ROLE OF OVERLOADING OF THE VACULAR APPARATUS IN CHANGES IN LIVER CELL LYSOSOMES IN ACUTE TOXIC HEPATITIS

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The physicochemical properties of the liver lysosomes were investigated in rats after administration of a single dose of the lysosomotropic agent Triton WR 1339 to intact rats and to rats with acute toxic hapatitis. Administration of the detergent to intact animals was followed by a decrease in the buoyant density of the particles, solubilization of the lysosomal enzymes, and reduced resistance of the particles in a hypotonic medium. Signs of overloading of the vacuolar apparatus with the detergent also were found in the liver cell lysosomes of rats with toxic hepatitis. The most marked solubilization of β-galactosidase, acid RNase, and cathepsin D was observed in the case of combined administration of CCl_4 followed by WR 1339 24, 48, and 72 h and 7 days later. The possible consequences of overloading of the vacuolar apparatus of the rat liver cells are discussed.

KEY WORDS: lysosomes; lysosomotropic preparations; Triton WR 1339; toxic hepatitis.

The isolation of a group of lysosomotropic agents (LTA) [6] has raised the question of the "control" of lysosomal functions in vivo, i.e., of acting on the cell for specific purposes through the lysosomes. Since these particles are concerned with the development of various pathological processes [3, 4, 6], it is interesting to investigate the effect of LTA under pathological conditions accompanied by disturbance of lysosomal functions [9]. Of the known LTA, the nonpolar detergent Triton WR 1339, which is selectively taken up by lysosomes of rat liver cells, has been studied in sufficient detail. Meanwhile the action of LTA themselves on lysosomes damaged as a result of the development of a pathological process has been investigated the least.

The object of this investigation was to assess the action of Triton on rat liver lysosomes during the development of acute toxic hepatitis, which is accompanied by labilization of these particles.

EXPERIMENTAL METHOD

Male Wistar rats weighing 220 ± 10.0 g were used. Acute toxic hepatitis was induced by peroral administration of CCl_4 in a dose of 0.15 ml 100 g body weight. The animals were killed 24, 48, and 72 h and 7 days after poisoning. Triton was injected intraperitoneally in a dose of 85 mg 100 g body weight, and in the series in which it was given in conjunction with CCl_4, the Triton was given 4 h before poisoning. Preparative and analytical procedures were carried out as in [1, 2]. Activity of β-galactosidase and cathepsin D was determined by Barrett's method [5], using 4-nitrophenyl-β-D-galactopyranoside (from Chemapol, Czechoslavakia) and hemoglobin (fron Reanal, Hungary) respectively as the substrates. Total activity of lysosomal enzymes was determined with 0.1% Triton X-100 [14]. The stability of the lysosomes was estimated from the degree of solubilization of lysosomes by the enzyme β-galactosidase present in the matrix, and the partially membrane-bound enzyme acid RNase and cathepsin D [3]. The results of determination of nonsedimented activity of lysosomal enzyme were expressed as percentages of total activity. The fragility of the lysosomes in hypotonic medium (HFL) was estimated by the method described previously [11]. The results were subjected to statistical analysis by means of Student's criterion.

To determine the buoyant density of Triton-loaded lysosomes, equilibrium centrifugation of the combined fraction of heavy and light mitochondria was carried out in separate experiments in a linear sucrose density gradient [14]. The model 12-75B Spinco–Beckman ultracentrifuge with SW65L rotor was used.

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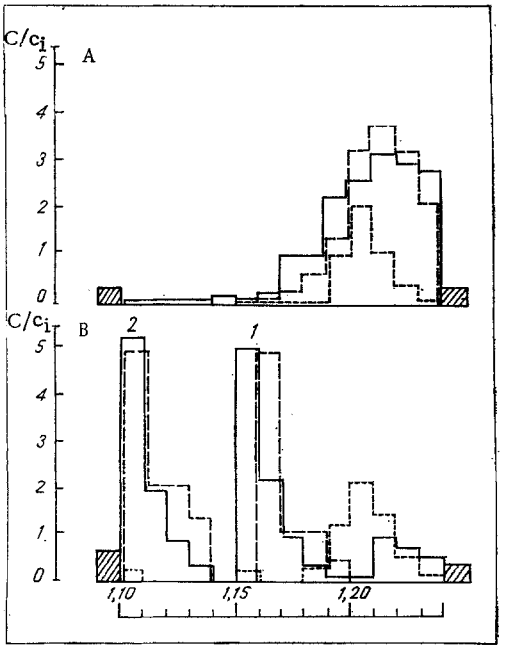

