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Effect of Cadmium and Aluminum on Bone Alkaline and Acid Phosphatases

Naoki Sugawara,* Terukazu Sadamoto, and Chieko Sugawara

Department of Public Health, Sapporo Medical College, S-1, W-17, Sapporo 060, Japan

Bone abnormalities caused by Cd have been found in industrial workers who inhaled Cd dust and fumes and in inhabitants exposed environmentally to the metal (FRIBERG et al.1974;TSUCHIYA 1978). The condition manifested by the latter is known as the Itai-Itai disease, which is identical to osteomalacia, often accompanied by osteoporosis. The bone abnormalities are believed to be caused by primary damage to kidney function due to Cd exposure. On the contrary, some investigators reported that Cd directly affected bone (YOSHIKI et al. 1975;KAWAI et al.1976).

It has recently been suggested that Al intoxication may induce osteomalacia in patients on regular hemodialysis (PLATTS et al.1977;WARD et al.1978;ELLIS et al.1979; PIERIDES et al.1980;MAYOR et al.1980). This assumption is supported by the observations that in such patients Al was mainly localized in bone between osteoid and calcified tissues (COURNOT-WITMER 1981), and that in rats given daily i.p. injections of $AlCl_3$ for 20 weeks, osteomalacia developed (ELLIS et al.1979).

Despite the large differences between Cd and Al in their physico-chemical properties, it is interesting that the two metals eventually induce osteomalacia. Therefore, we attempted to investigate in vitro the effects of Cd and Al on acid and alkaline phosphatases in rat calvarium, and in addition, to measure serum alkaline phosphatase in adult animals given Cd or Al.

MATERIALS AND METHODS

Calvaria from 5-day old rats (Wistar Strain) were removed, care being taken not to damage the periosteum. Bones were washed with chilled 0.9 % NaCl solution to remove blood and then were blotted gently with filter paper. About 1.8 g of bone were added to 6 ml of 0.25 M sucrose-50 mM N-tris-(hydroxymethyl)-2-aminoethane sulfonic acid (TES) buffer, pH 7.4, and then were homogenized with a Polytron (Type PT 10-35) at 2 ℃.

* To whom correspondence should be addressed.

The resulting homogenate was centrifuged at 1,000 g for 10 min at 2 °C. The supernatant was further centrifuged at 5,000 g for 10 min. The final supernatant was used as bone extract for the assay of alkaline (EC.3.1.3.1) and acid (EC.3.1.3.2) phosphatases.

Phosphatase activities were measured by the method of BESSY et al. (1946). For the assay of alkaline and acid phosphatases, 0.1 M glycine buffer, pH 10.5, and 0.1 M acetate buffer, pH 4.8, were used, respectively. P-nitrophenylphosphate was used as substrate for both phosphatases. Cd as cadmium chloride or Al as aluminum lactate was added directly into the reaction mixture. After incubation at 37 °C for 30 min, the released p-nitrophenol was measured at 410 nm. The measurement of protein was carried out according to the method of LOWRY et al. (1951).

Mature Wistar strain male rats and Hartley strain male guinea pigs were used for measurement of serum alkaline phosphatase and phosphorus. Ex-1 was composed of 5 control rats, and 6 rats receiving drinking water containing 300 ppm Cd for 30 days. Ex-2 was composed of 8 control guinea pigs, and 7 pigs given a diet containing 2 % $AlCl_3$ and, in addition, injected daily with Al (3.8 mg) as aluminum lactate for 21 days. The control animals were given tap-water and a diet not supplemented with metals. Measurement of serum alkaline phosphatase was carried out by the method described above. Phosphorus was measured by the method of ALLEN (1940).

RESULTS AND DISCUSSION

Fig.1 and 2 indicate the effect of Cd and Al on the two phosphatase activities. Cd showed an inhibitory effect on alkaline phosphatase from 10^{-7}M, and at 10^{-6}M decreased this activity to about 1/2 of the control. Al did not show any stimulation or inhibition on the two enzyme activities from $10^{-8}$to 10^{-4} M. An assay above 10^{-4} M was not performed, since the buffer action collapsed due to the acidity of Al solution.

We previously demonstrated that Cd inhibited the alkaline phosphatase of rat intestinal brush border in vitro and in vivo (SUGAWARA & SUGAWARA 1975). From this previous observation, the in vitro action of Cd on bone alkaline phosphatase was substantially expected. LIEBERHERR et al.(1982) recently reported that acid and alkaline phosphatases in a bone extract obtained from the cultivation of rat calvarium was stimulated by the addition of $3\times10^{-11}$to 1.5×10^{-6} M Al as aluminum chloride or sulfate. When Al was added to the cultivation mixture at 3×10^{-6} M, this stimulation was no longer found.

