COMPARATIVE MORPHOMETRIC STUDY OF HEPATOCYTE ULTRASTRUCTURE IN VARIOUS LINES OF MICE ACUTELY POISONED WITH CCl_4

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A comparative morphometric investigation of the ultrastructural organization of hepatocytes from intact CBA and C57BL mice revealed significant differences between them as regards several parameters of the cytoplasmic components. Injection of CCl_4 into mice of the different lines in identical doses and conditions led to more severe damage to the liver in the C57BL and in the CBA mice. This is probably because of genetically determined differences in detoxication enzyme systems in the mice of the two lines. Activity of intracellular reparative regeneration in the residual hepatocytes depends directly on the degree of liver damage. However, because of differences in the degree of liver damage by CCl_4 these data cannot be used to compare the ability of the hepatocytes of these mice to carry out intracellular reparative regeneration and regeneration of the liver as a whole, as they can in experiments with measured mechanical injury.

Different lines of inbred mice differ in several biological characteristics and also in their response to experimental and, in particular, to therapeutic procedures [3, 12-14, 16]. The pharmacodynamics of most substances, including CCl_4, is also known to be determined by the degree to which they are metabolized in the liver [1, 7, 17, 20].

The object of this investigation was to use a morphometric method to study the reaction of the hepatocytes at the ultrastructural level to acute CCl_4 poisoning in mice of line CBA and C57BL, which differ in several biological parameters.

EXPERIMENTAL METHOD

Experiments were carried out on 26 male mice aged 30 days and kept under identical conditions. Six mice of each line were used as the control, and the other animals of both lines were poisoned simultaneously with CCl_4 (by inhalation in a single dose of 0.1 ml/100 g body weight). Samples were taken from the liver 24 h later for electron-microscopic investigation and fixed in 1% OsO_4 solution. This material was subsequently treated in the usual way. Parallel investigations of the material were made in the light microscope. The technique of morphometry was as follows. The picture obtained of the cell sections were projected from the photographic negatives magnified on paper and the outlines of the components were traced with colored inks. On the outline plans thus obtained [8], the areas of the components to be studied were measured. The necessary measurements were made by means of a linear integrator (LI-5) working on the principle of measurement of the perimeters and areas by the method of random sections [5].* The dimen-

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TABLE 1. Indices of Ultrastructural Organization of Hepatocytes of Two Lines of Mice, Intact and 24 h After CCl_4 Poisoning (M ± m)

Line of mice	Index of cell class	ΣSR_{gr}	ΣSM	S_mM	KM	ΣSGL	ΣSLp	ΣSGo	ΣSL	K_n
1. CBA	1A	39,8±1,64	17,4±1,90	0,20±0,02	5,88±0,76		5,50±2,60	5,87±0,17	0,65±0,03	69,4±3,90
	2A	24,3±1,95	20,1±1,05	0,33±0,04	4,50±0,70	10,3±2,20	2,00±0,50	2,68±0,62	0,25±0,03	59,7±0,80
	3A	17,5±1,62	20,0±0,84	0,33±0,03	3,47±0,38	3,87±0,90	1,02±0,30	3,37±0,35	0,31±0,03	46,0±1,12
	4A	31,1±0,65	23,6±3,10	0,31±0,01	3,69±0,21	7,89±0,83	0,77±0,22	1,77±0,36	0,31±0,01	66,5±0,77
	5A	20,7±1,06	21,2±0,37	0,30±0,02	4,46±0,43	11,7±0,45	1,11±0,23	1,73±0,09	0,28±0,02	55,6±2,54
	Dark hepatocyte:									
	C	28,3±0,84	21,4±1,22	0,30±0,06	3,22±0,03	9,12±0,90	4,05±0,45	2,30±0,37	0,30±0,09	68,0±2,42
	E	34,5±2,30	22,3±0,72	0,23±0,03	3,36±0,32	4,45±1,90	0,70±0,22	1,02±0,45	0,16±0,06	62,4±1,28
	Average hepatocyte:									
	C	20,4±0,55	23,3±0,11	0,44±0,03	2,47±0,14	12,7±0,88	3,40±0,26	1,70±0,16	0,29±0,02	61,6±0,93
	Average hepatocyte:									
	C	15,4±0,52	17,6±0,86	0,30±0,04	3,73±0,22	34,1±0,57	1,70±0,18	1,52±0,21	0,22±0,02	71,5±0,77
2. C57BL	1B	30,1±2,40	23,6±0,90	0,20±0,04	5,20±1,10		2,20±0,49	2,15±0,06	0,62±0,19	64,5±2,80
	2B	13,9±2,26	17,7±2,50	0,26±0,03	5,00±0,08	11,0±3,00	0,90±0,40	1,40±0,26	0,53±0,04	47,0±3,40
	3B	32,3±0,56	15,5±0,44	0,14±0,01	7,77±0,89	5,32±0,44	4,42±0,45	3,27±0,22	0,92±0,12	61,4±0,98
	4B	27,0±2,00	23,8±1,29	0,33±0,01	3,17±0,26	7,45±1,35	2,00±0,50	1,75±0,40	0,96±0,11	64,0±1,07
	P_{1-2}	<0,001	<0,001	<0,01	<0,001	<0,001	<0,001	>0,05	>0,05	<0,001

