Effects of Preadministration of Phenobarbital and Zyxorin on Repair of Rat Liver Parenchyma after Ischemic Injury

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Optic and electron microscopy and morphometry demonstrate that phenobarbital aggravates postischemic injury of the liver to a greater degree than Zyxorin. Repair regeneration processes proceed in induced liver much more intensively, but are most marked after Zyxorin.

Key Words: *liver; monoxygenase induction; ischemia; repair regeneration*

Stimulation of repair processes in the liver after various pathological states, ischemia included, is a current topic in hepatology. One of the possible approaches to the solution of this problem may be the creation of a functional load on liver cells [4,6,7,11], including that produced by induction of monoxygenase system enzymes, the more so as this system is involved in ischemia [1]. Since repair processes unfold at the subcellular level under such conditions [5,7], a study of their ultrastructural manifestations appears to be of interest.

We studied subcellular changes in hepatocytes occurring in various periods after ischemia and after preinjection (before ischemia) of the monoxygenase enzyme inductors sodium phenobarbital (reference drug), better studied in this respect, and Zyxorin, as the capacity of both these drugs to stimulate plastic processes in hepatocytes in health has already been demonstrated [2].

MATERIALS AND METHODS

Male Wistar rats weighing 200 to 250 g kept on a standard laboratory diet were used. Two-hour ischemia of the left and central lobes of the liver was used as a model of acute ischemia; it was attained by clamping with a microclamp the vascular pedicles of the relevant lobes with preliminary separation of the bile duct [9]. The rats were divided into 3 groups: group 1, "pure" ischemia; group 2, intraperitoneally injected phenobarbital before ischemia (50 mg/kg once a day for 4 days); and group 3, intragastrically administered Zyxorin in a dose of 80 mg/kg in a 20% solution of Tween-80 once a day for two days [3]. The operation (two-hour ischemia) was performed under phenobarbital narcosis (40 mg/kg), in groups 2 and 3 it was performed 24 h after the regular drug administration. The rats were decapitated under light ether anesthesia 2, 24 h, and 3 days after ischemia. Liver samples from 5 intact rats were controls. Liver samples from 5 animals were examined in each period of follow-up. For histological studies they were fixed in a 10% solution of neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin. Slices 5-6 μ thick were stained with hematoxylin and eosin and used to assess the volume of necrosis using a test system of squares at 200-fold magnification. Liver samples for electron microscopy were fixed in 1% OsO_4 in phosphate buffer and embedded in Epon. Slices 1 μ thick were stained with toluidine blue and used for morphometry. Ultrathin sections were examined under a JEM-100S electron microscope. For

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morphometry 20 negative images of hepatocyte cytoplasm sites per animal were used. Stereometry was carried out using multipurpose closed test systems as was previously described [12]. Morphometric studies of hepatocytes outside necrotic zones were performed. Differences between the compared means were considered reliable at $p<0.05$ (Student's t test).

RESULTS

Analysis of the results of morphometry of necrosis zones showed that preinjection of monoxygenase enzyme inductors exacerbated postischemic injury to the liver, but after Zyxorin the increase of necrotic tissue volume was much less pronounced than after phenobarbital (Fig. 1).

The intensity of plastic processes realized at the subcellular level during the formation of repair response was estimated by using the index of total concentration of cytoplasmic organoid membranes in hepatocytes (μ^2/μ^3) [8], as well as the number of free and adhered ribosomes and of free polysomes.

After 2-h recirculation these parameters virtually did not differ from the control in all three groups (Table 1). At the same time, the total area of cytoplasmic organoid membranes per hepatocyte (Fig. 2) was increased in comparison with the control by 49, 134, and 66% in groups 1, 2, and 3, respectively. The increase of this parameter in this period cannot be attributed to the activation of plastic processes. It was probably due to stretching of the organelle membranes owing to edema, which was seen during visual examination of electronograms and was indicated by an increase, vs. the control, of the volumetric density of organoids by 25.4, 39.8, and 37.8% in groups 1, 2, and 3, respectively (Table 1). This led to an increase of the absolute volume of hepatocyte cytoplasm by 46, 121, and 58.4% vs. the control, respectively (Fig. 3).

After 24-h recirculation (period of necrosis formation) the absolute volume of hepatocyte cytoplasm outside necrosis zones did not noticeably differ from that in the control in any of the groups (Fig. 3). The total volumes and concentrations of cytoplasmic organoid membranes in the "pure" ischemia group did not differ from those in the control either. Repair processes in hepatocytes of this group were confined to the production of plastic material "needed" by hepatocytes, this being confirmed by the higher (in comparison with the control) counts of free ribosomes and polysomes by 36.2 and 103.8%, respectively. However the 26% decrease of adhered ribosomes (Table 1) appears to reflect a reduction of hepatocyte synthetic function.

