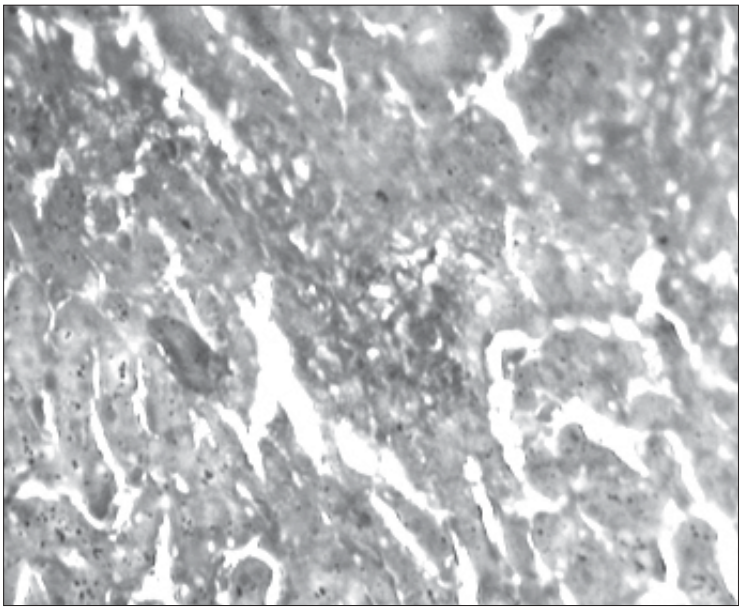

Fig. 3. Accumulation of acidophilic necrotic mass in the center of colliquation necrosis of hepatocytes after 72-h reperfusion.

Rare foci of coagulation necroses with deposition of eosinophilic tissue detritus were seen in foci of colliquation necrosis. The extension of areas of discomplectation of hepatocyte cords increased after 72 hours of reperfusion in comparison with that after 4-h ischemia. The cord structure was retained mainly in the centrolobular zones of hepatic lobules in the presence of hydropic hepatocytes.

Numerous DH were seen in the visual fields after 72 hours of reperfusion. Accumulations of neutrophils and lymphocytes were seen in the sinusoid capillaries. Neutrophilic cells formed chains along the sinusoids.

Infiltration persisted in the stroma and portal tracts. In addition to these, areas of damaged endothelium were seen in the portal vessels with signs of increased vascular permeability for blood cells in 67% cases.

In group 2, KCI of hepatocytes decreased 3-fold, while the number of DH increased compared to the corresponding values in group 1. The appearance of LILP was noted (Table 1).

After 72-h reperfusion, KCI of hepatocytes decreased significantly compared to groups 1 and 2. This was paralleled by an increase in the numbers of DH and LILP elements (Table 1).

Hence, despite visual signs of circulation improvement after 72-h reperfusion of the ischemic limb, the changes developed during 4-h acute ischemia progressed. More pronounced vascular spasm

in the portal tracts and edema between the cords were noted, the number of degenerative hepatocytes and elements of the inflammatory infiltration per visual field increased.

The appearance of signs of focal lipofuchsinosis in the liver tissue after 72-h reperfusion of the ischemic limb and the persistence of hydropic changes in hepatocytes indicate augmenting instability of the lysosomal membranes in hepatic cells. Moreover, disorders in the organ circulation (inflow in the afferent and outflow in the efferent microcirculatory zones of acinuses) augmented in the liver tissue after 72-h reperfusion.

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