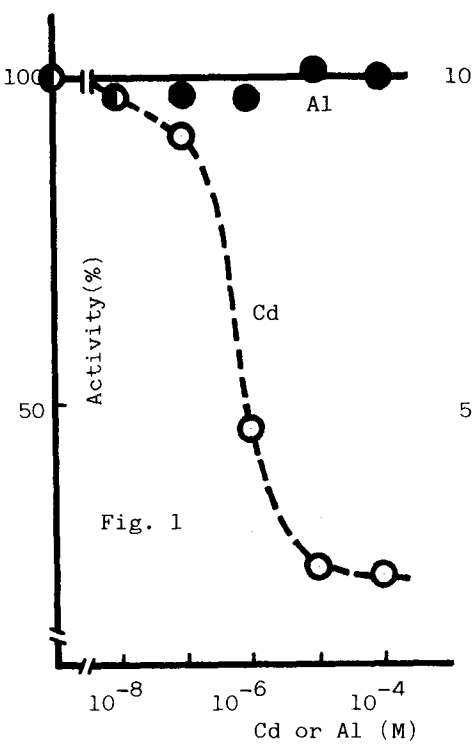

Fig. 1

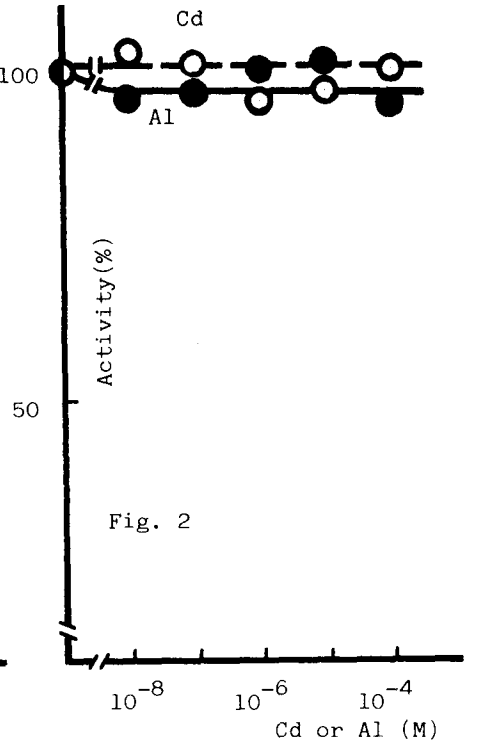

Fig. 2

Figures 1 and 2. Effect of Cd and Al on alkaline and acid phosphatases. Values showing mean of 3-5 trials are expressed as % of the control assay (100 %) without Cd or Al. In alkaline enzyme assay (Fig. 1), the control released $(1.76\pm0.07)\times10^{-2}$ μmoles p-nitrophenol/mg protein/30 min. In acid enzyme assay (Fig. 2), the control released $(0.34\pm0.03)\times10^{-2}$ μmoles p-nitrophenol/mg protein/30 min.

These results differed completely from our observatins described above.

To date, the role of alkaline phosphatase in bone is still under discussion, although previous studies have provided some insight (ROBINSON & SOAMES 1924;FLEISCH & NEUMAN 1961). It is well known that the enzyme is localized in the microvilli of the intestinal mucosa, in the syntrophoblasts of the placenta or in the brush border of the renal proximal tubuli. These facts suggest that this enzyme is involved in the transport of inorganic substances. Such a hypothesis may fit in with the role of the enzyme in hard tissue.

KIMURA et al.(1974) reported that Cd accumulated at 1 ppm level in the bone of rats receiving a diet contain-

ing 300 ppm Cd for 3 weeks, and the Cd did not exist in the form of metallothionein, but associated loosely with other macromolecules. From their results, it may be deduced that Cd may directly influence the enzyme in vivo.

ELLIS et al.(1979) reported that bone Al was 90-124 ppm (ppm ash) in rats injected with $AlCl_3$ (total dose of 33-38 mg Al) for 52 days. In related work, COURNOT-WITMER et al.(1981) proposed that the Al concentration was 57-130 ppm in bones obtained from the patients on regular hemodialysis with histologic severe osteomalacia. However, our results (Fig. 1) do not support the possibility that bone Al directly influences alkaline phosphatase.

Table 1 indicates serum alkaline phosphatase and phosphorus in rats and guinea pigs receiving Cd or Al. These two metals both significantly decreased the enzyme activity, although they showed differences in the enzyme action (Fig. 1). Generally, in osteomalacia, serum alkaline phosphatase increases with a concomitant elevation of bone type isoenzyme. In this study, it could not be clarified whether or not osteomalacia developed in their bones, since, unfortunately, their bones were not followed histologically.

Table 1. Serum Alkaline Phosphatase and Phosphorus

	Ex-1		Ex-2	
	ALPase	Pi	ALPase	Pi
Control	2.02±0.35(5)	5.5±0.5(5)	6.45±0.38(5)	12.6±4.9(8)
Exposure	[a]0.99±0.25(6)	[a]3.9±1.0(6)	[a]4.33±0.53(4)	[b]8.9±1.4(7)

Values are expressed as mean±SD(Bessy-Lowry Unit for alkaline phosphatase, and mg/dl for phosphorus). The number of animals is given within parentheses. ALPase; alkaline phosphatase, Pi; phosphorus. a; $p<0.01$, b; $p<0.05$, by the Student's t-test.

In hypophosphatasia, manifested by rickets in infants and children and by osteomalacia in adults, low serum alkaline phosphatase activity is observed with a concomitant elevation of serum phosphorus (ITOKAWA et al. 1974). In our study, serum phosphorus significantly decreased in the animals receiving Cd or Al (Table 1). Therefore, their condition is not manifested by the general hypophosphatasia described above. The low serum phosphatase induced by Cd or Al may be a transitional condition. The cause of the phenomenon remains obscure.

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