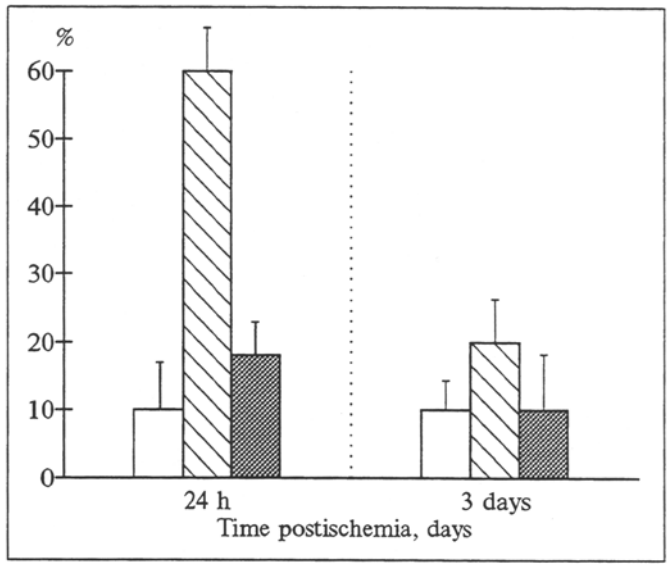

Fig. 1. Results of morphometry of necrosis zones in the liver after 2-h ischemia. Ordinate: Volume density of necroses, % of volume of liver tissue. Here and in Figs. 2 and 3: light bars: groups of rats after "pure" ischemia; cross-hatched bars: phenobarbital administration before ischemia; grey bars: Zyxorin administration before ischemia.

In groups preadministered phenobarbital and Zyxorin hyperplasia of cytoplasmic organoids was observed in this period of follow-up: the total volume and concentration of organelle membranes increased by 29.7 and 22.5% (group 2) and by 27.2

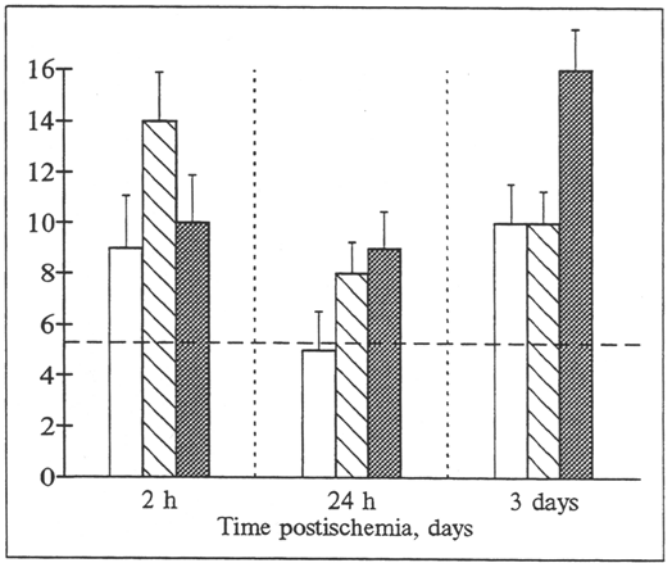

Fig. 2. Results of measurements of total area of cytoplasmic organoid surface per hepatocyte. Ordinate: total area of membrane surface of cytoplasmic organoids: external and internal membranes of mitochondria, granular and agranular endoplasmic reticulum, Golgi apparatus (in $\mu\times10^4$). Here and in Fig. 3 the broken line shows the value of the respective parameter in intact animals.

TABLE 1. Results of Morphometry of Hepatocyte Ultrastructure in Various Periods after Ischemia and after Preinduction with Phenobarbital and Zyxorin (mean±SEM)

Group of rats	Time postischemia	Parameters				
		ΣV_v	ΣS_v	adhered ribosomes (N_A)	free ribosomes (N_A)	polysomes (N_A)
Control	–	51.2±1.5	15.3±0.4	36.6±2.8	26.8±1.9	5.2±0.4
1	2h	64.3±3.2*	15.6±0.2	40.4±2.7	29.9±2.1	4.6±0.4
2		71.6±1.9*	16.2±0.2*	45.8±3.1*	27.4±1.7	5.3±0.4
3		70.4±2.0*	16.1±0.5	36.2±2.5	27.9±1.7	4.9±0.5
1	24h	52.3±1.5	16.2±0.5	27.0±1.9*	36.6±2.6*	10.6±1.4*
2		66.5±2.3*	19.4±0.7*	28.5±2.8*	37.8±3.2*	7.5±0.7*
3		62.8±1.9*	18.7±0.6*	31.5±2.6	25.4±1.7	4.5±0.3
1	3 days	62.7±1.8*	17.6±0.5*	57.0±2.8*	48.5±3.1*	8.3±0.6*
2		63.4±1.8*	19.1±0.6*	53.6±3.5*	40.4±2.5*	6.7±0.5*
3		64.2±2.3*	21.4±0.7*	51.9±3.5*	43.4±3.7*	7.8±0.6*

