Effects of Zinc on the Mineralization of Bone Nodules from Human Osteoblast-like Cells

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ABSTRACT

Zinc is an important mineral that is required for normal bone development. However, the direct effects of zinc on the mineralization of bone cells of human origin are not clear. The objective of this study was to determine the effects of zinc on the differentiation of SaOS-2 human osteoblast-like cells and the formation of mineralized bone nodules. Cells were cultured for 8 d and then transferred to zinc-free medium and treated with varying concentrations (0–50 µM) of zinc. Alkaline phosphatase (ALP) activity was used as a measure of osteoblast differentiation, and bone nodules were detected by von Kossa staining. After 4, 6, and 8 d of treatment, zinc increased ALP activity at 1 and 10 µM, but decreased activity at 50 µM. After 9 d of treatment, zinc increased both the number and area of mineralized bone nodules at low concentrations (1 and 10 µM), but decreased both at higher concentrations (25 and 50 µM). These findings demonstrate that zinc has biphasic effects on the differentiation and mineralization of human osteoblast-like cells.

Index Entries: Zinc; osteoporosis; SaOS-2 cells; bone; alkaline phosphatase.

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INTRODUCTION

Osteoporosis is a major cause of morbidity and mortality and is a growing public health concern. The causes of osteoporosis are multifactorial and include both genetic and environmental factors (1,2). Some of the modifiable risk factors include diet, smoking, physical inactivity, and alcohol consumption (3). Along with other essential nutrients, minerals such as zinc play a pivotal role in the formation of the bone matrix.

Although the human body contains approx 1.5–2.5 g zinc (4), which is stored in bone and muscle, the available pool, estimated at 157–183 mg zinc (5), is too small to provide a metabolic buffer. Kinetic modeling experiments with human subjects and animals have shown that the liver, pancreas, kidney, and spleen have very high rates of zinc turnover, whereas the nervous system and bone have slow turnover rates (5). Because there appears to be little zinc available as a stored reserve, deficiency can occur rapidly when dietary intake decreases (6).

Zinc plays an important role in the growth of humans and deficiency has been associated with abnormalities in bone growth, formation, and mineralization (7–9). Low zinc intake has been reported to be associated with low bone mass in women (10) as well as reduced serum zinc concentrations and increased urinary zinc excretion in women with osteoporosis (11).

Animal studies have shown that zinc deficiency causes low bone mass (12,13). Insufficient dietary intake of zinc causes a decrease in the number of osteoblasts and chondrocytes in animals bone (14), and in vitro studies show that zinc increases the number of murine osteoblastlike cells (15). Zinc is also involved in the stimulation of collagen production in rat femur and calvaria (16). Studies have shown that zinc has an inhibitory effect on bone resorption (16) as well as a stimulatory effect on bone formation and mineralization in osteoblastic cell cultures (17).

Alkaline phosphatase (ALP) activity has been used as a measure of bone formation in healthy and osteoporotic subjects (18) and requires zinc for its activity (17). ALP is produced by the osteoblast, especially during the bone formation phase (19), as a result of its key role in the formation and calcification of hard tissue (20). The enzyme is attached to the external surface of plasma membranes by phosphoethanolamine bound to oligosaccharides (21), where it hydrolyzes phosphate esters to increase the phosphate concentration and mineralization of the extracellular matrix (21). ALP is also a zinc metalloenzyme and contains two molecules of zinc that results in loss of activity when removed (17).

The aim of the present study was to investigate the direct effects of zinc on osteoblastic bone formation in a time- and dose-dependent manner. We performed this study using SaOS-2 human osteoblast-like cells, because this cell line has characteristics of human osteoblasts, including high steady-state levels of ALP activity (17).