INCREASED PERMEABILITY OF LYSOSOMAL MEMBRANES IN RABBITS POISONED WITH HEXACHLOROBENZENE

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Administration of the fungicide hexachlorobenzene (HCB) to experimental animals causes damage to the liver parenchyma [4, 7, 12-15, 18], accompanied by autophagy and by increased formation of secondary lysosomes [7, 15, 18]. In previous experiments on rabbits [6] with porphyria induced experimentally by administration of HCB for 20 days the writers showed an increase in β-N-acetylglucosaminidase and α-mannosidase activity in the blood serum. There is no information in the literature on changes in the stability of the lysosomal membrane in rabbits during brief and more prolonged adminstration of this fungicide.

The object of the present investigation was to study the permeability of the lysosomal membrane during the first days of HCB poisoning and also during chronic poisoning.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 34 male chinchilla rabbits. The 15 animals (weighing 1080-1300 g) used in series I were divided into four groups. Intact animals of group 1 (four rabbits) served as the control. Animals of the experimental groups received a single dose of HCB of 280 μmoles/kg body weight (diluted in 0.4 ml sunflower oil) by gastric tube and were killed 16 h (group 2) and 48 h (group 3) later. The animals of group 4 received the same dose of HCB for 1 week, after which they also were killed.

In the experiments of series II 19 rabbits weighing 2050-2950 g were used and were divided into two groups: control (six rabbits) and experimental (13). The experimental animals received HCB in a dose of 105 μmoles/kg body weight for 50 days.

The animals were decapitated and the blood serum obtained. Activity of β-N-acetylglucosaminidase (β-AGA) (E.C. 3.2.1.30) and of α-mannosidase (α-MS) (E.C. 3.2.1.24) was determined by Ockerman's method [16] and expressed in nanocatals/ml serum (i.e., in nanomoles 4-nitrophenol split off per second per liter of serum). In the experiments of series II total (after treatment with triton X-100 in a final concentration of 0.1%) and free activity [9] in the homogenate and unsedimented [5] activity (in the supernatant after centrifugation of the freshly prepared homogenate at 100,000g for 30 min) of β-AGA were determined by the spectrophotometric method of Bradley and Tappel [5]. Free and unsedimented activity was expressed as percentages of total activity, and served as an indicator of permeability of the lysosomal membranes.

EXPERIMENTAL RESULTS

Activity of the various enzymes studied in the blood serum in the animals of the experiments of series I was unchanged 16 and 48 h after administration of a single dose of HCB (Fig. 1). In response to administration of the above-mentioned dose of HCB for 1 week activity both of β-AGA ($P < 0.02$) and of α-MS ($P < 0.05$) was increased compared with the control. In an experiment of this duration no abnormalities of porphyrin metabolism could be found.

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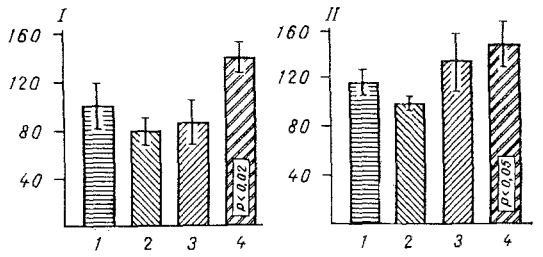

Fig. 1. Changes in activity of serum lysosomal enzymes during brief administration of HCB. 1) Control; 2 and 3) experimental animals killed 16 and 48 h, respectively, after administration of HCB; 4) experimental animals killed 7 days after daily administration of HCB, P) significance of differences compared with control. Ordinate: I, II) activity of α-AGA and β-MS, respectively (in nanocatals/ml serum; M ± m).

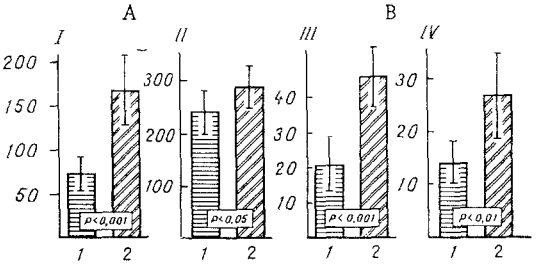

Fig. 2. Changes in activity of lysosomal enzymes in blood serum and liver of rabbits poisoned with HCB for 50 days. 1) Control; 2) experiment. A) Blood serum; B) liver. Ordinate: I, II) activity of β-AGA and α-MS, respectively (in nanocatals/ml serum); III, IV) free and unsedimented β-AGA activity respectively (in % of total activity).

