ACTION OF SODIUM AUROTHIOMALATE IN ACUTE TOXIC HEPATITIS

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The effect of the lysosomotropic compound sodium aurothiomalate on the character of the liver damage and changes in the lysosomes in acute toxic hepatitis was studied. After the combined action of this compound and CCl_4 the extent of spread of necrosis was reduced and the area of zones with degenerative changes in the parenchymatous cells of the liver was increased. The degree of solubilization of acid RNase was reduced. Nonsedimented β-galactosidase and cathepsin D activity was the same as in CCl_4 hepatitis.

KEY WORDS: rat liver lysosomes; toxic hepatitis; lysosomotropic substances.

Sodium aurothiomalate (SA) belongs to the recently distinguished group of "intralysosomal modifiers," with the aid of which direct action on the cell through changes in the lysosomes is possible [10]. If administered in vivo, SA is assimilated by the lysosomes, accumulates in them, and sharply reduces the rate of proteolysis [8, 9]. Such changes in the functional state of the lysosomes, it might be supposed, would act favorably during the development of liver damage.

In the investigation described below the effect of SA on liver damage in acute toxic hepatitis was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 35 male Wistar rats weighing 180-220 g. Intact rats (group 1) served as the control. The animals of group 2 received an intraperitoneal injection of SA (gold sodium thiomalate, Aldrich Chemical Co. Inc., Milwaukee, USA) in a dose of 25 mg/kg body weight. Given in this dose, the compound depresses the rate of proteolysis in the lysosomes but has no significant effect on destruction of the particles [8]. In the rats of group 3 acute toxic hepatitis was produced by intragastric administration of CCl_4 in a dose of 0.15 ml/100 g body weight, and the animals of group 4 received SA by intraperitoneal injection simultaneously with the CCl_4 as indicated above. In the experiments of all groups material was taken 24 h after administration of the various substances.

The integrity of the lysosomes was studied by determining the liberation of β-galactosidase [3, 4], acid phosphatase, acid RNase, and cathepsin D into the supernatant fraction. The preparative procedures and determination of acid phosphatase and acid RNase activity were carried out by methods described previously [1, 2]. To determine β-galactosidase, β-glucosidase, and cathepsin D activity Barrett's method [7] was used. The results of determination of the nonsedimented activity of the lysosomal enzymes were expressed as percentages of total activity.

Parallel samples of material were taken for histological and electron-microscopic investigation. The liver samples were prepared by the usual method.

EXPERIMENTAL RESULTS

Administration of SA was followed by solubilization of β-galactosidase and an accompanying decrease in nonsedimented activity of acid RNase and cathepsin D (Table 1). The total activity of cathepsin D (and also of acid RNase, β-galactosidase, and β-glucosidase; Fig. 1), it must be noted, not only was not reduced, but was

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TABLE 1. Changes in Nonsedimented Activity of Lysosomal Enzymes (in % of total activity) after Administration of SA to Rats with CCl_4 Hepatitis

Group of animals	β-Galactosidase	Acid RNase	Cathepsin D
Intact	4,6±0,53	9,1±0,99	11,2±0,65
SA	19,6±1,67	6,0±0,94	8,7±0,10
CCl_4	7,6±0,51	40,3±8,70	20,6±4,42
SA + CCl_4	11,2±1,57	8,3±0,63	14,9±1,94
P_{1-2}	<0,001	<0,05	<0,05
P_{1-3}	<0,05	<0,05	<0,05
P_{1-4}	<0,05	—	—
P_{2-4}	<0,05	—	—
P_{3-4}	<0,05	<0,001	—

