Measurement of Dehydrogenase Activity in Hepatocytes in Massive Pulmonary Embolism

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Experimental massive pulmonary embolism in dogs induces structural and metabolic changes in the liver. The severity of these changes depends on the duration of the postembolization period and the development of heart failure.

Key Words: *hepatocyte; dehydrogenases; pulmonary embolism*

Massive pulmonary embolism (MPE) poses a real threat for patient's life due to the development of acute cardiovascular insufficiency. In light of this, changes in the heart and pulmonary vessels are extensively studied [2,7,9]. However, other organs are also altered in MPE, in particular, liver dysfunction plays an important role in the pathogenesis of postembolization syndrome.

The aim of the present study was to evaluate structural and metabolic changes in dog liver in MPE accompanied and not accompanied by heart failure.

MATERIALS AND METHODS

Experiments were carried out on 37 mature mongrel dogs weighing 18-20 kg. The animals were fed standard vivarium chow and received no chow 12 h before study. The experiments were carried on closed chest and under conditions of natural respiration. The animals were narcotized with sodium thiopental (20 mg/kg, intravenously) after promedol premedication (10 mg/kg). Longitudinal fragments of canine sartorial muscle were used for embolization. MPE modeling and catheterization of the heart and vessels were described previously [1]. Heart rate (HR), ECG, respiration rate, and blood pressure in the aorta were recorded.

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The animals were divided into 4 groups: compensated MPE for 1 and 6 h (MPE without heart failure, euthanasia) and uncompensated MPE for 1 and 6 h (death from cardiovascular insufficiency within 1 and 6 h after MPE modeling). In two control groups the animals were immobilized and narcotized after premedication, the heart and vessels were catheterized, and the animals were euthanized after 1 and 6 h by sodium thiopental overdose.

Liver samples were frozen and stored in liquid nitrogen. On parallel cryostat sections, activities of succinate dehydrogenase (SDH), 3-hydroxybutyrate dehydrogenase (BDH), glucose-6-phosphate dehydrogenase (G6PD), lactate dehydrogenase (LDH), and NADH and NADPH diaphorases were histochemically assayed [4]. Enzyme activities were evaluated using a Microvideomat TV image analysis system (Opton). Paraffin sections were stained with hematoxylin and eosin. The data were processed statistically.

RESULTS

Microscopic examination of control liver samples revealed minor plethora of sinusoidal vessels and vacuolar degeneration of some hepatocytes. Metabolic profile of hepatocytes assayed by histoenzymatic methods was characterized by a peculiar relationship between oxidation enzyme activities. Dehydrogenase activities in group 2 dogs (6 h) slightly differed from those in group 1 (1 h), in particular, G6PD activity in group 2

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TABLE 1. Hepatocyte Dehydrogenase Activity in Dogs with MPE (arb. units, *M±m*)

Group	Duration of MPE, h	SDH	BDH	G6PD	LDH	NADH diaphorase	NADPH diaphorase
Control	1	603±25	718±28	368±15	671±28	807±33	831±28
	6	570±28	692±29	392±16	688±28	793±26	803±24
MPE							
compensated	1	407±19	702±28	349±15*	788±34	782±28*	768±26
	6	701±31	697±25*	423±20*	755±29*	756±30*	830±39*
decompensated	1	462±19	681±27	393±18*	696±29*	742±33*	761±26
	6	418±18	614±29*	303±13	707±28*	714±24	739±34*

Note. All values except for those marked with asterisks (*) significantly differ from the control ($p<0.05$).

surpassed that of group 1 by 6.5% ($p>0.05$), which was probably associated with immobilization and heart catheterization.

In the liver of animals with MPE, the degree of structural and metabolic changes depended on the presence of heart failure. Dilatation of portal vessels and sinusoids, erythrocyte aggregation, vacuolar degeneration, and monocellular necroses were found primarily in the perivenular (centrolobular) areas of the liver acini. In dogs with MPE complicated by heart failure, these changes in the liver were accompanied by microcirculatory plethora, dilatation of Disse spaces and perivenular necroses (6 h after MPE modeling). These changes corresponded to those observed in patients died of MPE [3].

More informative data were obtained in quantitative histoenzymatic assay of the liver tissue (Table 1) allowing to evaluate the intensity of metabolic processes in hepatocytes. One hour after MPE modeling activity of all enzymes except for LDH decreased, but to a various degree. For instance, SDH activity (marker of the Krebs cycle) was below the control level by 32.5%. Cell energy was primarily utilized for cell function at the expense of restriction of biosynthetic processes, which was confirmed by increased NAD/NADP ratio (1.07 *vs.* 0.97 in the control). A 17.4% increase in LDH activity indicates activation of glycolytic processes.

In dogs with MPE complicated with heart failure, SDH, BDH, and NADH and NADPH diaphorase activities decreased in comparison with the control, the decrease in NADH diaphorase activity being most pronounced (by 8.1% *vs.* 3.1% in dogs with uncomplicated MPE). Activity of G6PD (marker of pentose phosphate pathway) increased by 12.6% and LDH by 3.7% in comparison with the control.

Six hours after MPE modeling, changes in dehydrogenase activities depended on the presence of heart failure. In dogs with compensated MPE only a 4.7% decrease in NADH diaphorase activity was observed. Activity of other enzymes was above the control level, especially SDH (by 23%), which attests to involvement of the liver into compensatory reactions of the organism. In dogs with heart failure, dehydrogenase and diaphorase activities were longer than in the control and dogs with compensated MPE. Activities of SDH (Krebs cycle), BDH (fatty acid metabolism), and G6PD (pentose phosphate pathway) were maximally suppressed: by 26.7, 11.3, and 22.7% in comparison with the control. LDH activity were increased by 2.8%.

Thus, structural and metabolic changes in hepatocytes 1 h after MPE are typical of nonspecific liver response and represent a shock reaction to MPE. Changes in dehydrogenase activities are most likely related to hypercatecholaminemia arising immediately after MPE modeling [5]. Partial recovery of enzyme activity 6 h after MPE suggests active involvement of the liver into compensatory reactions of the organism. The observed inhibition of oxidative enzymes in dogs with decompensated MPE after 6 hours is probably related to uncoupling of respiration and oxidative phosphorylation in mitochondria and microscopy data suggest disruption of adaptation of metabolic systems. Taking into account the distribution of hepatocyte degeneration and necrotic foci in the acini, we assume that hypoxia (observed in 72% patients with MPE [5]) was the main damaging factor. Similar changes in various metabolic pathways in MPE can reflect the mechanism of hypoxic coordinated metabolic regulation [8].

Thus, the development of MPE is accompanied by morphofunctional changes in the liver. This should be taken into account when planning the pathogenetic treatment of postembolization disturbances.

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