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AGGRAVATION OF INJURY TO LIVER LYSOSOMES OF RATS WITH CCl_4 HEPATITIS BY PRELIMINARY PROLONGED ADMINISTRATION OF CHLORPROMAZINE

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Preliminary prolonged administration of chlorpromazine (5 mg/kg for three weeks) aggravated the injury to liver lysosomes of rats with acute CCl_4 hepatitis. Similar marked changes were observed in lysosomes sedimented with "heavy" and "light" mitochondrial fractions.

KEY WORDS: heterogeneity of liver lysosomes; toxic hepatitis; chlorpromazine.

The developmental disturbances of liver function during chlorpromazine therapy suggests that this drug is a hepatotropic substance, with an effect at the cellular and ultrastructural levels [8, 10, 12]. During repeated administration of chlorpromazine, the drug remains for a long time in the liver and brain [12] and it probably accumulates in the lysosomes, subcellular structures capable of accumulating various cationic substances [11]. The changes in the properties of the lysosomes taking place under these circumstances, it can tentatively be suggested, may aggravate the injury to the liver caused by other factors.

In this investigation the degree of changes in the liver lysosomes was assessed in rats with CCl_4 hepatitis developing after preliminary administration of chlorpromazine. Considering that the formation of acute toxic hepatitis is accompanied by an increase in the specific acid hydrolase activity of the "heavy" mitochondria [1], a separate study was made of the integrity and vulnerability of the lysosomes sedimented with the "light" and "heavy" mitochondrial fractions.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male Wistar rats weighing 150-180 g. Acute toxic hepatitis was induced by intraventricular injection of CCl_4 in a dose of 0.15 ml/100 g body weight. The animals were killed 24 h later. Chlorpromazine was injected subcutaneously in a dose of 5 mg/kg daily for three weeks and the

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rats were decapitated 72 h after the last injection of the drug. In the case of CCl_4 poisoning of the rats after repeated administration of chlorpromazine, the poison was given 48 h after the last injection of chlorpromazine and the animals were decapitated a further 24 h after the beginning of CCl_4 poisoning.

The liver was fractionated by the method of De Duve et al. [7]. The integrity and vulnerability of the particles [3, 5, 6] were determined in fractions sedimented at 3500 and 16,000g (designated in the "heavy" and "light" lysosomes, respectively) [9].

In all the groups of rats a morphological investigation was made of liver sections by the usual histological method. Glycogen was revealed by Schiff's reaction with periodic acid. The results were subjected to statistical analysis by the use of Student's criterion.

EXPERIMENTAL RESULTS

The development of acute CCl_4 hepatitis was accompanied by increased free acid phosphatase activity of the "heavy" and "light" lysosomes and by increased vulnerability of the particles to treatment at pH 5.0 and 37°C; solubilization of acid phosphatase was observed (Tables 1 and 2; Fig. 1). This indicates considerable disturbance of the integrity and increased vulnerability of the lysosomal subfractions studied. After the end of chlorpromazine administration minimal changes were found (an increase in free acid phosphatase activity of the "light" lysosome fraction).

TABLE 1. Effect of CCl_4 Poisoning on Total and Nonsedimented Acid Phosphatase Activity of Liver of Rats after Preliminary Prolonged Chlorpromazine Administration (M ± m)

Group of animals	Acid phosphatase activity of liver homogenate	
	total, μg Pi/10 min/mg protein	nonsedimenting, % of total
1 (intact)	18,7±1,48	4,4±0,32
2 (receiving chlorpromazine)	23,1±2,03	4,8±0,09
3 (administration of CCl_4)	18,5±0,71	7,2±0,71
4 (administration of chlorpromazine + CCl_4)	16,2±1,62 $P_{1-3}<0,001$ $P_{1-4}<0,001$	12,6±1,80 $P_{2-4}<0,01$ $P_{3-4}<0,05$

TABLE 2. Effect of CCl_4 on Integrity and Vulnerability of "Light" Liver Lysosomes of Rats after Repeated Administration of Chlorpromazine (M ± m)

Group of animals	Total activity, μg pI/10/mg protein	Free activity, % of total activity				
		basic index	during treatment with hypotonic solution	Heat treatment, pH 5.0		
				15 min	30 min	60 min
1 (intact)	47,0±2,82	10,0±0,92	24,4±1,54 (14,4)	22,4±1,92	37,5±3,12	65,4±4,84
2 (repeated administration of chlorpromazine)	36,3±4,21	13,6±0,96	25,5±1,39 (11,9)	22,8±2,16	38,9±1,04	80,7±5,39
3 (administration of CCl_4)	38,9±4,28	14,1±0,84	27,1±2,54	43,8±3,30	63,0±2,39	61,8±4,57
4 (repeated administration of chlorpromazine + CCl_4)	31,6±4,26 $P_{1-4}<0,01$	24,7±2,98 $P_{1-2}<0,05$ $P_{1-3}<0,01$ $P_{1-4}<0,001$ $P_{2-4}<0,01$ $P_{3-4}<0,01$	29,7±3,45 (5,0)	66,2±12,07 $P_{1-3}<0,001$ $P_{1-4}<0,001$ $P_{3-4}<0,1$	80,3±5,73 $P_{1-3}<0,01$ $P_{1-4}<0,001$ $P_{2-4}<0,001$ $P_{3-4}<0,005$	79,0±9,57 $P_{1-2}<0,1$