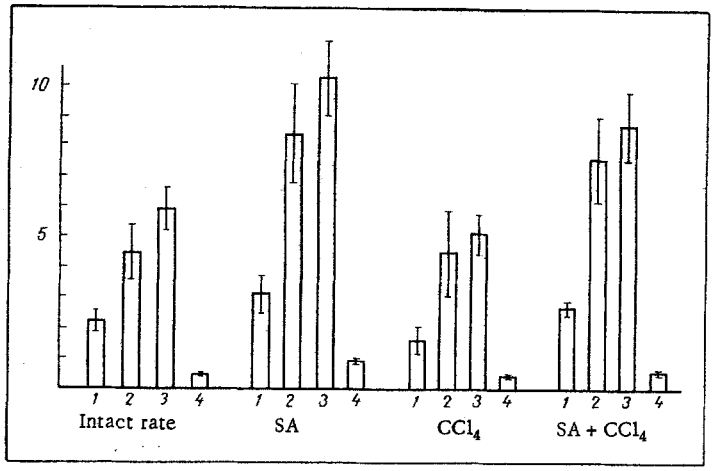

Fig. 1. Changes in total lysosomal enzyme activity in liver of rats with CCl_4 hepatitis following administration of SA: 1, 4) activity of β-galactosidase and β-glucosidase, respectively (in μ moles p-nitrophenol/min/mg protein); 2) activity of acid RNase (in μg RNA/min/mg protein); 3) activity of cathepsin D (in μg tyrosine/min/mg protein).

actually increased although, according to Davies [9], after administration of SA the digestive power of the lysosomes with respect to albumin-^{125}I is reduced by almost two-thirds. This is a result of blocking of thiol-dependent lysosomal enzymes belonging to the cathepsin B group. Under the experimental conditions used, the organization of the hepatocytes differed only a little from that in intact rats. Some increase was observed in the number of small and large vacuoles of the Golgi complex and lysosomes compared with the control. The lysosomes were mainly secondary and contained granules measuring 20-25 nm (Fig. 2).

The development of acute toxic CCl_4 hepatitis was accompanied by solubilization of all the lysosomal enzymes studied. The changes in nonsedimented acid RNase activity were particularly marked. Meanwhile, the degree of solubilization of β-galactosidase was less than after administration of SA. The decrease observed in the total β-galactosidase activity could evidently be regarded as an indication of a decrease in the number of primary lysosomes, which are rich mainly in this enzyme [4]. Morphological investigation at this period showed the presence of centrilobular foci of necrosis. These affected about one-fifth of the area of the hepatic lobules. Zones in which the cells were in a state of balloon degeneration could be clearly distinguished. Some cells of the intermediate zones of the hepatic lobules and hepatocytes located at their periphery had an unchanged structure of their cytoplasmic organoids. In zones of degenerative changes many of the hepatocytes were reasonably isomorphic in their ultrastructural organization. The changes consisted of increased translucency of the ground substance of the cytoplasm, an increase in the number and size of the lipid inclusions, and the absence of glycogen granules. The granular reticulum was fragmented and vacuolated, and the majority of the attached ribosomes were lost. The mitochondria were smaller than in the control, their matrix was