Note. ΣV_v: total volumetric density of organoids: mitochondria (MC), peroxisomes, primary and secondary lysosomes, autophagosomes, granular (GER) and agranular endoplasmic reticulum (AER), and Golgi apparatus (GA) (in % of cytoplasm volume); ΣS_v: total concentration of organoid membranes, external and internal membranes of MC, GER, AER, and GA (in μ^2 per μ^3 of cytoplasm); N_A: number of structures (per μ^2 of cytoplasm section area); asterisk: $p<0.05$ vs. control.

and 22.2% (group 3) in comparison with the control, respectively (Table 1). The total area of organoid membrane surface per hepatocyte was also markedly increased in these groups, surpassing the same parameter in the "pure" ischemia group by 57.1% after phenobarbital and by 79.5% after Zyxorin (Fig. 2), this possibly indicating a higher functional potential of induced hepatocytes. Assessment of the "quality" of intracellular regeneration in these groups of rats showed that both after phenobarbital induction and after "pure" ischemia synthetic processes prevailed in hepatocytes, aimed at meeting the "requirements" of cells to the detriment of external synthetic functions: an increase

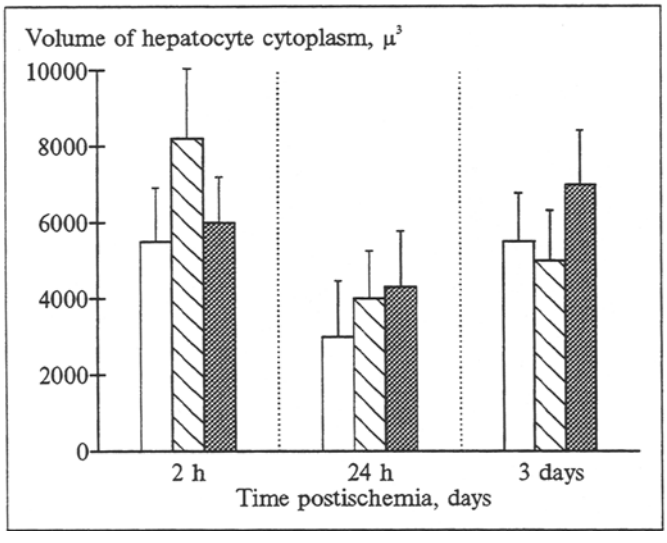

Fig. 3. Results of measurements of hepatocyte cytoplasm volume.

of free ribosomes and polysomes by 40.7 and 46.3%, respectively, and a decrease of adhered ribosomes by 22% vs. the control (Table 1). Such a reorientation of plastic processes appears to reflect a specific pattern of stages in the development of repair processes: first a structural basis is created for increasing the functional "power" of surviving hepatocytes, and then extrasecretory functions are performed at a higher level. After Zyxorin induction the numbers of all types of ribosomes were the same as in the control, but the total volume and concentration of organoid membranes and the latter value per hepatocyte were higher than in the control, this indicating an increased level of plastic processes as a manifestation of repair regeneration.

After 3 days of recirculation (period of necrosis resorption) repair processes in hepatocytes of the test groups were manifested by cellular hypertrophy (Fig. 3) due to ultrastructural hyperplasia (Table 1, Fig. 2). The degree of hepatocyte hypertrophy and the level of plastic processes assessed by the size of organoid membrane surface area calculated per hepatocyte were higher in rats preadministered Zyxorin.

Hence, these data indicate that the processes of intracellular repair regeneration following ischemia after preinduction with monoxygenase system enzymes are characterized by a higher level of plastic processes in hepatocytes, thus providing for their higher functional activity. However only Zyxorin may be regarded as a promising drug for the stimulation of repair processes in the liver, if

we bear in mind the tendency of phenobarbital to lower the liver's resistance to ischemia and its known sedative effect.

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Localization and Fate of Thymic Epitheliocytes of Endodermal Origin Synthesizing Cytokeratin 18

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A reaction in the cytoplasm and processes of some cells of the epithelial reticulum is revealed by indirect immunofluorescence with monoclonal antibodies to cytokeratin 18 in the cortical zone of human thymus. In the medullary zone the reaction is observed in spherical elements similar in shape and size to intestinal goblet cells.

Key Words: *epithelial cells of the thymus; endodermal origin; cytokeratin 18*

Tissue structures characteristic of many different organs are described in fundamental works on the morphology of the thymus which are frequently cited [3,7,8]. The presence in the thymus of spheroid cells resembling in their tinctorial properties young muscle elements and of cells similar to intestinal mucosal epithelium goblet cells, in which mucin has been detected [8], is quite perplexing. By now myoid cells of the thymus have been studied in detail [1,6]. Experiments with polyclonal antibodies have revealed in them the synthesis of contractile proteins of the heart, skeletal muscles, and smooth muscles. Reports on thymic goblet cells as heteroorgan structures are far less numerous [8], although many authorities [2,5,6] are persuaded that heteroorgan elements of the thymus are directly involved in the formation of natural immunological tolerance to the organism's own antigens.

In the present study we examined the localization and fate of goblet cells in human thymus using monoclonal antibodies (MAB) to protein characteristic of the cytoskeleton of epitheliocytes of endodermal origin.

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