SUBCELLULAR DISTRIBUTION OF ACID HYDROLASES IN THE RAT LIVER IN TOXIC HEPATITIS

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Changes taking place in a population of lysosomes were estimated from the results of investigation of the intracellular distribution of lysosomal marker enzymes: acid phosphatase and acid ribonuclease (RNase). Acute (pure CCl_4, 0.15 ml/100 g body weight, by gastric tube) and chronic (inhalational poisoning by the scheme of Rabinovici and Wiener) toxic hepatitis are accompanied by increased specific activity of the enzymes in the heavy mitochondrial fraction, indicating changes in the sedimentation properties of the lysosomes. An increase in the "unsedimented" acid RNase activity in chronic toxic hepatitis is a sign of injury to the lysosomal membranes.

KEY WORDS: lysosomal enzymes; acute and chronic hepatitis; carbon tetrachloride.

The most widely used methods of assessing the state of lysosomes in biochemical research are those based on enzymic identification of subcellular particles and the determination of the degree of their latency, as suggested by De Duve et al. [2]. These methods, with the addition of data on the sedimentation velocity of the particles, give an indication of the dimensions of the lysosomes.

The intracellular distribution of acid phosphatase and acid RNase – marker enzymes of lysosomes – was studied in acute and chronic toxic hepatitis caused by administration of CCl_4.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-180 g were used. Acute toxic hepatitis was produced by giving pure CCl_4 by gastric tube in a dose of 0.15 ml/100 g body weight (group 1). The animals were killed 24 h after administration of the poison. Inhalation poisoning of the second group of animals with CCl_4 was carried out by the scheme of Rabinovici and Wiener [4] twice a week for 4 h each time, over a period of three weeks. The rats were killed 18 h after the last exposure, and the animals of both groups were deprived of food for 12 h before sacrifice.

The liver tissue was fractionated by the scheme described by De Duve et al. [2]. The total activity of the lysosomal marker enzymes – acid phosphatase and acid RNase – was determined in the five fractions thus obtained: nuclear (N), heavy mitochondria (M), light mitochondria (L), microsomal (P), and supernatant (S). Liberation of the enzymes was facilitated by the presence of the nonpolar detergent Triton X-100 in the sample. Acid phosphorus activity was determined by the method of De Duve et al. [2], inorganic phosphorus set free was determined by the method of Weil-Malherbe and Green [6], and acid RNase activity at pH 5.8; the RNA concentration in the sample was 1 mg/ml. RNA and proteins were precipitated with lanthanum chloride solution in 80° alcohol with 1 N HCl [1]. The protein content was determined by the method of Lowry et al. [3].

The protein distribution was expressed as percentages of the total protein content in each fraction and the distribution of enzymes as values of relative specific activity, as described by De Duve et al. [2].

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TABLE 1. Intracellular Distribution of Protein and Acid Hydrolases of Rat Liver (M ± m)

Group of animals	N	M	L	P	S
			Protein		
Intact	24,3±1,9	20,1±2,3	6,8±1,4	22,4±2,2	25,7±1,1
Poisoning with CCl_4, sacrificed after 24 h	36,3±2,8	7,9±1,0	6,6±0,12	16,4±0,9	32,4±2,1
P	<0,01	<0,001	>0,05	<0,05	<0,02
Poisoning with CCl_4, sacrificed after 3 weeks	37,9±3,3	9,6±0,9	7,4±1,3	16,2±1,2	29,0±1,8
P	<0,001	<0,001	<0,5	<0,05	<0,1
			Acid phosphatase		
Intact	0,28±0,06	0,4±0,08	11,8±1,2	1,1±1,1	0,25±0,03
Poisoning with CCl_4, sacrificed after 24 h	0,1±0,02	2,9±0,4	6,7±0,9	1,1±0,1	0,3±0,08
P	<0,05	<0,01	<0,01		>0,05
Poisoning with CCl_4, sacrificed after 3 weeks	0,28±0,07	1,9±0,2	7,96±2,4	1,1±0,17	0,4±0,1
P		=0,05	>0,1		>0,1
			Acid RNase		
Intact	0,3±0,07	0,38±0,1	13,2±1,6	0,5±0,04	0,13±0,05
Poisoning with CCl_4, sacrificed after 24 h	0,2±0,04	4,6±1,1	6,8±0,6	0,6±0,04	0,2±0,05
P	>0,05	<0,001	<0,001	>0,05	>0,05
Poisoning with CCl_4, sacrificed after 3 weeks	0,22±0,04	2,7±0,3	7,7±2,5	0,86±0,1	0,47±0,1
P	>0,25	<0,001	>0,05	<0,01	<0,01

Differences between means were taken as significant when $P \leq 0.05$.

EXPERIMENTAL RESULTS

The distribution of protein and enzymes of the subcellular fractions (Table 1) of the liver of intact rats agreed in general with the results given by De Duve et al. [2]

Analysis of the results shows that acute and chronic liver damage by CCl_4 led to identical changes in the intracellular distribution of protein and lysosomal enzymes: a redistribution of protein of the nuclear fraction and heavy mitochondrial fraction took place, so that the former became preponderant. This change could evidently be attributed to the mass of debris and the population of Kupffer cells. In the case of liver damage caused by a single dose of CCl_4, an increase in the protein content of the supernatant fraction also was observed.

The study of the distribution of the two lysosomal marker enzymes gave some idea of the effect of acute and chronic liver damage on the state of the two different classes of lysosomes [5]. As Table 1 shows, a common feature of both classes in these situations was the appearance of particles containing acid hydrolases in the fraction of heavy mitochondria, as shown by the increase in specific acid phosphatase and acid RNase activity in fraction M. Meanwhile, the increase in "unsedimented" acid RNase activity in chronic poisoning indicates a reduction in the resistance of the membranes of this class of lysosomes and is a sign of their injury. The change in the sedimentation properties of the lysosomes was more marked in acute toxic hepatitis.

In both acute and chronic toxic hepatitis changes thus take place in the size and sedimentation properties of the subcellular particles containing acid hydrolases. This was evidently connected with the fact that in response to injury a large number of secondary lysosomes, with sedimentation rates close to those of heavy mitochondria, appear in the lysosome population. The increase in "unsedimented" acid RNase activity in chronic hepatitis may also be evidence of activation of lysosomes of the Kupffer cells, which are known to be richer in this enzyme than the lysosomes of hepatocytes [7].

LITERATURE CITED

1. G. A. Nechaeva, Proceedings of the Fourth All-Union Conference on Biochemistry of the Nervous System [in Russian], Tartu (1969), p. 323.
2. C. De Duve, N. C. Pressman, R. Gianetto, et al., Biochem. J., 60, 604 (1955).

3. O. H. Lowry et al., J. Biol. Chem., 193, 265 (1951).
4. N. Rabinovici and E. Wilner, Gastroenterology, 40, 416 (1961).
5. Y. E. Rahman, S. F. Howe, et al., Biochem. Biophys. Acta, 146, 484 (1967).
6. H. Weil-Malherbe and R. H. Green, Biochem., J., 49, 286 (1952).
7. R. Wattiaux, P. Baudhuin, et al., Biochem. J., 63, 608 (1956).