FLUORIDE AND ALKALINE PHOSPHATASE

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SUMMARY: Since serum alkaline phosphatase increases in fluoride therapy for osteoporosis, it is generally accepted that fluoride stimulates bone formation. However, histochemical studies have shown that alkaline phosphatase is also increased in resorbing osteocytes. Fluoride is toxic to metabolically active bone cells, alkaline phosphatase is released, and serum alkaline phosphatase increases. We propose that the increased serum alkaline phosphatase following fluoride therapy may reflect a toxicity of fluoride for both osteoblasts (bone forming cells) and resorbing osteocytes.

When cells are injured their first response is to initiate repair processes and if this repair fails the cell dies. An increase of serum alkaline phosphatase and increased bone mass following fluoride therapy represent a failed repair response involving an initial increase in both bone formation and resorption. This repair response to cell injury results in pathological bone formation. Furthermore, as the repair process fails there is a toxic death of resorbing osteocytes and a decrease in bone resorption. Osteoclasia of fluorotic bone may result in secondary toxic effects of fluoride on osteoclasts, and contribute to decreased bone resorption.

The increased amount of trabecular bone in fluoride therapy is claimed to be the morphologic expression for fluoride as a stimulus for bone formation. We propose that the increased amount of trabecular bone results from pathological bone formation by injured osteoblasts and decreased bone resorption by resorbing osteocytes and osteoclasts. Both resorptive processes are required for the remodeling of trabecular bone into compact bone.

Fluoride has only negative effects on bone cell metabolism. Fluoride should be avoided, especially in osteoporosis.

Key words: Alkaline phosphatase; Bone; Cell injury; Fluoride therapy; Osteoporosis.

Fluoride therapy in osteoporosis has been shown to increase bone mass. Increased bone mass can result from increased bone formation or from decreased resorption of bone. Since serum alkaline phosphatase (SAP) increases with fluoride therapy, it has been proposed that fluoride stimulates bone apposition. An example: "Fifty-six post menopausal women with overt osteoporosis were treated for up to three years with NaF, 33 subjects receiving 100 mg NaF/day and 23 subjects 50 mg NaF/day" and : "After eight months of NaF treatment, alkaline phosphatase reached a peak of +40% above base-line value in the 50 mg NaF group (P< 0.05) and of +94% in the 100 mg NaF group (P< 0.001)".

1. Alkaline phosphatase as an enzyme of bone apposition

In 1923 Robison published his now classical paper: "The possible significance of hexosephosphoric esters in ossification." Alkaline phosphatase (AP) has ever since been considered an enzyme of bone apposition. SAP is higher in growing subjects and is greatly increased in osteosarcoma, a neoplastic proliferation of osteoblasts.

2. Alkaline phosphatase as an enzyme of bone resorption

In 1951, two extensive papers on AP as an enzyme of bone resorption were published. The papers have, unfortunately, not received deserved attention. AP was studied by histochemical methods in bone of man and rabbits. One of the many illustrations (reproduced in Figure 1) describes "... the life cycle of human bone cells..."
concerning alkaline phosphatase.” Osteoblasts and newly formed osteocytes are rich in AP. However, this enzyme disappears from older osteocytes, until deep seated bone resorption is initiated. At the beginning of osteocytic bone resorption the AP reappears in the osteocytes, reaches a maximum, and then disappears in the dying osteocytes (Figure 1). The process evolves in 18-36 hours in areas of rapid deep seated bone resorption.

Figure 1. The life cycle of human bone cells concerning alkaline phosphatase.

In Stage I, the osteoblasts show a rich concentration of alkaline phosphatase. In Stage II, the osteoblasts are rich in alkaline phosphatase and so is, to a lesser degree, the recently trapped osteocyte. The cell on top of the bone surface is a bone marrow cell. Stage III shows a more deep-seated, resting osteocyte with no alkaline phosphatase. In Stage IV, the beginning of osteocytic osteolysis, alkaline phosphatase reappears in the osteocyte and reaches maximum (Stage V). The alkaline phosphatase is now as prominent as in the osteoblasts (Stage I). At the end stage (VI), the dying osteocyte shows no alkaline phosphatase. (Reproduced from Reference 3.)

von Recklinghausen⁵ used the term onchosis to refer to the “necrobiotic resorption of bone tissue.” Mäjno and Rouiller³ and Rutishauser and Mäjno⁴ considered onchosis to be a regressive process (necrobiosis), as well as a form of bone resorption: pericytic osteolysis. This defines a deep-seated bone resorption mediated by osteocytes. This concept was brought to wide, if not general, acceptance by the contributions by Bélangér⁶ who introduced the now more common term “osteocytic osteolysis”. This is an active metabolic process that results in increased synthesis of alkaline phosphatase and metalloproteinases required for bone resorption, and culminates in apoptosis or “programmed cell death” of osteocytes. This activation, or increase in metabolic activity, of resorbing osteocytes may be initiated by any of the normal physiological, nutritional and/or endocrine mechanisms that regulate increases in bone turnover, and it may be more important than osteoclasia.⁷,⁸ This activation can also be initiated by cell injury that initiates a repair response in both surface osteoblasts and deep seated osteocytes that have been activated metabolically. The repair response in both types of injured bone cells contributes to the increase in SAP. This increase in SAP is undoubtedly further augmented by the death of injured osteocytes when the fluoride toxicity overwhelms the repair process.