Legend. Increase in activity relative to free activity, by the method of Neely et al. [13], shown in parentheses.

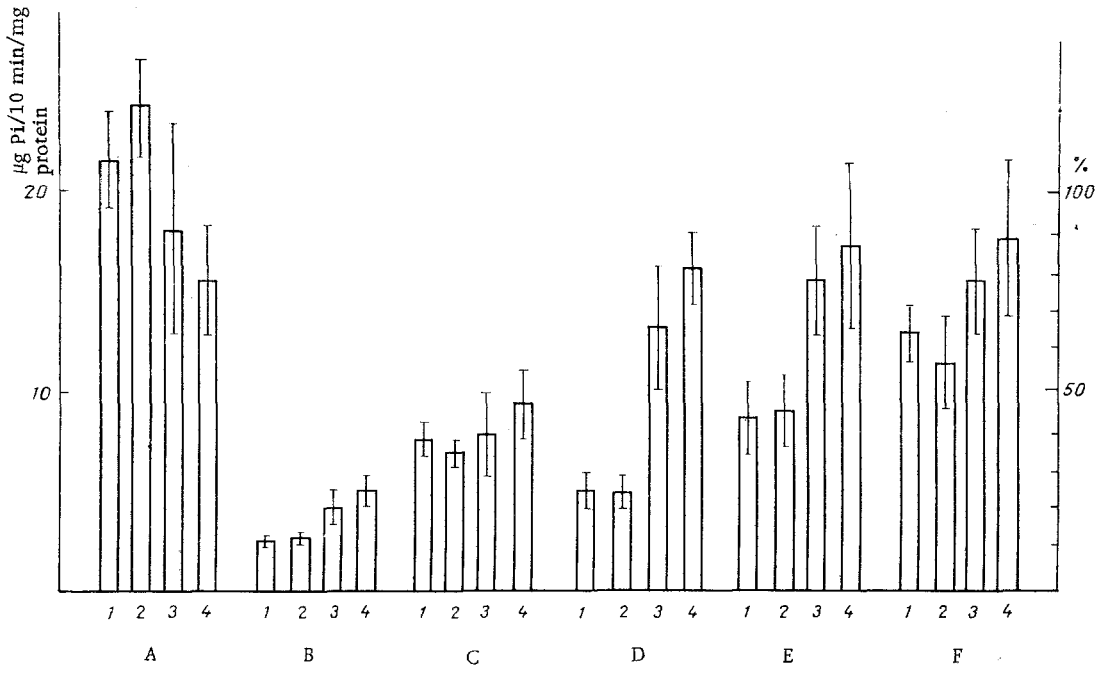

Fig. 1. Changes in "heavy" liver lysosomes of rats receiving a combination of chlorpromazine and CCl_4: 1) intact animals; 2) chlorpromazine; 3) CCl_4; 4) chlorpromazine + CCl_4. A) Total acid phosphatase activity (in μg Pi/10 min/mg protein); B) free activity (in percent of total); C) the same after treatment in hypotonic medium; D, E, F) the same after treatment at pH 5.0 and 37°C for 15, 30, and 60 min, respectively.

The question arises whether the changes in the lysosomes, which were already sufficiently marked in acute CCl_4 hepatitis, could be aggravated. During the combined action of chlorpromazine and CCl_4 the greatest changes were found in the liver lysosomes of the rats, including marked disturbances of integrity of the "light" and "heavy" lysosomes and increased vulnerability of the particles to heat treatment. In this series of experiments a high level of nonsedimenting acid phosphatase activity was observed; the changes were significant compared with the corresponding data for administration both of chlorpromazine and of CCl_4.

The degree of solubilization of the enzyme reached almost the maximal values found on lethal poisoning of rats with an extract of A. phalloides [5]. Compared with CCl_4 hepatitis, during the combined action of CCl_4 and chlorpromazine the fractions of "heavy" and "light" lysosomes showed a tendency toward an increase in free activity and increased vulnerability on heat treatment (37°C) at pH 5.0. During the combined action of the two substances the "light" lysosomes almost lost their ability to swell in hypotonic medium (the changes were 5.0%). The fraction of "heavy" lysosomes gave the same increase [13] as in intact animals, although the absolute values in the hypotonic solution were greater than those for the intact animals.