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 conventional area of cytoplasm; ΣSGL) total area of glycogen; ΣSLp) total area occupied by lipids; ΣSGo) total area of Golgi apparatus; ΣSL) total area of lysosomes; K_n) coefficient of saturation of cytoplasm with its components; C) control, E) experiment.

sionless indices of the ultrastructures were calculated as follows: total areas of the granular endoplasmic reticulum (GER), of lipids, glycogen, lysosomes, and mitochondria, their mean size and number per unit conventional area of the cytoplasm, and the coefficient of saturation of the cytoplasm with its components (K_n). All indices were expressed as percentages of the area of cytoplasm.*

EXPERIMENTAL RESULTS

The results of the investigation of the liver of the control animals agreed basically with detailed morphological descriptions of the ultrastructural organization of the cytoplasmic components of mouse hepatocytes given by other workers [4, 9, 19]. The morphometric investigation enabled the organization of an "average hepatocyte" to be described quantitatively for the control mice of each line and differences to be revealed between them with respect to several indices of the cytoplasmic components (Table 1). These data reflect structural and functional differences between the hepatocytes of the two lines of mice and they are probably determined genetically. The higher content of GER in the hepatocytes of the CBA than in the C57BL mice could be evidence of a higher level of protein synthesis in the mice of that line. This conclusion is confirmed by determination of the serum protein concentrations in these mice [18]. Differences in protein metabolism probably determine differences in carbohydrate and lipid metabolism, as reflected in different concentrations of glycogen and lipids and also in variations in the characteristics of the mitochondria in the hepatocytes of these animals.

Areas of centrilobular necrosis were present 24 h after CCl_4 poisoning in the hepatocytes of both lines of mice, as described by other workers [10, 20]. However, the degree of spread of the necrosis was much more marked in the C57BL mice. Investigation of the hepatocytes of the two lines of mice revealed differences in their structure, related to their topographic position in the lobe of the liver. In accordance with these features the hepatocytes belonging to the mice of each line were grouped into classes. Their organization could then be described quantitatively and a comparative analysis made of their structural and functional state. Class 1A hepatocytes were found chiefly near zones of necrosis and they were few in number. Changes in their ultrastructures consisted of clarification of the basic matrix of the cytoplasm, a decrease in the number of free ribosomes, and considerable vesiculation, fragmentation, and degranulation of elements of the GER, increasing the area which they occupied by comparison with the hepatocytes of intact mice. They contained virtually no glycogen, they had an increased content of lipids, and their mitochondria were small, with a condensed matrix and with few cristae. The number of mitochondria was increased by comparison with the hepatocytes of the other classes and in the control. The Golgi apparatus was composed of structures distended with vacuoles. These hepatocytes contained many lysosomes, mainly secondary [11] (Fig. 1). No hepatocytes with this type of organization were found in the liver of C57BL mice. Hepatocytes of classes 2A, 3A (CBA) and 1B, 2B (C57BL) were located in the central and, to some extent, the intermediate zones of the lobules. The qualitative changes in their structures were similar to those in the class 1A hepatocytes but were much less marked. They differed from each other in various qualitative and quantitative features of their subcellular organization. The hepatocytes of these classes in the mice of the two lines thus suffered different degrees of visible damage interpreted as degenerative. The hepatocytes of classes 4A and 3B of the intermediate zones of the liver lobules and of class 4B located at their periphery contained many GER tubules with numerous ribosomes, many mitochondria with numerous cristae, and extensive collections of ribosomes (Fig. 2). These observations indicate the development of processes of intracellular compensatory hyperplasia of the ultrastructures in these hepatocytes, for this condition is based on an increase in the number of organoids [6] (Table 1). It was most marked in hepatocytes of the C57BL mice. For example, class 5A hepatocytes in the peripheral zones of the hepatic lobules were virtually indistinguishable from the control, whereas in the class 4B hepatocytes the GER content was considerably increased. These differences in the manifestation of processes of intracellular compensatory hyperplasia are probably due to the more severe damage to the liver in C57BL mice (more extensive areas of necrosis).

