SUBCELLULAR MANIFESTATIONS OF THE CORRECTING EFFECT OF α-TOCOPHEROL ON POSTISCHEMIC CHANGES IN THE RAT LIVER PARENCHYMA

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The main cause of cell death from ischemia is membrane damage connected with activation of lipid peroxidation [1]. This defines the ultrastructural level of morphological investigations of an organ in order to study the consequences of ischemia and the use of antioxidants as means of correcting it. It has been shown that α-tocopherol stimulates proliferation of the liver cells after partial hepatectomy [6]. The subcellular aspects of the influence of α-tocopherol on the parenchymatous cells of the liver during repair after ischemia have not been adequately studied.

In the investigation described below the effect of α-tocopherol was studied on structural organization of the hepatocytes during recovery after ischemic liver damage.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar rats weighing 180-200 g. The operation on the liver was performed under intraperitoneal pentobarbital (40 mg/kg body weight) anesthesia. At each stage of the investigation five animals were used. The control consisted of intact animals. In experiments 1 and 2, ischemia of the liver for 2 h was created by clamping the vascular pedicle of the central and left lobes of the liver, after preliminary separation of the bile duct. In experiment 2, α-tocopherol was injected intraperitoneally into the rats 12 h before application of the clamps, in a dose of 100 mg/kg body weight [2]. In both experiments the animals were decapitated 2 and 24 h and 7 days after restoration of the blood flow in the liver.

The left lateral lobe of the rat liver was used for histological investigation. The tissue was fixed in a 10% solution of neutral formalin and embedded in paraffin wax. Paraffin sections were stained with hematoxylin and eosin. The bulk density of the zones of necrosis in the liver sections was determined by means of a closed test system of 25 points after 24 h of recirculation [3]. Samples of liver were prepared for electron-microscopic investigation and subjected to morphometry by the method in [5]. Differences between mean values were considered to be significant at the $P < 0.05$ level by Student's test.

EXPERIMENTAL RESULTS

Ischemia of the liver for 2 h led to death of 24.90 ± 1.76% of the rats' liver tissue. Preliminary injection of α-tocopherol did not reduce the volume of the necrotic changes in the liver (the loci of necrosis accounted for 28.11 ± 2.13%). Consequently, α-tocopherol did not prevent the development of irreversible changes in the liver.

In experiment 1 (ischemia without correction) the numerical density of the mitochondria was increased by 26% after 2 h of recirculation (Table 1). The bulk and surface density of the mitochondria later decreased gradually, while the numerical density of the mitochondria remained unchanged (compared with the control). Consequently, the mitochondria in the hepatocytes were reduced in volume. This was accompanied by a decrease in surface area of the inner mitochondrial membrane. On the 7th day of recirculation the surface density of the mitochondrial cristae gradually increased but did not reach the control level. During the first week of recirculation amorphous electron-dense formations were found in the mitochondria (Fig. 1) and

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TABLE 1. Results of Morphometry of Rat Hepatocyte Mitochondria ($\bar{X} \pm S_{\bar{x}}$)

Parameter	2 h of ischemia				2 h of ischemia + correction by α-tocopherol			
	control	time after removal of clamp			control	time after removal of clamp		
		2h	24 h	7 days		2 h	24 h	7 days
V_V	36,56± 1,19	41,05± 1,14*	28,43± 1,18*	26,40± 1,05*	29,50± 1,0	43,50± 1,60*	35,80± 1,30*	28,40± 1,0
S_V								
outer membrane	1,77± 0,05	1,91± 0,06	1,32± 0,06*	1,51± 0,06*	1,45± 0,05	2,27± 0,08*	1,75± 0,06*	1,48± 0,05
inner membrane	7,80± 0,37	6,75± 0,31*	4,48± 0,29*	5,70± 0,31*	6,90± 0,42	11,73± 0,53*	8,99± 0,35*	7,11± 0,42
N_V	0,340± 0,017	0,430± 0,023*	0,330± 0,028	0,371± 0,025	0,263± 0,015	0,512± 0,029*	0,383± 0,020*	0,360± 0,018*

Legend. V_V) Bulk density of ultrastructures (in % of volume of cytoplasm), S_V) surface density of ultrastructures (in μ^2/μ^3 of cytoplasm); N_V) numerical density of ultrastructures (number per μ^3 of cytoplasm). Asterisk indicates significant difference from control.

