Laboratory Investigations

Inhibition of In Vitro Mineralization by Aluminum in a Clonal Osteoblastlike Cell Line, MC3T3-E1

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Summary. The direct effect of aluminum on mineralization was examined using an osteoblastlike cell line, MC3T3-E1. The mineralization process was quantitated by measuring 45Ca accumulation into the cell and matrix layer of MC3T3-E1 cells in culture. The accumulation of 45Ca into the cell and matrix layer increased dramatically after 13 days of culture without a parallel change in the DNA content of these cells. Because nodular clusters of cells appear around the same period in which a massive mineralization occurs, the marked increase in 45Ca accumulation after the 13th day of culture appears to represent deposition of 45Ca into the extracellular matrix. Thus, this culture system offers a useful model for making a quantitative estimation of osteoblast-mediated mineralization in vitro. When aluminum was added to this system, the accumulation of 45Ca into the cell matrix layer was inhibited in a dose-dependent manner: 10^{-6} M aluminum reduced 45Ca accumulation to 40.8 \pm 2.7\% of that in nontreated cells without affecting alkaline phosphatase activity or the DNA content of these cells. Because the concentration of aluminum used in this study is well within the range of serum aluminum levels seen in chronic dialysis patients, the direct effects of aluminum on osteoblast-mediated mineralization shown in the present study may underlie the development of so-called aluminum-induced "osteomalacia" in certain dialysis patients.

Key words: Aluminum — Mineralization — MC3T3-E1 cells — Osteoblastlike cell line — Hemodialysis-associated osteomalacia.

In some chronic dialysis patients who show histologic evidence of "osteomalacia," deposition of aluminum at the interface of osteoid and mineralized bone (mineralization front) has been demonstrated [1–4], with the severity of osteomalacia correlating with the content of aluminum in bone [4, 5]. Based on these and other observations, it has been suggested that aluminum may interfere with the normal mineralization process and thus cause osteomalacia with accumulation of unmineralized osteoid, aluminum-induced osteomalacia. However, Goodman et al. [6] reported that short-term aluminum administration impaired new bone and matrix formation but did not cause classic osteomalacia in the cortical bone of rats. In addition, Quarles et al. [7] demonstrated that, although bone aluminum content increased by aluminum administration in vitamin D-deficient dogs, vitamin D repletion resulted in healing of osteomalacia despite continued administration of aluminum. From these observations, they suggested that aluminum deposition at the mineralization front is secondary to increased osteoid, but does not impair bone mineralization. It has also been shown that the secretion of parathyroid hormone (PTH) is impaired in dialysis-associated osteomalacia patients [8–10], and that aluminum may inhibit PTH release from parathyroid gland [11]. Therefore, a relative deficiency of PTH may also contribute to the pathogenesis of aluminum-induced osteomalacia [12]. Nevertheless, it remains unclear whether deposition of aluminum at the mineralization front is causally related to the development of dialysis-associated osteomalacia and thus whether aluminum per se can impair bone mineralization process.

A clonal osteoblastlike cell line, MC3T3-E1, established from newborn mouse calvaria by Kodama et al. [13], has high alkaline phosphatase activity, is capable of collagen synthesis, has receptors specific for PTH and 1,25(OH)_{2}D_{3}, and is shown to increase cyclic AMP production in response to PTH [14–16], properties all characteristic of osteoblasts.
Furthermore, these cells form matrix vesicles and collagen-rich matrix, and the matrix can be mineralized in vitro [17]. Therefore, MC3T3-E1 cells can be a good in vitro model to investigate the bone mineralization process. The present study was undertaken to examine the direct effect of aluminum on mineralization using MC3T3-E1 cells in culture. The results demonstrate that aluminum directly inhibits mineralization in these cells at concentrations often seen in some chronic dialysis patients.

Materials and Methods

Cells and Culture Conditions

MC3T-E1 cells were kindly donated by Dr. H. Kodama of Tohoku Dental University, Fukushima, Japan. The cells were plated at a density of 1 × 10^5 cells/ml in 24-well plastic dishes and grown in Eagle’s alpha minimal essential medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (M. A. Bioproducts, Boston, MA) and 5 mM β-glycerophosphate at 37°C under 5% CO₂ in humidified air. The medium was replaced 1 day after seeding and was changed every other day thereafter. Accumulation of ⁴⁵Ca was assessed during each 2-day period until the 15th experimental day.

Assay of ⁴⁵Ca Accumulation

The cells were cultured in the medium containing 0.5 μCi/ml of ⁴⁵CaCl₂ for 48 hours at the indicated time after seeding. When the effect of aluminum on ⁴⁵Ca accumulation was examined, various concentrations of aluminum chloride were added to the culture medium throughout the experiments. After 2 days of culture with ⁴⁵CaCl₂, the medium was removed and the cell and matrix layer was washed twice with phosphate-buffered saline, scraped into minivials containing 0.1 ml of perchloric acid, and vortexed vigorously. Then, 0.2 ml of hydrogen peroxide was added to the vials, and they were incubated for 60 minutes at 80°C. After incubation, the mixture was dissolved in 0.3 ml of ethyleneglycol monoethyl ether, vortexed vigorously, and the radioactivity was counted by liquid scintillation counting with 4 ml of ACS-II (Amersham Corp., Bucks, England).

Assay of Alkaline Phosphatase Activity

After cells were washed with phosphate-buffered saline, they were scraped in 1 ml of distilled water and stored at −20°C. The cell suspensions were thawed, homogenized with a Dounce homogenizer, and the homogenate was assayed for alkaline phosphatase activity. The assay mixture contained 10 mM p-nitrophenyl phosphate in 0.1 M sodium carbonate buffer (pH 10.0) supplemented with 1 mM MgCl₂ in a total volume of 1 ml. The mixture was incubated at 37°C for 30 minutes, and reaction was stopped by adding 2 ml of 0.1 M NaOH. The amount of p-nitrophenol liberated was measured by spectrophotometer at 410 nm. One unit of enzyme was defined as the activity causing the release of 1 nmol of product per minute.

Assay of DNA Content

At the indicated time after seeding, the medium was removed and the cells were washed twice with phosphate-buffered saline, scraped into glass tubes containing 2 ml of 5% trichloroacetic acid, and left for more than 10 minutes at room temperature. They were then centrifuged for 10 minutes at 4,000 × g, and the supernatant was discarded. The above procedure was repeated...