In the intact animals the "heavy" lysosomes were more vulnerable to injury in a hypotonic medium than the "light" fraction. This difference in the osmotic behavior of the particles reflects the easy reversibility of metabolic changes in the lysosomes [13] and is probably connected with the relative predominance of heterolysosomes in the "heavy" fraction. The same pattern was observed in all groups of experimental animals. The "light" lysosomes of the intact animals, almost like the "heavy," were susceptible to the harmful action of heat treatment. In the experimental rats a tendency was observed for the "heavy" lysosomes to be more vulnerable to treatment at pH 5.0 and 37°C. The possibility cannot be ruled out that in the less highly purified ("heavy") fraction the products of peroxidation injure the lysosomes more actively. Observations of this sort have been obtained in investigations of fractions of different degrees of purity [4].

It is interesting to compare these facts showing increased vulnerability of lysosomes after preliminary repeated administration of chlorpromazine to animals with CCl_4 hepatitis with the protective effect of other phenothiazine derivatives [2, 15]. Administration of promethazine simultaneously with CCl_4 prevents the development of necrosis and reduces the intensity of changes in the lysosomes to some extent [15]. We tried as far as possible to prevent direct interaction between the chlorpromazine and CCl_4 by not giving the two agents at the same time, and the greatest changes were thereupon observed in the lysosomes.

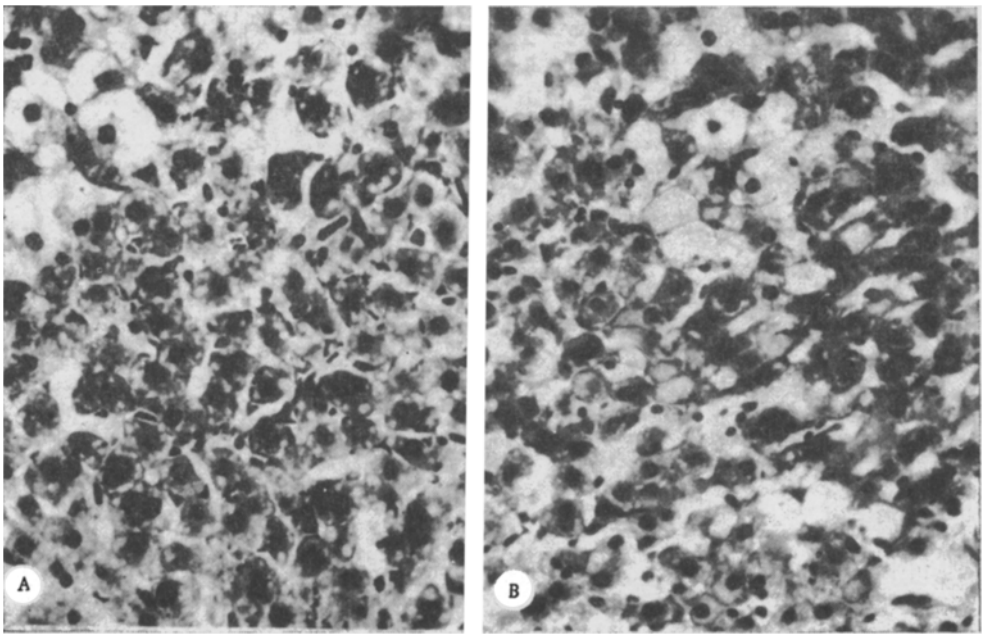

Fig. 2. Effect of preliminary prolonged administration of chlorpromazine on morphological picture of liver of rats with acute CCl_4 hepatitis. A) Single dose of CCl_4. Areas of fatty degeneration and destruction of hepatocytes; B) combined administration of chlorpromazine and CCl_4. Zone of necrosis with intracellular edema and fatty degeneration of hepatocytes, 400×. Hematoxylin–eosin.

Morphologically, compared with CCl_4 hepatitis, after combined administration of CCl_4 and chlorpromazine the foci of necrosis and the destructive lesions were larger. Some cells showed cloudy swelling, but the predominant form was fatty degeneration (Fig. 2). The distribution of glycogen was mosaic in character.

Combined administration of CCl_4 and chlorpromazine thus aggravates the degree of injury to the liver and changes the character of the lesion. During combined administration of CCl_4 and chlorpromazine the features of liver injury observed after administration of each separately are probably combined. The phenothiazines have been shown to potentiate changes in lysosomes produced by other harmful factors, in this case CCl_4. Although chlorpromazine is widely used in psychiatric practice, the frequency of hepatic complications is relatively low [14]; treatment with this substance must be regarded as a potentially serious background factor contributing to the severer course of other liver diseases.

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