TABLE 2. Results of Morphometry of Ultrastructures of Rat Hepatocytes ($\bar{X} \pm S_{\bar{x}}$)

Parameter	2 h of ischemia				2h of ischemia + correction by α-tocopherol			
	control	time after removal of clamp			control	time after removal of clamp		
		2 h	24 h	7 days		2 h	24 h	7 days
Polysomes (N_A)	1,28± 0,22	0,22± 0,07*	0,77± 0,12*	1,50± 0,19	2,09± 0,27	1,58± 0,35	2,51± 0,38	2,41± 0,31
Free ribosomes (N_A)	33,05± 2,55	19,50± 1,10*	19,0± 1,20*	23,80± 1,54*	34,24± 2,79	34,14± 3,88	31,84± 2,45	31,44± 2,32
Adherent ribosomes (N_A)	8,9± 1,0	11,9± 1,0*	13,9± 1,3±	16,9± 1,1*	11,9± 1,2	13,7± 1,3	13,3± 1,5	15,8± 1,5*
Membranes of organoids (ΣS_V)	13,70± 0,43	12,41± 0,36*	8,74± 0,34*	11,38± 0,38*	10,62± 0,45	16,78± 0,57*	12,89± 0,38*	11,51± 0,45

Legend. N_A) Numerical density of ultrastructures (number per μ^2 of cytoplasm); ΣS_V) total surface density of organoid membranes (inner and outer membranes of mitochondria, membranes of rough and smooth endoplasmic reticulum, in μ^2/μ^3 of cytoplasm). Asterisk indicates significant difference from control.

were evidently a denaturation product of mitochondrial protein [7]. The structural changes observed in the mitochondria in the postischemic period are evidence of depression of their functional activity [4]. In experiment 2 (corrosion by α-tocopherol) the numerical density of the hepatocyte mitochondria was virtually doubled after 2 h of recirculation (Table 1), and this was accompanied by an increase (by 70% compared with the control) in the surface density of the cristae, and an increase of 47% in the bulk density of the mitochondria. After 24 h of recirculation the numerical density of the mitochondria was 46% higher than the control level, the bulk and surface densities of the mitochondria were increased by 21%, and the surface area of the cristae was increased by 30%. By the 7th day of restoration of the blood flow the numerical density of the mitochondria was still 37% higher than in the control animals, but the bulk and surface densities had fallen to their initial values.

Preliminary administration of α-tocopherol before ischemia of the liver thus facilitated activation of adaptive structural changes in the mitochondrial compartment, and this was evidently accompanied by intensification of energy formation.

In experiment 1 the numerical density of the free ribosomes and polysomes was reduced relative to the control, but the numerical density of the adherent ribosomes gradually increased (Table 2).

Preliminary administration of α-tocopherol enabled the numerical density of the free ribosomes and polysomes to be kept at the control level throughout the postischemic period. After ischemia the numerical density of adherent ribosomes increased gradually and by the 7th day of recirculation it was 35% higher than in the control (Table 2), indicating an adequate structural basis for synthetic process to meet the intrinsic needs of the cells and the intensification of their external secretory functions. This is confirmed by the increase in total concentration of membranes of the organoids studied (the sum of the surface densities of the membranes of the mitochondria and rough and smooth endoplasmic reticulum) by 58% after recirculation for 2 h and by 21% after recirculation for 24 h. By the 7th day of recirculation this parameter was equal to the control value (Table 2).

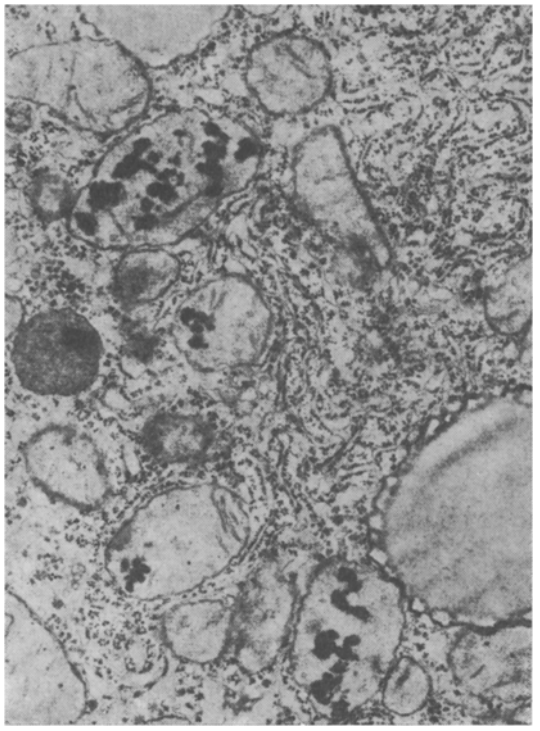

Fig. 1

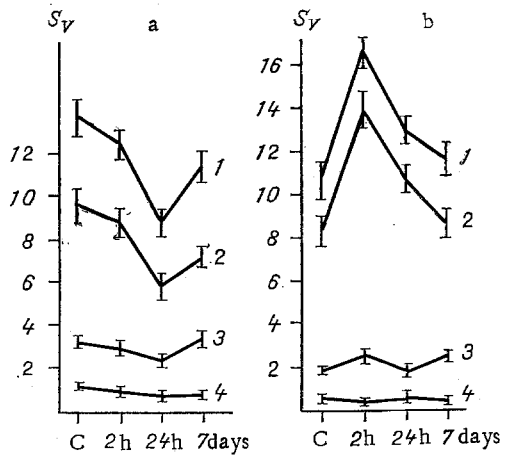

Fig. 2

Fig. 1. Accumulation of electron-dense material in hepatocyte mitochondria after 24 h of recirculation, following 2 h of ischemia of the rat liver. 17,500×.

