EFFECT OF LOADING THE LIVER LYSOSOMES WITH TRITON WR 1339 ON THE DEVELOPMENT OF CHRONIC TOXIC HEPATITIS

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Preliminary administration of Triton WR 1339 has a favorable effect on the course of chronic toxic hepatitis. The zones of necrosis are reduced, the development of connective tissue is delayed, and liver function is improved. The liver lysosomes of animals poisoned with CCl_4 after preliminary administration of the detergent are more stable when exposed to harmful procedures in vitro.

KEY WORDS: liver lysosomes; toxic hepatitis; Triton WR 1339.

The participation of lysosomes in the development of a pathological process is associated primarily with changes in their autophagous and heterophagous functions, which may be intensified or deficient. Under these conditions the additional administration of substances selectively accumulated in lysosomes (Triton WR 1339,* dextran) can lead to considerable disturbances of the structural organization and function of the cell. Administration of Triton WR 1339, for instance, has been shown to give rise to a marked increase in the intensity of necrosis in the liver induced by administration of a single dose of CCl_4 [7].

The effect of administration of Triton WR 1339 on the development of chronic toxic hepatitis was studied.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-200 g were used. Group 1 consisted of animals with toxic hepatitis induced by inhalations of CCl_4 for 3 weeks [10]; the concentration of CCl_4 in the chamber was 20 mg/liter. The control to this group (group 2) consisted of intact rats. Group 3 included animals treated with CCl_4 by the scheme described above, but 4 h before the first inhalation they were given a single intraperitoneal injection of Triton WR 1339 in a dose of 85 mg/100 g body weight. Group 4 (control) consisted of animals receiving a single injection of Triton WR 1339 but not poisoned with CCl_4.

The liver lysosomes were thus loaded for 21 days with the detergent, and according to data in the literature, it remains in the liver lysosomes for up to 30 days after a single injection [12]. The animals were deprived of food for 12 h before sacrifice, which took place 18 h after the last inhalation of CCl_4.

A suspension of lysosomes isolated by the method of De Duve et al. [5] was subjected to injury in vitro [12]. The degree of injury to the lysosomal membranes was estimated from the ratio between free and total activity of marker lysosomal enzymes: acid phosphatase and acid ribonuclease. Free activity was expressed as a percentage of total activity. Acid phosphatase was determined by the method of De Duve et al. [5], and acid ribonuclease as described previously [3]. The protein content was determined by the method of Lowry et al. [8].

*Triton WR 1339: a quaternary hydroxyethylated octylphenolpolymethylene polymer (Ruger Chemical Co. Inc.).

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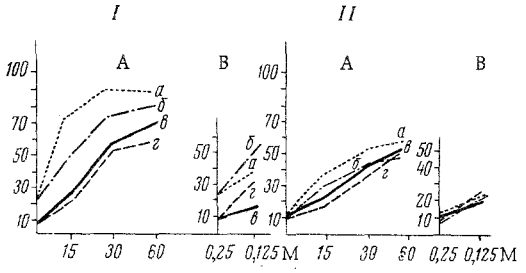

Fig. 1

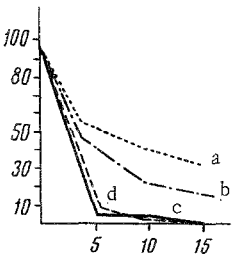

Fig. 2

Fig. 1. Effect of administration of Triton WR 1339 on free activity of acid phosphatase (I) and acid ribonuclease (II) of the liver lysosomes of rats with chronic toxic hepatitis. Fraction of light mitochondria, rich in lysosomes, was incubated for 15, 30, and 60 min at pH 5.0, 37°C (A) or treated with 0.125 M sucrose solution at 0°C for 15 min (B) as described by Wattiaux [12]. Here and in Fig. 2: a) rats with chronic toxic hepatitis; b) rats with chronic toxic hepatitis receiving injection of Triton WR 1339; c) intact animals; d) rats receiving a single injection of Triton WR 1339. Abscissa, incubation time (in min); ordinate, free activity of enzymes (in % of total activity).

