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Studies on the Influence of Certain Heavy Metals on Acid Phosphatase Activities

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수종 중금속에 의한 Acid Phosphatase의 영향에 관한 연구

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적 요

$HgCl_2$ 5 mg/kg body wt., 10 mg/kg body wt. 그리고 20 mg/kg body wt.와 $CdCl_2$ 10 mg/kg. body wt., 15 mg/kg body wt.와 20 mg/kg body wt.를 mouse의 복강에 주사한 후 각각 24시간, 48시간 그리고 72시간 후에 간을 적출하여 disodium p-nitrophenyl phosphate를 기질로 하고 Mundry 비색법으로 acid phosphatase 활성도를 측정하여 다음과 같은 결과를 얻었다. 즉 $HgCl_2$는 5 mg/kg body wt.를 처리한 후 24시간 경과 후 3.47 mg Pi/ml/0.5 hr이었고, 72시간 경과 후에는 5.00 mg Pi/ml/0.5 hr이었고, 20mg/kg body wt.에서는 24시간 경과 후 6.79 mg Pi/ml/0.5 hr로, 72시간 경과 후에는 3.47 mg Pi/ml/0.5 hr로 나와 대조군의 8.3 mg Pi/ml/0.5 hr의 약 0.5배로 나타났다. 또한 $CdCl_2$는 15 mg/kg. body wt.와 20 mg/kg body wt.는 모두 죽었으나, 10 mg/kg body wt.에서는 거의 50% 정도가 살았고, 24시간, 48시간 그리그 72시간 경과 후에 거의 모두가 11.2 mg Pi/ml/0.5 hr로 나와 대조군의 8.63 mg Pi/ml/0.5 hr보다 1.5배 정도 높게 나타나 activate시킴을 보여 주었다.

INTRODUCTION

Rapid industrial development inadvertently contributes toward environmental pollution, and evidently results in deterioration of the public health. The presence of heavy metals among industrial wastes particularly creates a serious ecological

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problem because they tend to accumulate in the living system. Certain aquatic organisms are immediately suffered by these heavy metals, which eventually reach the human level throuh the food chain system. Pharmaceuticals, agricultural chemicals and industrial wastes are well known sources of mercury in the environment in inorganic as well as organic forms.

There are many investigations under way concerning effects of mercury on the biological system. Wojtalik (1971) reported his work on distribution and accumulation of mercury in various organisms and its mode of action. Localization of mercury in contaminated cells was studied with labelled mercury (Bergstrand *et al.*, 1958; Norseth, 1968). Acid phosphatase of the kidney lysosome is known to be activated by application of a sublethal dose of mercury but reduced to the normal level upon removal of the poison through the tubular release (Taylor, 1965). Effects of mercury poisoning on changes in the fine structure of the rat kidney were reported (Gritzka, 1968). Fowler (1972) observed increased rough endoplasmic reticulum, deformation of mitochondria, and increased in the number of cytolysome in the rat kidney by CH_3HgCl. Structural changes are known to occur in response to mercury even in the nucleus where karyolysis and a loss of the chromatid were observed in the mouse kidney (Ganote, 1974) and in the liver as well (Mizuhira, 1975).

Cadmium, another heavy metal well known as an industrial pollutant, is reported to be responsible for the "Itai-Itai" disease in Japan. Clinical investigations revealed that workers exposed to cadmium had high levels of the metal in the liver and the kidney and developed a kidney disorder (Friberg, 1950, 1959; Piscator, 1962). Symptoms for the chronic cadmium poisoning are reported to include pulmonary emphysema, proteinuria, and renal damages (Friberg, 1959). Vander (1962) analyzed cadmium contents in various tissues of infected animals. Accumulation and distribution of cadmium in animal tissues were also investigated with a radioisotope (Gum and Gould, 1957; Berlin and Ullberg, 1963). According to these investigations, cadmium is known to be accumulated in the kidney, liver, spleen, pancreas, adrenal gland and the testes. In the rat liver and kidney, Singhal (1974) reported increased activities of pyruvate carboxylase, phosphopyruvate carboxylase and hexosediphosphatase, and a reduced level of the liver glycogen by the cadmium treatment.

In order to elucidate the actions of mercury and cadmium, we have investigated, in the present work, acid phosphatase activities in the mouse liver from experimentally treated animals.

MATERIALS AND METHODS

Materials

Mice (ICR) of both sexes in the range of 15 to 20 g body weight (average body weight, 17.5 g) were raised with mixed feed at room temperature (18~20° C) and divided into the control and experimental groups.

Disodium p-nitrophenyl phosphate (MW. 371.15) was purchased from Tokyo Kasei. Kokyo, and was used as the substrate for the assay of acid phosphatase.

