ACTIVITY OF SERUM LYSOSOMAL ENZYMES IN RATS WITH EXPERIMENTAL LEAD POISONING

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The effect of experimental lead poisoning on the permeability of the lysosomal membrane was investigated in albino rats. Activation of two lysosomal enzymes, α-mannosidase and β-acetylglucosaminidase, was found in the blood serum as early as on the third day of daily administration of lead acetate (20 mg/kg) to the rats. Injury to the lysosomal membrane evidently plays an important role in the pathogenesis of lead poisoning.

KEY WORDS: experimental lead poisoning; lysosomal enzymes; membrane permeability.

Some heavy metals, including lead, are known to accumulate in the lysosomes [8, 11]. This may perhaps be explained by their intracellular transport and excretion. A similar role of the lysosomes has been demonstrated for iron salts [6]. Lead has a toxic action on the cell and mitochondrial membranes [9, 10, 13]. It is interesting to determine the effect of this heavy metal on the permeability of the lysosomal membrane because lysosomes contain numerous hydrolytic enzymes [4], the liberation of which is a potential danger to the cell [2, 5]. Acid phosphatase activity in the blood serum of guinea pigs is known to rise after administration of lead salts. Acid ribonuclease activity is unchanged under these conditions [14].

The object of this investigation was to study the effect of lead on the permeability of the lysosomal membrane in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 49 male Wistar albino rats weighing initially 150 ± 10 g. The animals were subdivided into 7 groups, each consisting of 7 rats. Two control groups were studied on the 1st and 20th days of the experiment. The animals of the experimental groups were given an aqueous solution of lead acetate (20 mg/kg) by mouth through a tube. Blood was taken for investigation after decapitation of the animals on the 1st, 3rd, 5th, 10th, and 20th days of the experiment.

The lead content in the liver was estimated after mineralization [3] with an LP-60 polarograph. The lead content was determined by the standard addition method. The activity of two lysosomal enzymes was determined in the serum of the animals: β-acetylglucosaminidase (EC 3.2.1.30) by the method of Koizumi et al. [12] and α-mannosidase (EC 3.2.1.24) by the method of Bradley and Tappel [7]. The results were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS AND DISCUSSION

Graphs showing changes in the lead concentration in the liver and in the activity of lysosomal enzymes in the serum in the course of the experiments are given in Figs. 1 and 2. The lead concentrations in the animals of the 1st and 2nd control groups was 0.081 ± 0.021 and 0.070 ± 0.078 μg/g wet weight of tissue respectively. It will be clear from Fig. 1 that on the 1st, 3rd, and 5th days after poisoning the lead concentration in the liver was considerably increased.

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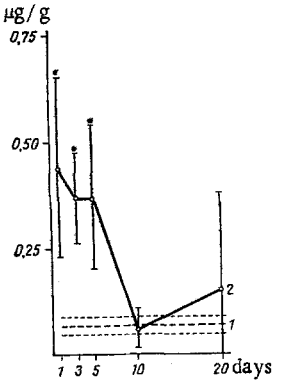

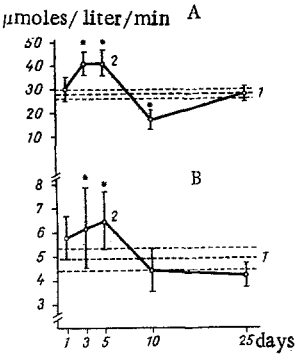

Fig. 1 Fig. 2

Fig. 1. Lead concentration in liver of animals with experimental lead poisoning: 1) control; 2) experiment. Values for which $P < 0.05$ marked by asterisk. Ordinate, lead concentration (in μg/g wet weight of tissue); abscissa, days of experiment.

Fig. 2. Serum β-acetylglucosaminidase (A) and α-mannosidase (B) activity in experimental lead poisoning. Ordinate, enzyme activity (in μmoles substrate/liter/min); abscissa, days of experiment. Remainder of legend as in Fig. 1.

