The Effects of Cadmium on a Clonal Osteogenic Cell, MC 3 T 3-E1

TATSURO MIYAHARA,^a HIROSHI YAMADA,^a

RYUJI ANDO,^a MIEKO TAKEUCHI,^a SATORU

NEMOTO,^a TOSHIYUKI KAJI,^a MASA-AKI MORI,^a

HIROSHI KOZUKA^a and HIROKO SUDO^b

Faculty of Pharmaceutical Sciences, Toyama Medical & Pharmaceutical University,^a 2630, Sugitani, Toyama 930-01, Japan and Tohoku Dental University,^b 31-1, Tomitamachi, Koriyama 963, Japan

There are several reports that Cd-exposed workers have bone lesions. Itai-itai disease, which occurred in Toyama Prefecture, is thought to be at least partly due to Cd exposure. Recently, this disease was also recognized in Nagasaki Prefecture. Most of the Itai-itai-diseased patients had osteomalacia, but it has often been overlooked that they also had osteoporosis. Cd-treated animals also have osteoporosis or osteomalacia or both. The etiology of this disease has been studied from many points of view. To examine the direct effect of Cd on bone we have studied the influence of Cd on bone metabolism using embryonic chick bone in a culture system. It was found that Cd caused a decrease in both bone mineral and collagen, which was histologically an osteoporosis-like change. On the other hand, Kodama *et al.* established a clonal osteogenic cell line, MC 3 T 3-E 1, which showed high mineralization activity. As very little research has been done on the effects of Cd in bone-cell cultures, the effects of Cd on bone formation were investigated in a culture of MC 3 T 3-E1 cells.

1. The Acute Effects of Cd on MC 3 T 3-E1 Cells and Metallothionein (MT)-Like Protein Synthesis

The nearly confluent cells were cultured with Cd at 0.03–1.0 ppm for 24 h. Cd at 1 ppm decreased the DNA and protein contents and alkaline phosphatase (ALP) activity. The formation of MT-like

protein was recognized in the cells treated with Cd at 0.3 ppm and above. Cd content in the high-molecular (HM) fraction increased with increasing level of Cd. The toxic effect of Cd may be at least partly due to the increased content of Cd in HM fraction.

2. The Effects of Cd on Collagen Metabolism

The effects of Cd on collagen metabolism were investigated at the cell stage before calcification. At 6 d after inoculation, Cd at 0.1–1.0 ppm was added to the medium and further cultured for 3 d. Cd at 1 ppm caused a significant decrease in cell content of hydroxyproline (Hyp). The incorporation of [³H]-proline (Pro) into collagenase-digestible protein (CDP) was also significantly inhibited by Cd at 1 ppm. The hydroxylation of [³H]Pro to [³H]Hyp in CDP was unaffected by Cd. Therefore, the decrease in Hyp content may be largely due to inhibition of procollagen synthesis without inhibition of its hydroxylation, though we have obtained a result suggesting that Cd might stimulate collagen degradation.

3. The Effect of Cd on Calcification

At 3 d after inoculation, Cd at 0.03–0.3 ppm was added to the medium containing 5 mM β -glycerophosphate, and culture was continued for 10 d. ⁴⁵Ca was added during the last 20 h of the culture period. The accumulation of ⁴⁵Ca decreased with increasing level of Cd at 0.03 and 0.1 ppm but there was a reversal in the effect of Cd at 0.3 ppm. On the other hand, calcification of cells at 8 d after inoculation was significantly inhibited by Cd at 0.3 and 0.4 ppm after 3 d of further culture. When the cell layer was stained by the von kossa method, Cd decreased the number of black spots compared with the control. The decrease in Hyp and DNA contents and ALP activity by Cd was much lower than that in calcification. These results suggest that Cd inhibits an initial process of calcification without causing marked cell damage.