Methods

1. Treatments

Physiologic saline (0.86% NaCl) containing $HgCl_2$ and $CdCl_2$ at various concentrations were used. The animals were divided into appropriate groups. and 0.2 ml of the solution containing 0, 5, 10 and 20 mg $HgCl_2$ per kilogram body weight, and 0, 10, 15 and 20 mg $CdCl_2$ per kilogram body weight was administered into the abdominal cavity with a syringe. The liver was taken from the animal at 24, 48 and 72 hrs after the abdominal administration.

2. Enzyme preparations

The liver was excised from the animal immediately upon killing. The tissue was homogenized with 0.1 M citrate buffer at pH 4.8 (1 ml/g fr. wt. tissue). The homogenates were diluted with the buffer and centrifuged at 3000×g for 30 min. The pellets were discarded, and the supernatant was used for the enzyme preparations. Ammonium sulfate (1.08 g) was added into 2 ml of the supernatant to make it 70% saturated. This was laid overnight, and the precipitate was obtained upon centrifugation at 3000×g for 30 min. The pellets were dissolved with 0.8 ml of citrate buffer at pH 4.8, and used as "crude enzyme". All the above mentioned procedures were carried out at 4° C.

3. Assay of acid phosphatase

The reaction mixture, which consists of 2.9 ml of 0.1 M citrate buffer at pH 4.8, 1 ml of 0.078% disodium p-nitrophenyl phosphate, and 0.1 ml of crude enzyme, were incubated at 37 °C for 30 minutes. At the end of this period, 1 ml of isobutanol was added to the reaction mixture. One ml of ammonium molybdate solution was added to react with the phosphate, and this was diluted again with 4 ml of isobutanol. Four ml of the seperated organic phase was mixed with 2.7 ml of $SnCl_2$-HCl for spectrophotometric measurements. A modified Mundry method (1958) was employed to assay the enzyme by measuring absorbance at 540 nm with a Beckman spectrophotometer DB-G.

RESULTS

Changes in acid phosphatase activities in the mouse liver following administration of the heavy metals.

1. Effects of $HgCl_2$

With the dose of 5 mg/kg body weight, the enzyme activities are reduced to 3.47 mg Pi/ml/0.5 hr at 24 hr after the treatment, but is slowly recovered to 5.00 mg Pi/ml/0.5 hr. However, with the higher doses (10 and 20 mg/kg body weight), the activities measured are 5.26 and 6.79 mg Pi/ml/0.5 hr respectively, and are further decreasing with time until 72 hr after injection to 4.00 and 3.47 mg Pi/ml/0.5 hr respectively. As illustrated in Fig. 1, mercury poisoning appears to result in reduced activities of the enzyme in the liver.

Figure 2 illustrates changes in the enzyme activities as a function of time. At 24 hr after the treatment, the activities measured are 3.47, 5.26 and 6.79 mg Pi/ml/0.5 hr for treatments with 5, 10 and 20 mg/kg body weight respectively, indicating that the activities increase with increasing doses at this period. At 48 hr, the activities measured for all three doses are around 4.6 mg/Pi/ml/0.5 hr, but the activities tend to decrease, at 72 hr, with increasing doses; these are 5.00 and 3.47 mg Pi/ml/0.5

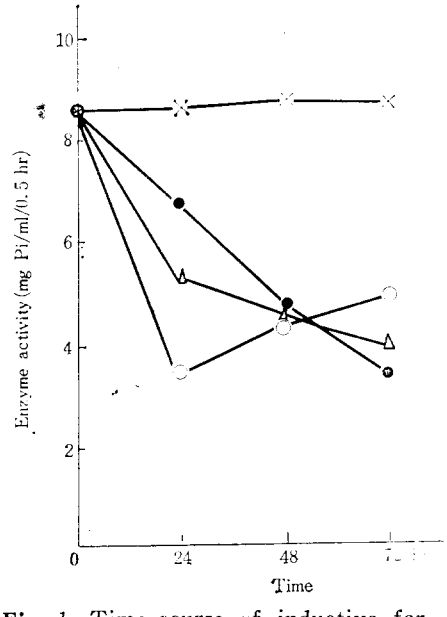

Fig. 1. Time course of inductive formation of acid phosphatase in the mouse liver treated with different $HgCl_2$ concentration.
× ; control,
○ ; 5 mg/kg body wt.,
△ ; 10 mg/kg body wt.,
● ; 20 mg/kg body wt.