Fig. 1. Distribution of acid phosphatase, acid DNase, and protein after centrifugation of fraction of heavy and light mitochondria from rat liver homogenate, 24 and 72 h after injection of Triton, in a linear sucrose density gradient (0.776-3.419 M) [14]. Abscissa, nos. of samples in order of taking (corresponding values of sucrose density, in g/cm^3, given in bottom part of figure); ordinate, ratio of concentration of indicator of given subfraction (C) to its initial concentration (C_i) in fraction of heavy and light mitochondria. A) Intact animals; B) injection of Triton: 1) 24 h, 2) 72 h after injection. Continuous line shows distribution of acid phosphatase, broken line distribution of acid RNase, dotted line distribution of protein. Ends of gradients shown by shaded columns.

EXPERIMENTAL RESULTS

Administration of Triton led to a decrease in the buoyant density of the lysosomes to 1.15 g/cm^3, which was observed as early as after 24 h (Fig. 1). Meanwhile increased fragility of the particles was found in hypotonic medium (Fig. 2A). Later, 48 and 72 h after injection of Triton, osmotic changes in the lysosomes were still more marked (Fig. 2B, C), and the buoyant density of the particles fell to 1.1 g/cm^3 (Fig. 1). Nonsedimented β-galactosidase (24, 48, and 72 h, 7 days), acid RNase (72 h), and cathepsin D (24 and 72 h, 7 days) activity was increased (Table 1). The combination of changes observed pointed to overloading of the vacuolar apparatus of the liver cells with Triton, such as has been observed for 30-35 days after administration of a single dose [14]. The intensity of the osmotic changes in the lysosomes after administration of Triton was to some extent a measure of the presence of the detergent in the particles.

The development of toxic hepatitis was accompanied by solubilization of the lysosomal enzymes β-galactosidase (24, 48, and 72 h, 7 days), acid RNase (24 h), and cathepsin D (48 and 72 h). The fragility of the lysosomes in hypotonic medium (24, 48 h) was reduced (Fig. 2), i.e., considerable loss of the osmotic properties of the particles detectable in the intact animals was observed. This was due, it can be tentatively suggested, to injury of the phospholipids of lysosomal membranes by lipid peroxides and free radicals, although these organelles are known to undergo lipid-peroxidation much more slowly than mitochondria and, in particular, than

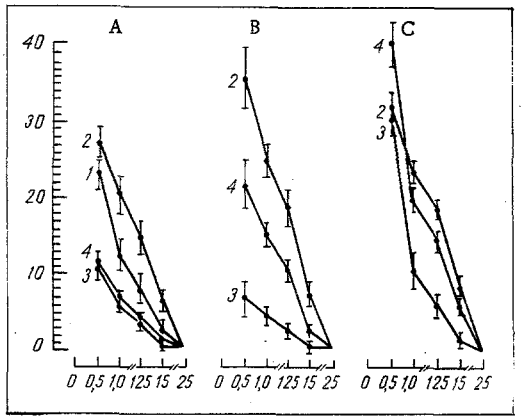

Fig. 2. Osmotic changes in lysosomes of liver cells in unfractionated homogenate of rat liver after injection of Triton into intact rats and rats with toxic hepatitis – 24 h (A), 48 h (B), and 72 h (C) after poisoning. Abscissa, molarity of sucrose; ordinate, increase in free acid phosphatase activity (in μmoles P_i/g wet weight of tissue/10 min [11]. 1) Intact, 2) Triton, 3) CCl_4, 4) Triton + CCl_4.