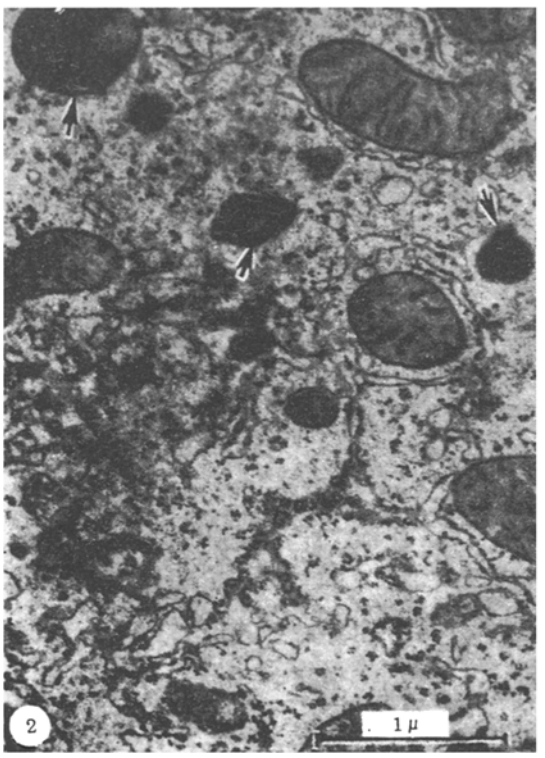

Fig. 2

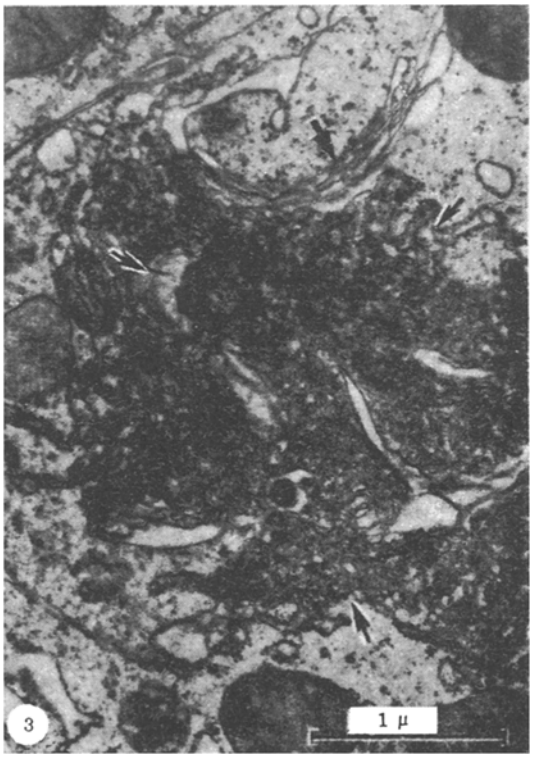

Fig. 3

Fig. 2. Ultrastructure of hepatocyte of rat 24 h after administration of SA. Arrows indicate lysosomes, 30,000×.

Fig. 3. Cytoplasm of centrilobular hepatocyte 24 h after combined administration of SA and CCl_4. Top arrows indicate cluster of smooth membranes, bottom arrow indicates Golgi complex, 28,600×.

denser, and they had fewer cristae. A characteristic feature of the Golgi complex was loss of the vesicular components (both large and small) at its poles. The number of lysosomes was somewhat smaller (than in the hepatocytes of the control animals) and secondary lysosomes predominated. Nearer to the zones of necrosis there were individual cells with degenerative changes which, unlike those described above, contained small clusters of smooth membranes. In the hepatocytes with an intact organization the number of large and small vesicles in the zone of the Golgi complex and also the number and size of the lysosomes were increased. Most of the lysosomes were secondary.

After combined administration of SA and CCl_4 the nonsedimented acid RNase activity was reduced compared with after CCl_4 alone and there was also a tendency toward a decrease in the nonsedimented cathepsin D activity. The values of nonsedimented β-galactosidase activity were higher than those in intact animals. After combined administration of SA and CCl_4 the total activity of the various lysosomal enzymes studied was higher than in CCl_4 poisoning alone. Just as in the experiment with CCl_4 alone, cells with necrotic changes were observed in the centers of the hepatic lobules, but the area of the zones of necrosis was much smaller. Besides cells in a state of balloon degeneration, hepatocytes with changes of the cloudy swelling type were observed. Besides a marked decrease in the area of the foci of necrosis, zones of cells with degenerative changes were larger, mainly on account of the cells of the central and, to some extent, of the intermediate zones in which, in the experiment in which CCl_4 alone was given, the hepatocytes underwent necrosis. The cells of these zones were characterized by a wide spectrum of structural changes: from prenecrotic to mild degenerative. Many degeneratively changed cells contained many large clusters of smooth membranes (Fig. 3). The number of lysosomes, chiefly secondary, in the cells with degenerative changes was on the whole smaller than in the hepatocytes of intact animals, but it varied considerably depending on the degree of injury to the ultrastructures of the hepatocytes. They were more numerous in the less damaged cells. Lysosomes in hepatocytes with an intact ultrastructure were chiefly secondary and, on the whole, they had the same quantitative and qualitative characteristics as similar cells in rats receiving CCl_4 alone. This polymorphism as regards the organization

of the cells of the central and intermediate zones of the hepatic lobules, and also the ratio between the volumes of zones with predominantly necrotic and degenerative changes in the hepatocytes were characteristic of the earlier stages (6 h) of the pathological process developing after administration of CCl_4 [5, 6].

If SA is administered together with CCl_4 to animals it thus considerably modifies the physicochemical properties of the lysosomes. Since in intact animals SA reduces the digestive power of the lysosomes, it has been suggested that the protective effect observed when administered together with CCl_4 could be associated with this property of the compound. However, after combined administration the values for the nonsedimented cathepsin D activity were the same as in the experiment with CCl_4 alone, and the total activity was actually higher. Consequently, the observed decrease in liver damage is difficult to explain by inactivation of cathepsin D. This effect could perhaps be due to a decrease in the nonsedimented acid RNase activity, which is known to be much higher in the lysosomes of Kupffer cells than in hepatocytes. The use of "lysosomotropic modifiers" in certain pathological situations thus appears to be genuine and calls for further study of the mechanisms of their action on cell function with special allowance for the possible late results of their administration.

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