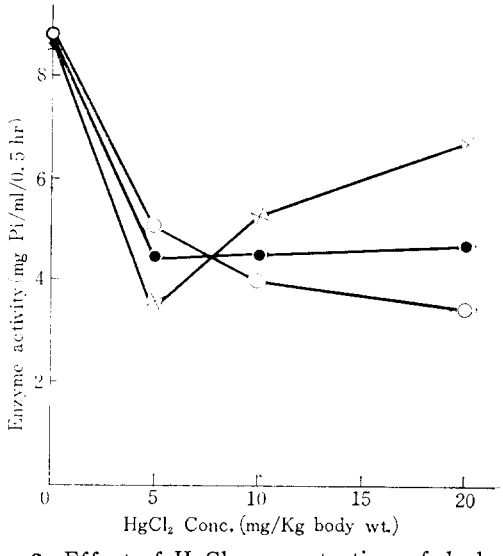

Fig. 2. Effect of $HgCl_2$ concentration of body weight on acid phosphatase activity in the mouse liver.
× ; 24 hrs,
● ; 48 hrs,
○ ; 72 hrs.

hr for 5 and 20mg $HgCl_2$/kg body weight respectively. Therefore, it can be concluded that the enzyme activities appear to decrease with increasing doses of $HgCl_2$ and with time following its administration (Table 1).

Table 1. Effect of $HgCl_2$ on the acid phosphatase in the mouse liver.

Hour	24			48			72		
Conc. of $HgCl_2$/body wt. (mg/kg)	Total liver wt. (gr)	No. of mouse	Enzyme activity mgPi/ml/ 0.5 hr	Total liver wt. (gr)	No. of mouse	Enzyme activity mgPi/ml/ 0.5 hr	Total liver wt. (gr)	No. of mouse	Enzyme activity mgPi/ml/ 0.5 hr
control	4.05	5	8.63	4.80	5	8.79	4.80	5	8.74
5	3.30	5	3.47	4.30	5	4.42	5.20	5	5.00
10	4.30	4	5.26	4.20	5	4.63	5.80	5	4.00
20	1.70	4	6.79	4.50	5	4.74	3.20	4	3.47

Table 2. Effect of $CdCl_2$ on the acid phosphatase in the mouse liver.

Hours	24			48			72		
Conc. of $CdCl_2$/body wt. (mg/kg)	Total liver wt. (gr)	No. of mouse	Enzyme activity mgPi/ml/ 0.5 hr	Total liver wt. (gr)	No. of mouse	Enzyme activity mgPi/ml/ 0.5 hr	Total liver (gr)	No. of mouse	Enzyme activity mgPi/ml/ 0.5 hr
Control	5.10	5	8.63	5.80	5	9.00	5.40	5	8.21
10	5.50	5	11.26	5.40	5	11.15	5.80	5	11.84

2. Effects of $CdCl_2$

With the dose of 10 mg/kg body weight, cadmium seems to activate the enzyme. At 24 hr, the activities measured are 11.26 mg Pi/ml/0.5 hr as against 8.63 mg Pi/ml/0.5 hr for the control(Table 2). At 48 hr, the activity is reduced to 11.15 mg Pi/ml/0.5 hr whereas the control value is increased to 9.00 mg Pi/ml/0.5 hr. At 72hr, the activity is increased to 11.84 mg/Pi/ml 0.5 hr, which is about 0.6 mg Pi/ml/0.5 hr higher than those at 24 and 48 hrs (Fig. 3). The higher doses (15 and 20 mg/kg body weight) all resulted in death of the animals.

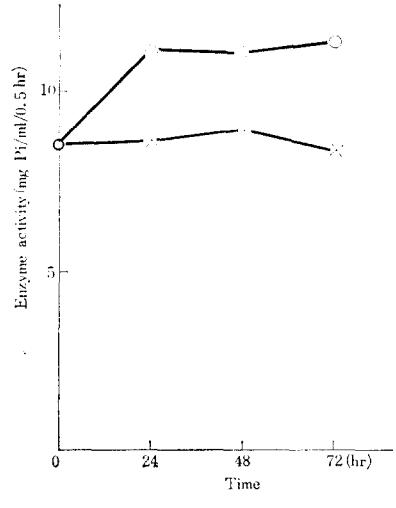

Fig. 3. Time course of inductive formation of acid-phosphatase in the mouse liver treated with different $CdCl_2$ concentration.
× ; control,
○ ; 10mg/kg body wt.

DISCUSSION

Widespread occurrence of mercury and other heavy metal compounds in our surroundings from industrial sources constitutes a major health

hazard, and impose a serious threat to the environment.