Fig. 2. Total surface density of membranes of hepatocyte organoids (1) and its components: surface density of mitochondrial membranes (2) and of membranes of rough (3) and smooth (4) endoplasmic reticulum. Abscissa: C) control, 2 and 24 h, 7 days) duration of recirculation after ischemia; ordinate: surface density of membranes (S_V, μ^2/μ^3); a) experiment 1, b) experiment 2.

The total concentration of organoid membranes (in μ^2/μ^3 of cytoplasm) reflects the level of intracellular plastic processes. In experiment 1 this level was lower by 10, 36, and 17% than the control after 2 and 24 h and 7 days of recirculation, respectively (Table 2). Before the 7th day of restoration of the blood flow, processes of destruction of the subcellular structures predominated in the liver, and it was only after 1 week of recirculation that intracellular reparative regeneration processes began to be activated, against the background of a general hypoplastic picture of the structural organization of the hepatocytes during the postischemic period studied.

The greatest contribution to the total concentration of hepatocyte organoid membranes was made by the surface density of the mitochondrial membranes (Fig. 2). Reduction of the total concentration of organoid membranes during the first 24 h of recirculation in experiment 1 was due to destruction of the mitochondrial membranes (Fig. 2a). This is evidence that the hepatocyte mitochondria are most sensitive to ischemia. The "point of application" of the corrective action of α-tocopherol is evidently the mitochondria. This is shown by the sharp increase (compared with the control and results obtained in experiment 1) in the surface area of the mitochondrial membranes in experiment 2, while the surface area of the membranes of the endoplasmic reticulum was virtually unchanged (Fig. 2b).

Preliminary (before ischemia of the liver) administration of α-tocopherol was thus not linked with a decrease in the volume of the necrotic foci in the liver, but it stimulated hyperplasia of the subcellular structures of the hepatocytes in the recovery period, thus providing a basis for an increase in functional "power" of the undamaged cells and compensation of the functions of the lost fraction of the parenchymatous cells.

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CHANGES IN HEPATOCYTE STRUCTURE IN OLD RATS WITH POSTHEMORRHAGIC ANEMIA

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Blood loss is accompanied by the development of processes aimed at replacing the lost plasma proteins. During aging the altered mechanisms of intracellular protein synthesis and its inadequate neurohumoral control [11] do not permit the initial plasma protein level to be fully restored [7]. Discovery of the factors constraining this process is therefore essential in the search for ways of correcting anemia and their particular features in old age.

The aim of this investigation was to discover age differences in ultrastructural changes effecting hepatocytes under conditions of posthemorrhagic anemia in order to determine the morphological substrate of the deficient protein-synthesizing function of the liver in old animals.

EXPERIMENTAL METHOD

Male laboratory albino rats of two age groups were used: 35 adult (aged 8 months) and 35 old (25 months) rats. The rats did not eat but had free access to water for 12 h before the experiment. Intact rats (group 1) served as the control. The animals were bled to the extent of 2% of body weight from the caudal artery once, and the liver was investigated after 15 min, 2.5, 5, 10, and 24 h, and 7 days later (animals of groups 2, 3, 4, 5, 6, and 7, respectively). Each group contained five adult and five old animals. Pieces of liver for light microscopy were fixed in acetic-alcohol-formalin [4]. Paraffin sections 7 μ thick were stained with hematoxylin and eosin; the number of binuclear hepatocytes per 1000 mononuclear cells was counted in them; the dimensions of the cytoplasm and nuclei were determined on a Leitz ASM instrument. Fixation with 3% glutaraldehyde in phosphate buffer (pH 7.4) and postfixation with 1% osmic acid solution were used for electron microscopy; the material was dehydrated and embedded in Epon-812. Sections cut on the LKB-111 ultrotome were stained by Reynolds' method [15] and examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

A distinguishing feature of the liver in old animals on light-optical microscopy (LOM) was the accentuation of the structure of the lobules due to the abundance of connective tissue, the larger number of binuclear cells (Fig. 1), and the increased average area of the hepatocytes (Fig. 2). On electron microscopy (EM) age differences were manifested as a decrease in the number of glycogen rosettes in the cytoplasm, clarification of its matrix, polymorphism of the mitochondria, an increase in the number of secondary lysosomes with lipofuchsin granules, and the appearance of postlysosomes, including myelin-like structures, in the old animals.

Under experimental conditions during LOM of the liver at different times after blood loss, changes such as anemia, disturbance of the complex structure of the hepatic trabeculae, and dystrophic changes in the hepatocytes, described previously [2 5], were discovered. Mean-

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