INTRACELLULAR DISTRIBUTION OF HYALURONIDASE IN ACUTE AND CHRONIC HEPATITIS AND REPARATIVE REGENERATION OF THE LIVER IN RATS

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The dynamics of the subcellular distribution of hyaluronidase was studied in the rat liver after acute and chronic hepatitis induced by CCl_4. In the early period of recovery an increase was found in the relative specific activity of the enzyme in the supernatant fraction but a decrease in the fraction of light mitochondria. Later this index increased in the fraction of heavy and light mitochondria, evidence of active processes of phagocytosis and digestion. After acute hepatitis the process of recovery at the subcellular level lasted much longer than at the morphological level.

KEY WORDS: *lysosomes; hyaluronidase; acute and chronic hepatitis; posttoxic regeneration.*

Liver damage, disturbing relations between parenchyma and stroma, is accompanied by changes in metabolism of the proteins and mucopolysaccharides of connective tissue [9]. Whenever complete recovery of the structure and function of the organ is observed as the outcome of experimental hepatitis, the newly formed connective tissue is completely absorbed. Lysosomes of hepatocytes, containing enzymes which hydrolyze acid mucopolysaccharides [3, 8, 10], possibly participate in its degradation. Hutterer [8] considered that the excess of mucopolysaccharides is removed by phagocytosis, followed by their digestion in the lysosomes. A disturbance of acid mucopolysaccharide metabolism develops in toxic injuries to the liver.

TABLE 1. Intracellular Distribution of Hyaluronidase during Spontaneous Recovery from Acute Liver Damage by CCl_4 (M ± m)

Duration of experiments	Hyaluronidase activity in liver homogenate (in μg N-acetylglucosamine/mg protein/4 h)	Hyaluronidase activity in fractions (in % of activity in liver homogenate)						
		homogenate	N	M	L	P	S	yield
Control	16,8±:0,7	100	28,9	16,4	24,9	16,3	3,6	90,5
1 day	7,4±1,2	100	33,8	13,1	24,0	27,0	5,9	103,8
2 "	8,5±1,7	100	24,4	16,8	13,1	20,2	6,4	80,8
3 "	12,4±1,5	100	32,6	14,9	16,1	17,9	10,4	91,9
1 week	17,2±1,2	100	22,3	17,4	13,9	18,8	6,2	78,6
2 "	24,3±2,2	100	23,2	17,2	17,0	22,0	7,2	86,6
3 "	22,7±1,4	100	27,0	18,3	25,6	12,4	3,0	76,3
4 "	21,1±0,5	100	20,7	15,1	35,7	20,6	3,5	105,5

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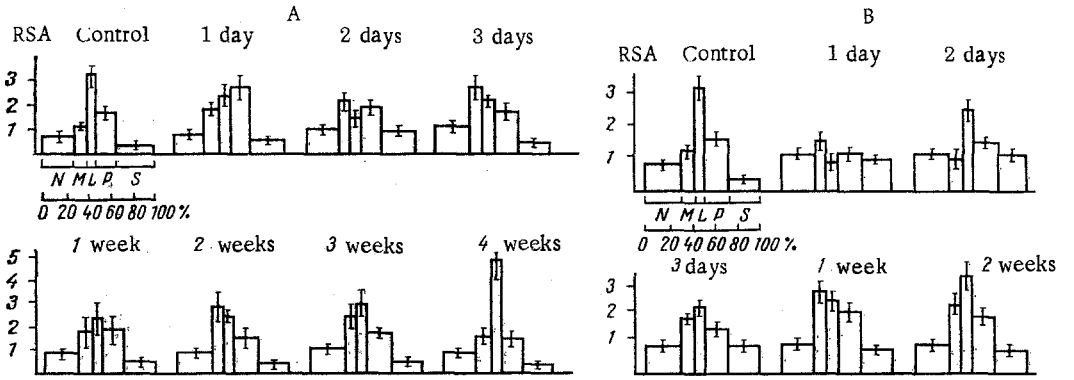

Fig. 1. Subcellular distribution of hyaluronidase in rat liver during spontaneous recovery from acute (A) and chronic (B) poisoning of animals with CCl_4. Abscissa, protein content in fractions (in %); ordinate, RSA of hyaluronidase.

It was accordingly decided to study the subcellular distribution of hyaluronidase during the process of recovery after toxic injury to the liver.

EXPERIMENTAL METHOD

Eighty male Wistar albino rats weighing 120-180 g were used. Acute toxic hepatitis was induced by oral administration of a single dose of 0.15 ml pure CCl_4/100 g body weight, and chronic hepatitis by inhalation of the poison for 3 weeks by the method of Rabinovici and Wiener [14]. Each experimental group consisted of 6-8 animals, and intact rats served as the control. The acutely poisoned animals were decapitated after 1-3 days and 1-4 weeks, the chronically poisoned animals after 1-3 days and 1 and 2 weeks. Complete differential centrifugation was carried out by de Duve's method [6].

Hyaluronidase activity was determined in five subcellular fractions: nuclei (N), light and heavy mitochondria (M and L), microsomes (P), and the supernatant fraction (S) by the method of Bowness et al. [5]. The composition of the incubation medium was as follows: hyaluronic acid from human umbilical cords in a concentration of 1 mg/ml, 0.15 M NaCl, and 0.1 M Na-acetate buffer, pH 3.8. Incubation continued for 4 h. Free N-acetylglucosamine was determined by the method of Reising et al. [15] and protein by the method of Lowry et al. [11].