The increase in the content of lysosomes, mainly secondary, in the hepatocytes of class 3B and 4B could be evidence of the active course of liberation of the ultrastructures damaged by CCl_4 from nonviable elements in these cells. The increased content of lipids in the class 3B hepatocytes probably also must be regarded as residual manifestations after toxic injury; CCl_4 poisoning is known to lead to the accumulation of lipids in the hepatocytes as a result of injury to the GER [17, 20]. These results suggest that the pro-

*The significance of the differences between the mean values compared was determined by Student's method; differences were taken as significant when $P < 0.05$.

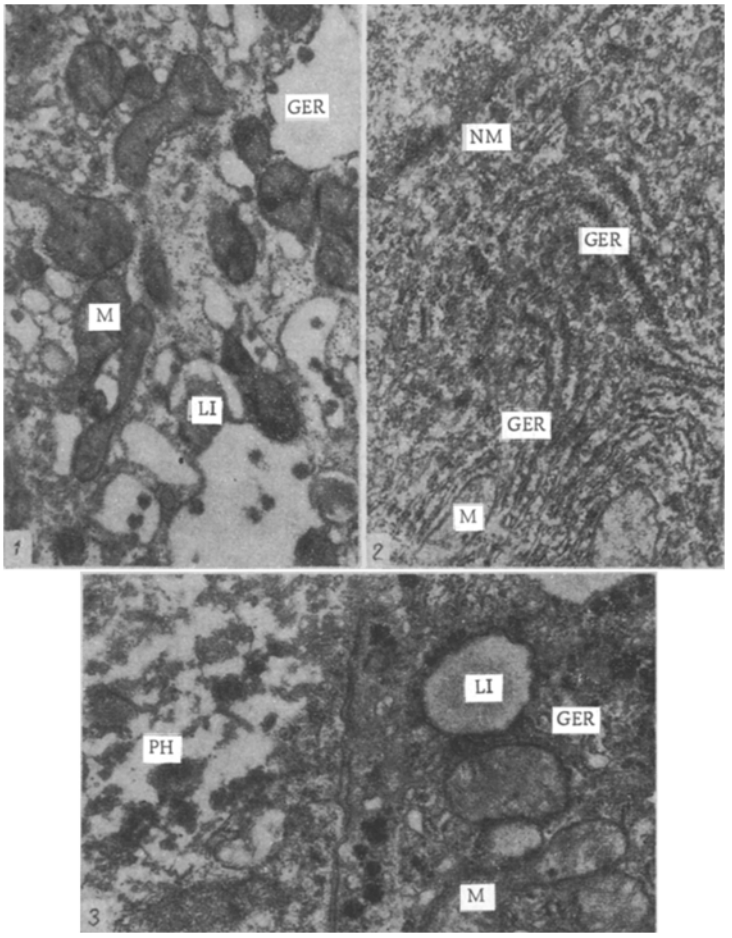

Fig. 1. Ultrastructural organization of cytoplasmic components characteristic of class 1A hepatocytes of line CBA. GER) granular endoplasmic reticulum; M) mitochondria; LI) lipid inclusion. Here and in Figs. 2 and 3, 30,000×.
Fig. 2. Organization of granular endoplasmic reticulum characteristic of class 4B and 3B hepatocytes of line C57BL. NM) nuclear membrane. Remainder of legend as in Fig. 1.
Fig. 3. Part of cytoplasm of a "dark hepatocyte" with intact structure of its cytoplasmic components (right) and part of cytoplasm of a cell in a state of necrobiosis (left). PH) parenchymatous hepatocyte in state of necrobiosis. Remainder of legend as in Figs. 1 and 2.

cesses of intracellular compensatory hyperplasia and regeneration of the intracellular structures after damage may develop parallelly and are interconnected.

In the peripheral and to some extent the intermediate zones of the hepatic lobules in the mice of both lines many "dark hepatocytes" were found; these are regarded by some workers as a manifestation of repair processes [2, 15]. These cells had no visible changes characteristic of the harmful action of CCl_4 (Fig. 3). The quantitative changes in these hepatocytes are illustrated by the results of their morphometric analysis in CBA mice, which show them to be a slightly different generation of cells from the control (Table 1).

It can thus be concluded that these differences in the reaction of the hepatocytes in mice of the two lines compared are probably determined genetically. They are possibly determined by differences in the intoxication enzyme systems in these animals, on account of which the metabolism of CCl_4 to free radicals takes place more actively in hepatocytes of the C57BL mice, leading to more extensive liver damage in this line. In turn, this factor probably determines the more marked processes of compensation.

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