Fig. 2. Dynamics of elimination of bromsulfalein by rats with chronic toxic hepatitis after injection of Triton WR 1339. Abscissa, time (in min); ordinate, concentration of dye in plasma (retention coefficient).

Pieces of liver were fixed in a 10% alcoholic solution of formalin and embedded in paraffin wax in the usual way. After dewaxing, liver sections were stained with hematoxylin and eosin and by Van Gieson's method [1].

The excretory and ingestive function of the liver was studied in parallel experiments by estimating the rate of excretion of bromsulfalein [4, 6]. The results were expressed in retention coefficients [2]. The numerical data were subjected to statistical analysis. Differences between the mean values compared were significant for which $P < 0.05$.

EXPERIMENTAL RESULTS

Injection of Triton WR 1339 led to increased sensitivity of the liver lysosomes of the rat to hypotonic conditions (Fig. 1). This was shown by an increase in the free acid phosphatase activity up to 30% compared with 16% in the intact animals. Meanwhile, a tendency was observed for the free acid phosphatase and acid ribonuclease activity to decrease during incubation of the lysosomes at pH 5 and 37°C, indicating some stabilization of the membrane of these organelles as the result of this treatment. Similar but less clearly marked changes in the lysosomal membranes were described by Wattiaux in the period of maximal accumulation of the detergent in the early period after administration of Triton WR 1339 [12].

Compared with the corresponding parameters for rats with chronic toxic hepatitis, after preliminary injection of the detergent the free acid phosphatase activity was reduced after incubation of the lysosomes for 15 min at pH 5 and 37°C; this is evidence of stabilization of the membranes: after 30 and 60 min only a tendency toward a decrease in acid phosphatase activity could be observed.

In liver sections of rats receiving Triton WR 1339 only, activation of the Kupffer cells was observed, as shown by an increase in their number and size. On microscopic examination of the liver of rats receiving Triton WR 1339 before the first inhalation of CCl_4, the zones of necrosis of the liver cells were narrower and less frequent than in animals receiving CCl_4 only. The proliferation of the collagen stroma was less marked. The rate and completeness of bromsulfalein excretion after injection of Triton WR 1339 were indistinguishable from those found in intact animals. During the development of chronic hepatitis marked retention of the dye in the blood was observed, evidence of a marked disturbance of the excretory and ingestive function of the liver (Fig. 2). After injection of Triton WR 1339 into animals subsequently poisoned with CCl_4, some improvement in liver function was observed, as shown by the lower value of dye excretion after 10 and 15 min.

It can accordingly be postulated on the basis of changes in the membranes, that the liver lysosomes of the intact rats and also of animals with chronic hepatitis induced by CCl_4 contained the detergent three weeks after its administration.

The morphological investigation of the organ and liver function tests showed that loading the lysosomes of the liver with Triton WR 1339 during the period of development of toxic hepatitis leads to a decrease in the severity of liver damage. This is reflected in a reduction in the area of necrosis, delayed development of connective tissue in the liver, and more complete elimination of bromsulfalein.

The two most likely interpretations of the protective effect of the detergent are as follows. Triton WR 1339 protects the membrane against the harmful action of treatment at pH 5.0 for 37°C through interaction with the lipoproteins of the lysosomal membranes [12]. Lysosomes isolated from the liver of rats with chronic toxic hepatitis become more sensitive to this procedure [3]. The possibility of the appearance of a mechanism of nonspecific protection in response to injection of the detergent cannot be ruled out, by analogy with other substances (trypan blue, charcoal), that are inducing agents for the reticuloendothelial system [9, 11]. Activation of the Kupffer cells with Triton WR 1339 probably leads to increased utilization of the necrotic masses on account of the more effective discharge of the contents by heterolysis.

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