QUANTITATIVE INVESTIGATION OF THE EFFECT OF ACUTE CARBON TETRACHLORIDE POISONING ON ULTRASTRUCTURAL ORGANIZATION OF HEPATOCYTES OF THE MOUSE LIVER

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The severity of changes in the endoplasmic reticulum (ER) and mitochondria of hepatocytes in the various parts of the liver lobule in acute CCl_4 poisoning depends on the degree of metabolic activation by its microsomal enzymes in each hepatocyte, and this in turn is determined by the structural and functional characteristics of these hepatocytes in the liver of intact animals. Changes in the mitochondrial population in each hepatocyte are largely responsible for the degree of damage to the ER. The fact that the hepatocytes vary in their vulnerability to CCl_4 suggests that the functions of some of the damaged hepatocytes can be restored by intracellular reparative regeneration.

Investigations have shown the heterogeneity of the hepatocytes in the liver of intact animals with respect to their ultrastructural organization and functional properties [10, 3, 15]. It is also known that the hepatotoxicity of CCl_4 is determined by the ability of the microsomal enzymes to metabolize it to free radicals and it is increased if an increase in the content of ER in the hepatocytes is first induced [12-14, 17]. In this connection it is assumed that hepatocytes with different ER contents will suffer different degrees of damage by CCl_4.

The object of the present investigation was to make a quantitative study of ER and the mitochondria in the hepatocytes of intact animals and in the early stages after CCl_4 poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on 12 male CBA mice aged 1 month. The animals were divided into two groups. Material taken from the mice of group 1 (six animals) was used to study the hepatocytes of the intact liver. The mice of group 2 were poisoned by inhalation of a single dose of CCl_4 (corresponding to 0.1 ml/100 g body weight). The material was embedded Araldite and fixed in 1% OsO_4 solution by the usual method. Sections were studied in the JEM-7A electron microscope. The technique of the morphometric investigation was that developed at the Institute of Biophysics, Academy of Sciences of the USSR [5]. A series of dimensionless indices of the ultrastructures, the total areas of the rough endoplasmic reticulum (RER), and of the mitochondria, their mean dimensions, and their number per conventional unit area of cytoplasm were studied. All indices were expressed as percentages of the area of the cytoplasm, taken as 100. Material for morphometric investigation from the animals of group 1 was photographed by the random cell selection principle, and in addition the so-called dark hepatocytes were studied separately. The liver hepatocytes of the group 2 mice were studied with allowance for their topographical position in the hepatic lobules. The numerical results were subjected to statistical analysis. The results of the quantitative investigation of the liver hepatocytes of the two groups of mice are given in Table 1.

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TABLE 1. Results of Morphometric Investigation of Liver Hepatocytes of Intact Mice and of Mice 6 h after CCl_4 Poisoning (M ± m)

Group of animals	Class of cells	ΣSR_{gr}	ΣSM	S_mM	KM
1 (control)	A	30,2±0,41	25,3±1,84	0,40±0,05	2,50±0,21
	B	25,9±0,56	21,7±0,76	0,38±0,02	2,85±0,13
	C	21,3±0,25	23,5±1,12	0,51±0,01	2,38±0,24
	D	16,2±0,33	22,8±0,86	0,41±0,01	2,38±0,27
	E	10,9±0,50	24,5±0,51	0,57±0,02	2,05±0,13
	Average hepatocytes	20,4±0,55	23,3±0,11	0,44±0,03	2,47±0,14
	Dark hepatocytes				
	Control	28,3±0,84	21,4±1,22	0,30±0,06	3,22±0,03
	Expt.	22,4±0,72	23,1±1,62	0,31±0,04	3,70±0,04
2	A_0	12,9±0,97	17,4±0,65	0,20±0,04	5,17±0,32
	B_0	58,5±3,41	11,5±0,74	0,14±0,01	7,44±0,75
	C_0	35,7±2,04	16,3±0,95	0,25±0,02	3,93±0,19
	D_0	15,6±0,96	21,8±1,42	0,29±0,03	3,40±0,27
	E_0	17,0±1,02	22,2±0,30	0,44±0,03	2,54±0,05

Legend: ΣSR_{gr}) total area of granular endoplasmic reticulum; ΣSM) total area of mitochondria; S_mM) mean size of mitochondria; KM) number of mitochondria per unit area of cytoplasm.