The distribution of hyaluronidase in the subcellular fractions was estimated from its relative specific activity (RSA) [6]. The results were subjected to statistical analysis with the aid of Student's criterion. Differences between means were taken to be significant when $P < 0.05$.

EXPERIMENTAL RESULTS AND DISCUSSION

Data on the distribution of hyaluronidase activity in the subcellular fractions as percentages of activity of the enzyme in liver homogenate during spontaneous recovery from acute hepatitis are given in Table 1. The profile of distribution among the fractions lies within the limits stipulated by de Duve et al. [6] for lysosomal marker enzymes.

RSA of hyaluronidase in the subcellular fractions of the liver at various times of recovery from acute and chronic poisoning is shown in Fig. 1 (A and B). A redistribution of protein in the subcellular fractions was observed 24 h after the end of acute and chronic poisoning: an increase in its concentration in the nuclear fraction and a decrease in the heavy mitochondria. The distribution of marker enzymes of the mitochondria and microsomes (succinate dehydrogenase and glucose-6-phosphatase) did not differ, according to these findings, from their distribution in intact animals. It will be clear from Fig. 1 that after the end of both types of poisoning RSA of hyaluronidase was increased in the supernatant fraction. After acute poisoning RSA fell rapidly, but after chronic poisoning it remained high for 2 weeks.

The increase in solubilization of the lysosomal enzymes of the liver after acute damage caused by injection of CCl_4 can be explained by an increase in permeability of the lysosomal membranes [2, 16]. Some workers [8, 10] consider that the source of the enzymes in the supernatant fraction is the destroyed cells which appear in the liver as a result of the action of the poison. Another possibility is that equilibrium is disturbed between the supply of hyaluronidase from the microsomal membranes into the lysosomes and cytoplasm of the cells [7]. This possibility is confirmed by the way in which the enzyme appears in the supernatant fraction after acute poisoning, which is preceded by an increase in its content in the microsomal fraction (Fig. 1A), and also by the prolonged maintenance of its increased activity after chronic poisoning. Meanwhile, after acute poisoning in the early stages of the experiment there was a decrease in the total activity of the enzyme in the liver homogenate (Table 1).

After the end of acute and chronic poisoning RSA of nyaluronidase fell in the light mitochondria and microsomes. Later during recovery it rose in these fractions and particularly considerably in the fraction of heavy mitochondria, consisting chiefly of secondary lysosomes [12]. The appearance of these lysosomes in the cells is characteristic of active phagocytosis and digestion of material taken up during regeneration [1]. By contrast with acute hepatitis, in the chronic form the distribution of hyaluronidase in the cell returned close to normal as early as after 2 weeks, but the process of recovery was still incomplete, for the number of secondary lysosomes containing hyaluronidase remained increased. Kalashnikova and Rubetskoi [1] found that this process did not end until after 4 weeks, when under the electron microscope they observed replacement of the lysosome population in the liver cells — disappearance of large particles and the appearance of numerous tinly particles, probably primary lysosomes. After acute injury, according to morphological observations recovery of the liver is complete within 1 week [13]. The subcellular distribution of the enzyme at this time differs from normal. The histogram of the enzyme is still not back to normal even after 4 weeks.

The results thus indicate that hyaluronidase can participate in both destructive and reconstructive processes in the liver.

LITERATURE CITED

1. M. M. Kalashnikova and A. S. Rubetskoi, Arkh. Pat., No. 3, 51 (1974).
2. A. E. Kondakova, T. A. Korolenko, G. M. Vakulin, et al., Tsitologiya, No. 11, 1382 (1973).
3. N. N. Aronson and E. A. Davidson, J. Biol. Chem., 240, PC3222 (1965).
4. N. N. Aronson and E. A. Davidson, J. Biol. Chem., 242, 437 (1967).
5. J. M. Bowness, N. Carpenter, and D. N. Barry, Canad. J. Biochem., 49, 12 (1971).
6. C. de Duve, B. C. Pressman, R. Gianetto, et al., Biochem. J., 60, 604 (1955).
7. B. Fiszer-Szafarz and D. Szafarz, Cancer Res., 33, 1104 (1973).
8. F. Hutterer, Biochim. Biophys. Acta, 115, 312 (1966).
9. T. Koizumi, N. Makamura, and H. Abe, Biochim. Biophys. Acta, 148, 749 (1967).
10. T. Koizumi, T. Suematsu, N. Iwabori, et al., Biochim. Biophys. Acta, 151, 628 (1968).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
12. J. L. Mego, Lysosomes Biol. Path., 3, 528 (1973).
13. G. Paumgartner, J. Longueville, and C. M. Leevy, Exp. Molec. Path., 9, 161 (1968).
14. N. Rabinovici and E. Wiener, Gastroenterology, 40, 416 (1961).
15. J. L. Reising, J. L. Strominger, and L. F. Leboir, J. Biol. Chem., 217, 959 (1955).
16. G. Ugazio, M. Artizzu, P. Pani, et al., Biochem. J., 90, 109 (1964).