The serum α-mannosidase activity of the control animals was 4.88 ± 0.44 μmoles p-nitrophenol/liter/min, whereas the β-acetylglucosaminidase activity was 28.77 ± 2.16 μ/liter/min. The α-mannosidase activity was increased (Fig. 2) on the 3rd ($P < 0.05$) days after daily administration of lead. The serum β-acetylglucosaminidase activity was increased on the 3rd ($P < 0.01$) and 5th ($P < 0.001$) days of poisoning but was lowered on the 10th day ($P < 0.001$).

Changes in the lead concentration in the liver on the first days of poisoning are evidence that lead accumulates rapidly in that organ, for by the method of administration used the whole of the lead must pass through the liver. Normalization of the lead concentration after the 10th day of the experiment can be explained by activation of the deposition of lead in the bones and its elimination with the urine and bile.

The increase in α-mannosidase and β-acetylglucosaminidase activity found on the 3rd day after lead poisoning is evidence that the permeability of the lysosomal membrane is disturbed very early during poisoning. The actual organ responsible for this increased serum activity could not be determined reliably before methods of determining organ-specific isozymes of lysosomal hydrolases had been devised. Since lead poisoning leads to an increase in the activity of some serum enzymes specific for the liver [14], the main source of lysosomal enzymes in the serum must be presumed to be the liver. This hypothesis was confirmed by the discovery that the highest activity of both lysosomal enzymes in the serum was observed after a maximal increase in the lead concentration in the liver. Some of the increased activity of the serum lysosomal enzymes may perhaps have been of renal origin, for lead also accumulates in the kidneys as early as on the first days after its administration [9].

The cause of the decrease in β-acetylglucosaminidase activity on the 10th day of poisoning could not be confidently determined. It can tentatively be suggested that lead has an inhibitory action on the activity of this enzyme, just as on the activity of another lysosomal enzyme, acid phosphatase [15]. This may probably be due also to the secondary action of lead on the system of possible inhibitors or activators of this enzyme in the serum or on the synthesis, degradation, and elimination of the enzyme.

In conclusion it must be emphasized that the model of lead poisoning chosen causes early changes in permeability of the lysosomal membrane in vivo, which are accompanied by a parallel increase in the lead concentration in the liver of the experimental animals.

LITERATURE CITED

1. O. N. Kassandrova and V. V. Lebedev, Analysis of the Results of Observations [in Russian], Moscow (1970).
2. T. A. Korolenko, V. G. Titova, S. G. Dobrovol'skaya, et al., Byull. Éksp. Biol. Med., No. 1, 21 (1975).
3. E. M. Mal'kov, U. S. Sultanova, and A. T. Stromberg, Lab. Delo, No. 3, 131 (1971).
4. A. A. Pokrovskii and V. A. Tutel'yan, Uspekhi Sovrem. Biol., 68, No. 3, 318 (1969).
5. A. A. Pokrovskii, V. A. Tutel'yan, L. E. Olifson, et al., Byull. Éksp. Biol. Med., No. 7, 38 (1974).
6. W. D. Bradford, J. G. Elchlepp, A. U. Arstila, et al., Am. J. Pathol., 56, 201 (1969).
7. D. W. Bradley and A. L. Tappel, Anal. Biochem., 33, 400 (1970).
8. A. Brun and U. Brunk, J. Histochem. Cytochem., 19, 820 (1970).
9. N. Castellino and S. Aloj, Brit. J. Ind. Med., 26, 139 (1969).
10. S. Goldfischer and J. Moskal, Am. J. Pathol., 48, 305 (1966).
11. H. Koenig, J. Histochem. Cytochem., 11, 120 (1963).
12. T. Koizumi, T. Suematsu, N. Iwabori, et al., Biochim. Biophys. Acta, 151, 628 (1968).
13. P. Michaux, H. L. Boiteau, and F. Tolot, Arch. Mal. Prof., 32, 2 (1971).
14. G. C. Secchi, L. Alessio, and F. Spreafico, Enzyme, 12, 63 (1971).
15. J. Sroczynski and K. Zajusz, Arch. Immunol. Ther. Exp., 14, 391 (1966).