TABLE 1. Effect of Triton WR 1339 on Nonsedimented Activity of Liver Lysosomal Enzymes of Rats with Acute Toxic Hepatitis (M ± m)

Enzyme	Group	Time after injection of CCl_4, h			
		24	48	72	168
β-Galactosidase	1. Intact		4,56±0,55		
	2. Triton	11,8±2,04	10,6±1,84	8,9±0,90	9,4±0,93
	P	<0,001	<0,01	<0,01	<0,001
	3. CCl_4	7,6±0,51	17,7±1,78	9,3±1,14	6,6±0,39
	P	<0,01	<0,001	<0,01	<0,05
	4. Triton + CCl_4	12,2±2,60	31,2±3,86	14,7±0,84	10,9±1,35
	P	<0,001	<0,001	<0,01	<0,01
			$P_{2,3}$<0,01	$P_{2,3}$<0,01	—
RNase	1. Intact	—	9,13±0,95	—	—
	2. Triton	14,4±3,89	15,7±4,05	16,1±2,16	8,9±1,56
	P	—	—	<0,05	—
	3. CCl_4	40,3±8,70	10,4±1,78	9,3±1,15	6,95±0,73
	P	<0,01	—	—	—
	4. Triton + CCl_4	7,9±0,84	14,3±3,19	16,2±2,91	14,1±1,97
	P	—	—	<0,05	<0,05
Cathepsin D	1. Intact	—	11,18±0,72	—	—
	2. Triton	23,5±4,74	15,8±1,82	21,8±1,90	16,2±1,10
	P	<0,05	—	<0,01	<0,05
	3. CCl_4	20,6±4,42	37,8±5,29	23,9±5,84	14,9±0,72
	P	—	<0,01	<0,05	—
	4. Triton + CCl_4	36,6±6,97	41,1±8,58	15,9±3,09	18,3±1,47
	P	<0,01	<0,01	—	<0,05

microsomes, which correlates with the lower lipid content in the lysosomes [13]. On the whole, the distinctive character of the changes in the lysosomes – namely an increase in solubilization of the enzymes with a decrease in osmotic fragility (24 h) – recalls the corresponding observations made during anoxia of the liver [10, 11]. The lysosomes had almost regained their osmotic properties 72 h after administration of CCl_4.

The most marked labilization of the liver lysosomes was observed after combined administration of Triton and CCl_4: an increase in nonsedimented β-galactosidase (at all times studied), acid RNase (72 h and 7 days), and cathepsin D (24 and 48 h, 7 days) activity. The values for β-galactosidase (48 and 72 h) were higher than the corresponding data obtained after administration of both Triton and CCl_4. The osmotic fragility of the lysosomes

following combined administration of Triton and CCl_4 was the same after 24 h as that obtained with CCl_4, and after 72 h as that obtained after administration of Triton.

First, therefore, the most marked features of injury to the lysosomes, as reflected in the degree of solubilization of their enzymes, were observed in the animals receiving a combination of Triton and CCl_4, and each factor separately caused similar but less marked changes in the particles. Solubilization of the matrix enzyme, β-galactosidase, was on a larger scale. Similar changes occurred in acid phosphatase [1, 2, 8].

Second, to judge from the increased HFL (48 and 72 h), signs of overloading of the vacuolar apparatus of the cells were present in lysosomes of the liver damaged by toxic hepatitis in response to injection of Triton. However, in the early periods (24 h) accumulation of the detergent in the liver cells of the poisoned animals took place more slowly than in intact rats. This may perhaps have been the result of the slower uptake of the polymer by "damaged" lysosomes. The possibility likewise cannot be ruled out that if the uptake of Triton was undisturbed, the liver lysosomes of rats with CCl_4-induced hepatitis are less able to retain the material taken up, i.e., by analogy with lysosomal enzymes, the possibility of a "leak" of macrotriton [7, 12] from the particles may also take place.

The absence of a parallel trend in the changes in nonsedimented lysosomal enzyme activity and HFL must be noted. For instance, after administration of Triton an increased HFL accompanied solubilization of the lysosomal enzymes; in CCl_4 hepatitis an increase in nonsedimented enzyme activity was accompanied by a sharply reduced HFL. The greatest degree of solubilization of lysosomal enzymes as a result of the action of Triton and CCl_4 was observed in the presence of reduced (24 h), "normal" (48 h), followed by increased fragility in hypotonic medium (72 h). On the whole this is evidence that the degree of enzyme solubilization and the fragility of the particles in hypotonic medium are independent, i.e., that these indices characterize different physicochemical properties of the lysosomes, as has been observed with other models [4, 10, 11].

The results show that LTA can exhibit their effect on "damaged" liver lysosomes also. The consequences of accumulation of LTA in altered liver cell lysosomes following injury to the organ and the possible phenomena resulting from increased labilization of lysosomes must therefore be taken into account.

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