EXPERIMENTAL RESULTS

Morphometric investigation of the liver of the mice of group 1 demonstrated the heterogeneity of the hepatocytes as regards their RER content. In accordance with this feature the cells studied were subdivided into five classes. The RER was represented in the hepatocytes of each class mainly by narrow tubules with numerous attached ribosomes. Most frequently it consisted of groups of tubules arranged parallel to each other, some of them lying next to the mitochondria and repeating their configuration. The cytoplasm contained many free ribosomes. The smooth endoplasmic reticulum (SER) was poorly developed in all the hepatocytes. The mitochondria in the hepatocytes of each class had numerous cristae and they differed widely in their shape and size. The dark hepatocytes had a denser ground substance of their cytoplasm, the RER was well developed, and it contained numerous attached and free ribosomes. Qualitatively the RER and mitochondria in these hepatocytes were indistinguishable from those in the hepatocytes described above. The results point to different levels of functional activity of the hepatocytes studied. The definite constancy of the total area of the mitochondria despite the considerable polymorphism, couples with differences in the RER content, may perhaps be connected with their considerable potential capacity and with their marked functional heterogeneity.

The study of the liver from the mice of group 2 revealed changes in the hepatocytes mainly in the central zones of the hepatic lobules and, to a lesser degree, in the intermediate zones. Differences in the degree of expression of the changes in RER in the different hepatocytes were characteristic. The changes consisted of degranulation, fragmentation, and vesiculation of its tubules. Corresponding to the degree of fragmentation and dilatation of the tubules of the RER and the increase in the area occupied by them, the number of attached and free ribosomes was reduced and the density of the basic matrix of the cytoplasm was increased.* Hepatocytes of classes A_0, B_0, and C_0 with the greatest changes in RER had an extremely transparent basic matrix, while in hepatocytes of classes B_0 and C_0 the cytoplasm was honeycombed in appearance (Fig. 1a, b) as the result of the considerable vesiculation of the RER. In the class A_0 hepatocytes located directly by the central vein the RER were fragmented and concentrated mainly around the mitochondria. Its components located near the mitochondria were less severely damaged and retained some of their attached ribosomes. A large quantity of SER was present only in these hepatocytes, in the form of concentrations of its membranes (Fig. 1c). The RER of some hepatocytes in the intermediate zones of the liver lobules (class D_0) showed changes in the form of slight vesiculation of its components. The organization of the RER in the hepatocytes located mainly at the periphery of the lobule was indistinguishable from that in the hepatocytes of the control animals. In the zones where cells of classes B_0, C_0, and D_0 were found there were always a few hepatocytes in which the organization of the RER and mitochondria was virtually indistinguishable from that of the hepatocytes in the control (Figs. 1 and 2). The changes in the mitochondria in the hep-

* The hepatocytes were classified on the basis of these features, the change in the population of mitochondria, and their topographical position in the lobule.