Clams in the Minamata Bay, Japan with high levels of organic mercury in the body were attributed to the Minamata disease, which affects the central nerve system, often with high fatalities (Irukayma, 1967). Cadmium, another pollutant of major concern, is known to cause the "Itai-Itai" syndrome (Hagino and Yoshioka, 1961). Gritzka (1968) administered 4~16 mg $HgCl_2$ per kilogram body weight to the rat, and reported degeneration of the epithelial cells of pars recta in the kidney, rupture of the cell membrane, formation of vacuoles, rupture of the ER membrane, dissociation of the ribosomes from the ER membrane, vacuolization of the Golgi body, formation of cytolysomes, mitochondrial swelling, loss of the granular materials from the mitochondrial matrix, and condensation of the chromatid in the nucleus. In the mouse treated with 1 mg $HgCl_2$ per kg body weight, Ganote *et. al.* (1974) observed partial necrosis in the proximal convoluted tubule of the kidney. As early morphological changes, they observed degeneration of the brush border, dispersion of the ribosomes, appearance of vacuoles, and clumping of the chromatid in the nucleus. Mitochondrial deformation was observed to occur in later stages. Similarly, Mizuhira (1975) reported, from his EM studies on the mouse liver and kidney following administration of 1.0 to 2.0 mg $HgCl_2$ per kg body weight, occurrence of enlarged and transparent nucleus, rupture of the plasma membrane and membranes of other organelles, increases in the number of lysosomes and of rough ER, mitochondrial swelling, and loss of the chromatid in cells around the hepatic vein, and occurrence of lysosomes with varying electron densities, condensation of the nucleus, and loss of the chromatid in the kidney.

In the case of cadmium poisoning, Nishizumi (1972) fed rats with cadmium-containing water for a long period, but found no appreciable changes either in the glomerulus or in the distal tubule. However, in the proximal tubule, he observed increased number of lysosomes and mitochondrial deformation. As the dose of cadmium increases, he noted further increases in the number of lysosomes, mitochondrial deformation from the normal elongated from to ovoid or spherical from accompanied with changes of the cristae, increases in the number of microbodies, partial increases in the rough ER, and occurrence of intranuclear inclusions. In quails fed with cadmium-containing water (75 mg/kg), Richardson (1974) found in the small intestine depression of cells in the mucous membrane layer, occurrence of large lysosomes and oil droplets, abnormal increases in the number of the goblet cells, abnormal secretion of mucus, contraction of organelles, enlarged smooth ER membranes in the mucous membrane layer, and appearance of granular materials in the lysosomes of the epithelial cells of the blood vessel.

For enzymatic work with regard to metal poisoning, Jackim (1970) studied activities of alkaline phosphatase, xanthine oxidase, catalase, ribonuclease, and acid

phosphatase in the liver of killifish infected with various heavy metals. Hilton (1975) reported reduced activities of acid phosphatase in the fish liver at 72 hrs after injection of CH_3HgCl_2. Results obtained in the present work indicate that acid phosphatase activities of the animals treated with 10 and 20 mg $Hgcl_2$ per kilogram body weight are lower than those for the controls and tend to decrease further with time. These results are somewhat in parallel with those of Taylor (1965) who reported that the number and size of lysosomes containing products of acid phosphatase increase initially but decrease after 36 hrs in $HgCl_2$-treated rats. The present results indicate, on the other hand, increased activities of the enzyme in cadmium-treated animals (10 mg/kg body wt.) suggesting their relation to the lysosome and its hydrolytic enzymes responsible for detoxification processes.

SUMMARY

Mice were dosed with $HgCl_2$ (5, 10 and 20 mg per kilogram body weight) and $CdCl_2$ (10, 15 and 20 mg per kilogram body weight) by the abdominal injection. Acid phosphatase activities of the liver at 24, 48 and 72 hours following the injection were measured by the Mundry colorimetric method using disodium p-nitrophenyl phosphate as the substrate, and the following results were obtained. The enzyme activities measured were 3.47 mg Pi/ml/0.5 hr at 24 hr and 5.00mg/ml/0.5 hr at 72 hr respectively following the injection of 5 mg/kg body weight of $HgCl_2$ and 6.79 mg Pi/ml/0.5 hr at 24 hr and 3.47 mg Pi/ml/0.5 hr respectively following the injection of 20 mg/kg body weight of the mercury compound, as compared with the activity of 8.3 mg Pi/ml/0.5 hr for the control. With the cadmium treatment, about 50% of the animals injected with 10mg/kg body weight, and none of the animals injected with 15 and 20mg/kg body weight, survived. Of the surviving animals, the sublethal concentration of cadmium was shown to activate the enzyme: the activities at 24, 48 and 72 hr following the injection were around 11.2 mg Pi/ml/0.5 hr as compared with 8.63 mg Pi/ml/0.5 hr for the control.

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