In the experiments of series II (Fig. 2) β-AGA activity after daily administration of HCB for 50 days in a dose of 105 μmoles/kg body weight was more than doubled ($P < 0.001$). α-MS activity also was increased ($P < 0.05$). Both the free ($P < 0.001$) and the unsedimented ($P < 0.01$) activity of the first enzyme in the liver was doubled in these experiments. No change was found in the excretion of ALA, porphobilinogen, uroporphyrin, and coproporphyrin with the urine and of uro-, copro-, and protoporphyrin with the feces.

Mathematical analysis showed positive correlation between β-AGA and α-MS activity in the blood serum ($r = 0.589$, $P < 0.01$), between β-AGA activity in the serum and the relative percentage of free β-AGA activity ($r = 0.788$, $P < 0.001$), between β-AGA activity in the serum and the relative percentage of its unsedimented activity ($r = 0.578$, $P < 0.01$), and between the relative percentages of free and unsedimented β-AGA activity ($r = 0.886$, $P < 0.01$). The results are evidence that the above parameters underwent parallel changes in the same direction. Similar correlations were observed in experiments in which thioacetamide was used as the hepatotoxic agent [1].

Increased β-AGA and α-MS activity in the serum, together with the increased percentage of free and unsedimented activity of β-AGA in the liver show that under the present experimental conditions HCB increases the permeability of lysosomal and cell membranes. This effect of HCB is manifested very early — by the 7th day of the experiment (Fig. 1). Similar

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The increase in permeability of lysosomal membranes during HCB administration can be explained by different factors: by the direct toxic action of HCB on the liver parenchyma, by a reduction in the ATP content in the liver [12], by an increase in the concentration of porphyrin metabolites in the tissues [10, 11] and also, possibly, by retention of Fe in the liver [17, 18]. In patients with porphyria cutanea tarda we also found increased serum β-AGA activity [3, 6]. The isolated or combined action of the above-mentioned factors leads to trophic changes in the liver parenchyma [13, 18]. Under these circumstances increased autophagy develops, with an increase in formation of secondary lysosomes [13, 15], the membrane of which is more labile.

Increased permeability of lysosomal membranes as a result of administration of HCB, observed in these experiments, is evidence that lysosomes and their acid hydrolases participate in the pathogenesis of this type of poisoning.

LITERATURE CITED

1. I. Apostolov, I. Goranov, and G. Semeonov, Probl. Khraneneto (Sofia), 1, 57 (1979).
2. I. Apostolov, E. Ivanov, V. Pramatarova, et al., Khig. Zdraveopazv. (Sofia), 24, No. 1, 63 (1981).
3. D. Adzharov and I. Apostolov, Suvr. Med. (Sofia), 31, No. 1, 34 (1980).
4. E. Ivanov and P. Dimitrov, Eksp. Med. Morfol. (Sofia), 19, No. 4, 200 (1970).
5. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
6. I. Apostolov, E. Ivanov, and D. Adzharov (D. Adjarov), Enzyme, 21, 289 (1976).
7. A. Böger et al., Arch. Pathol. Anat. A, 382, 127 (1979).
8. D. W. Bradley and A. L. Tappel, Anal. Biochem., 33, 400 (1970).
9. C. De Duve, in: Subcellular Particles (T. Hayshi, ed.), New York (1958), pp. 128-159.
10. E. D. Ivanov et al., in: Porphyrins in Human Disease (M. Doss, ed.), Basel (1976), pp. 438-444.
11. E. Ivanov et al., Enzyme, 21, 8 (1976).
12. E. Ivanov (E. Iwanow) et al., Acta Hepato-Gastroent., 20, 39 (1973).
13. A. Medline et al., Arch. Pathol., 96, 61 (1973).
14. C. E. Mendoza, D. L. Grant, and J. B. Shields, Environ. Physiol. Biochem., 5, 460 (1975).
15. H. M. Mollenhauer et al., Am. J. Vet. Res., 36, 1777 (1975).
16. P. A. Öckerman, Clin. Acta, 20, 1 (1968).
17. C. A. Seymour and T. J. Peters, Br. J. Haemat., 40, 239 (1978).
18. A. H. Timme et al., S. Afr. Med. J., 48, 1833 (1974).