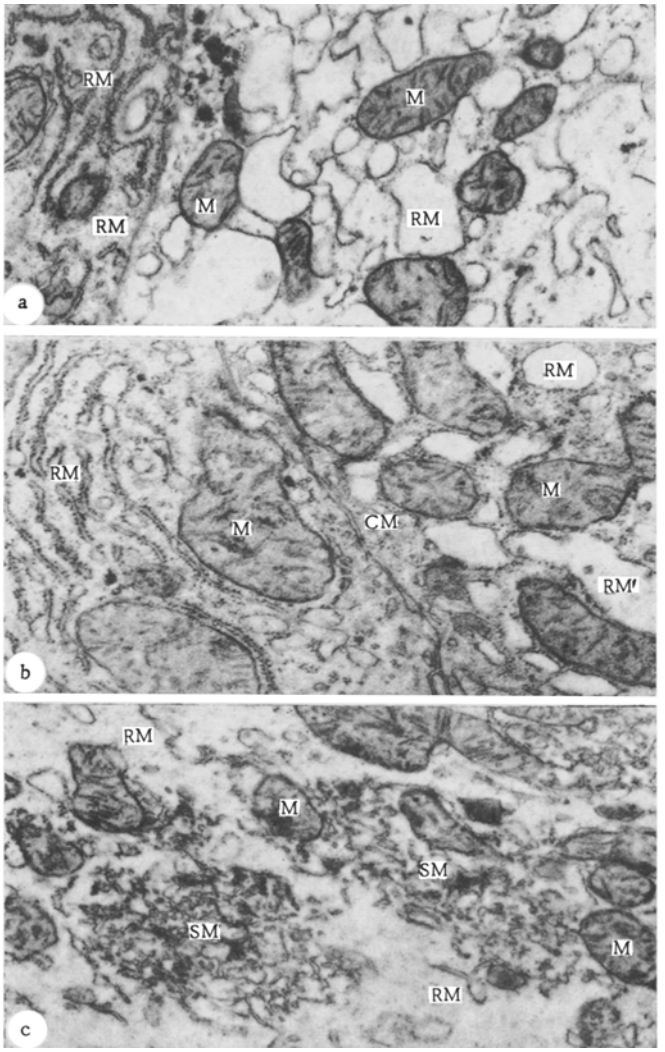

Fig. 1. Types of changes in organization of endoplasmic reticulum and mitochondria in hepatocytes of mice 6 h after CCl_4 poisoning, 30,000 ×: a) on right, part of cytoplasm of class B_0 hepatocyte, b) on right, part of cytoplasm of class C_0 hepatocyte. On left, part of cytoplasm of hepatocyte with unchanged cytoplasmic organoids, c) part of cytoplasm of class A_0 hepatocyte. M) mitochondria; RM) rough membranes of endoplasmic reticulum; SM) smooth membranes of endoplasmic reticulum; CM) cell membrane.

atocytes of classes A_0, B_0, C_0, and D_0 were similar in type. They consisted of uniformity of shape and a decrease in size by comparison with the control, condensation of the matrix, shortening of the cristae, and a decrease in their number. They differed in the different classes of hepatocytes only in the degree of expression of these features. Dark hepatocytes were found frequently in zones with considerable changes in the surrounding cells, but their cytoplasmic components had no visible qualitative changes.

The organization of the hepatocytes of the experimental animals points to differences in their vulnerability to the action of CCl_4. This fact is probably connected with differences in the content of RER in the hepatocytes of the intact animals. Investigations have shown that the hepatotoxicity of CCl_4 depends on its metabolic activation by microsomal enzymes of the hepatocytes and the effect is intensified after preliminary induction of an increase in the ER content in the hepatocytes [14, 19, 16]. Consequently, it might be expected that in some hepatocytes with the highest content of RER the damage would be more severe. How-

ever, the structures of the cytoplasmic organoids were unchanged in the dark hepatocytes with the greatest degree of hyperplasia of the RER. Only the quantitative content of RER was changed, being closer to its mean value in some of the hepatocytes of the control mice. This fact probably indicates that dark hepatocytes are resistant to the action of CCl_4. It may be linked with the functional characteristics of these particular hepatocytes. It has been shown that in hibernating sousliks most of the parenchymatous cells are dark hepatocytes, the number of which falls sharply when the animals awaken. The decrease in the RER content may be due to transformation of the dark hepatocytes into light, as several investigations have shown [2]. Carbon tetrachloride is known to cause an increase in the blood corticosteroid level [6]. Presumably, the decrease in the RER content in the dark hepatocytes is connected with an increase in the blood corticosteroid concentration.

Investigations have shown an increase in the toxicity of CCl_4 is accompanied by exposure to stress factors or administration of certain steroid hormones, as well as sex and age differences in the reaction to its administration as reflected in liver damage [8, 16, 12, 17]. The velocity of biochemical reactions has been shown to be determined by the activity of enzymes which can be controlled by various hormones [5].

It can be postulated on the basis of the facts described above that the toxicity of CCl_4 for different hepatocytes is determined not only by the presence of enzymes in these cells capable of metabolizing CCl_4, but also largely by their activity determined by hormonal induction and by the ability of the cell to respond adequately to it [5]. The degree of damage to the liver as a whole will be limited by the number of cells capable of metabolizing CCl_4. A special example of metabolic activation of CCl_4 in the liver is characteristic of many pharmacological agents, the action of which depends on their transformation in the liver [9, 18] and it may be determined by similar mechanisms. The functions of ER and the mitochondria are known to be closely connected [1, 7, 11]. The severity of the changes in the mitochondria in the hepatocytes of each class may probably be determined by the degree of disintegration of intracellular metabolism and, above all, by damage to ER, as the results in Table 1 confirm. The decrease in size of the mitochondria is perhaps connected with the decrease of their functional activity. The increase in the number of mitochondria may be a reflection of a compensatory reaction aimed at increasing their contact with ER, a matter of great importance for the normal function of the mitochondria. Not all the changes observed in the different hepatocytes must be regarded as potentially irreversible. In some cells with less severe changes restoration of the structure and function of their organoids can be expected. Investigations have shown that restoration of cell function can take place through intracellular reparative regeneration [4]. The differences in the degree of vulnerability of the hepatocyte to CCl_4 in this case may provide a basis for such a phenomenon.

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