Effects of cadmium, copper or zinc on formation of embryonic chick bone in tissue culture

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In Itai-itai disease, which occurred in Toyama Prefecture of Japan, the bone lesion was osteomalacic. The osteomalacia has been thought to be due to kidney damage caused by a chronic exposure to cadmium. In cadmium-treated animals, however, only osteoporotic change has been observed. We postulated that the etiology of Itai-itai disease may result from a simultaneous exposure to cadmium and some heavy metals including zinc, copper and lead because these heavy metals have been found in cadmium-polluted rice and bones of patients with Itai-itai disease. In the present study, we describe the effect of cadmium, copper or zinc on bone formation by biochemical and histological methods using embryonic chick bone in a culture system.

[Materials and Methods] Femurs from 9-day-old chick embryos were cultured for 6 days by the roller-tube method. An aqueous solution of cadmium chloride (2.5, 5.0, 10 and 20 μM), copper sulfate (2.5, 5.0, 10 and 20 μM) or zinc chloride (25, 50, 100 and 200 μM) was added to the medium. The medium was BGJb–HW2 supplemented with 5 mM β-glycerophosphate.

[Results] Cadmium (5.0 μM and above) and copper (2.5 μM and above) each caused a decrease in hydroxyproline (Hyp) content of cultured bone (Fig. 1). This decrease was mainly due to inhibition of Hyp synthesis. In addition, cadmium and copper each showed a tendency to inhibit an increase in calcium content of diaphysis, where intraperiosteal ossification can be observed (Fig. 2). Alkaline phosphatase (ALP) activity in diaphysis was also decreased by cadmium or copper. On the other hand, zinc at 50 μM and above inhibited an increase in calcium content of diaphysis (Fig. 2) without a marked decrease in both the collagen content (Fig. 1) and ALP activity of diaphysis. The accumulation of cadmium or copper was similar in diaphysis and epiphysis, but that of zinc was 5–10 times more in diaphysis than in epiphysis. Histological observations revealed that cadmium and copper each decreased both calcified and uncalcified osteoid tissue at 2.5 μM, while zinc at 100 μM decreased calcified tissue but increased uncalcified osteoid tissue (Fig. 3). Zinc was deposited at the edge of the calcified tissue.

[Discussion] In this study, we found that cadmium caused a decrease in both calcified and uncalcified osteoid tissue accompanied with a damage of mesenchymal cells in periosteum and osteoblasts around the trabecula. This suggests that cadmium harmed these cells, resulting in a striking decrease in collagen content and diaphysial ALP activity. Thus, it was considered that the primary effect of cadmium on bone formation is an inhibition of bone matrix formation and not an inhibition of calcification. The toxic effects of copper were quite similar to those of cadmium in this study. On the other hand, zinc inhibited calcification rather than Hyp synthesis and caused an increase in uncalcified osteoid tissue. Since zinc accumulated particularly in diaphysis and deposited at the edge of calcified tissue, it was suggested that zinc inhibited calcification physicochemically rather than biochemically. It is concluded that cadmium or copper would induce bone damage ensued on osteoporosis and zinc would induce osteomalacia.
Fig. 1. Effect of cadmium, copper or zinc on hydroxyproline content in diaphysis (A) and epiphysis (B) of the bone cultured for 6 days. Significantly different from control; *P<0.05, **P<0.01, ***P<0.001.

Fig. 2. Effect of cadmium, copper or zinc on the increase in calcium content in diaphysis of the bone cultured for 6 days. Significantly different from control; *P<0.05, **P<0.01.

Fig. 3. Microscopic findings of the central part of diaphysis in the femur cultured for 6 days. (A) control bone, (B) cadmium (2.5μM)-treated bone, (C) copper (2.5μM)-treated bone, (D) zinc (100μM)-treated bone. von Kossa/Hematoxylin/Ponceau-acid fuchsin stain. ×100
PO, periosteum; OS, uncalcified osteoid tissue; CT, calcified trabecula.