3. Alkaline phosphatase in nutritional secondary hyperparathyroidism

Young horses were fed a ration with normal calcium but with 3.6 times that high in phosphorus.⁹ The high dietary phosphorus induced hyperphosphatemia which, in accord with the mass law equation, induced hypocalcemia, which is the stimulus for parathyroid hyperactivity. With the resulting increase in bone resorption, the hypocalcemia was corrected to nearly normal levels. SAP showed an inverse correlation to serum calcium: when serum calcium decreased, SAP increased and vice versa. In the second part of the experiment, weeks 23 through 42, dietary phosphorus was increased to 5.2 times dietary calcium. The sequences in serum calcium and in SAP were repeated. In similar studies in pigs,¹⁰ serum calcium and SAP recordings were in
agreement with those in the horse study. The results can mean only one thing, viz. that SAP in nutritional secondary hyperparathyroidism reflects the fact that AP is also an enzyme of bone resorption.

4. Serum alkaline phosphatase in human populations

SAP was studied in 257 men and 195 women of various ages. In the 20-30 age group, SAP was higher in men than in women. It increased in men in the 60-65 age group and was still higher in men older than 70 compared to the 20-30 age group. In women more than 70 years of age, SAP was higher than in ages 50-70. Similarly, in a series of 117 subjects, it was reported that SAP increased progressively after 50 years of age. This rise in SAP at higher ages cannot be explained by increased bone formation. On the other hand, this will be the expected consequence of increased osteolysis, which results from the dietary calcium deficiency that is prevalent in most countries.

5. Alkaline phosphatase in nutritional hypercalcitoninism

Bulls at an artificial insemination station (about 300 bulls) were fed calcium at 3.5 times recommended levels in young bulls and this was increased progressively to 5.9 times in older bulls. The high dietary calcium induced hypercalcemia, a stimulus for the calcitonin producing C cells of the thyroid gland. Calcitonin is the antagonist of parathormone and decreases bone resorption. Serum calcium was reduced and reached hypocalcemic levels. High concentrations of calcium in the gastrointestinal tract stimulate the gastrin producing G cells of the gastrointestinal mucosa. Gastrin stimulates the C cells regardless of serum calcium. The high dietary calcium eventually caused hypocalcemia and osteopetrosis, because of increased calcitonin production. Because of the resulting decrease in bone resorption SAP decreased and remained low in old bulls (more than 12 years of age). Thus, this osteopetrosis resulted from decreased bone resorption. If bone production had increased to produce the osteopetrosis, SAP would have increased.

In experimental hypercalcitoninism in growing dogs, Great Dane puppies were fed ad libitum a diet high in protein, energy and calcium (2.05% compared to the recommended 1.0% on a dry matter basis). Control dogs of the same litter and sex were restricted to 2/3 of the amount of food consumed by the dogs fed ad libitum. In the ad libitum fed dogs an initial hypercalcemia was converted into isocalcemia after 30 weeks and at 60 weeks there was a pronounced hypocalcemia. Serum phosphorus went from hyperphosphatemia to hypophosphatemia, a parathormone effect. During the entire experiment, weekly recordings of SAP were consistently lower in ad libitum fed dogs. Electron microscopy studies of C cells indicated that there was a marked stimulation of C cells which would be consistent with hypercalcitoninism. Radiography, micro-radiography, fluorescence microscopy and histopathology all indicated that bone formation was increased in ad libitum fed dogs, causing hypertrophic osteodystrophy and osteopetrosis, with delayed remodeling of lamellar bone into osteonic bone. Delayed bone resorption was further documented with analysis of fluorochrome incorporation into forming bone, and by the presence of failure of modeling of the femoral neck in the ad libitum fed dogs. We now have before us a condition of increased bone formation, which would be reflected in increased SAP and decreased bone resorption, which would be reflected in decreased SAP. Since SAP was always lower in ad libitum fed dogs, the decrease in SAP resulted from the decreased bone resorption.
DISCUSSION

Fluoride is a potent enzyme poison. The concept that fluoride is a specific stimulus for bone formation is preposterous. Instead, fluoride induced cell injury in both osteoblasts and osteocytes initiates a repair response and results in increased SAP production in both of these cell populations. The repair response in osteoblasts results in increased proliferation, matrix production and SAP production. When the repair process in osteoblasts fails, the osteoblast undergoes either apoptosis or necrosis, and is replaced by proliferation of osteoprogenitor cells. These new osteoblasts will then be injured and the cycle of increased repair and cell death would be repeated. This activation of a repair response in osteoblasts would contribute to increased SAP.

Activation of repair in resorptive osteocytes will increase their production of AP, collagenase and other degradative enzymes. Recent studies have shown that the collagenase-1 that is required for native collagen degradation is produced by osteoblasts/osteocytes, while the gelatinase-B that degrades denatured collagen is produced by osteoclasts. When the repair process in these activated osteocytes fails, osteonecrosis occurs and there is a decrease in osteocytic osteolysis and an increase in the release of AP from dying osteocytes. This decrease in bone turnover may be further enhanced by a secondary fluoride induced injury of osteoclasts that are formed to degrade the necrotic bone. If these injured osteoclasts die, new osteoclasts will form from monocytes, so secondary injury of osteoclasts will not be expected to result in a paucity of osteoclasts on the surface of fluorotic bone (Figure 2). Furthermore, because fluoride injures all of the cells involved in bone formation and degradation, it is not the surprising that a poor quality of bone accumulates in patients treated with fluoride.

Figure 2. Photomicrograph of mandibular cortex of a 3-year-old cow, in which triplicate assays showed that the fluoride content of bone ash was 4733 ± 67 ppm. Many lacunae are empty because of death of osteocytes. Matrix is also being degraded, especially in the upper right. Osteoclasts and Howship's lacunae indicate that the surface of the devitalized bone is undergoing osteoclasia. Hematoxylin and eosin, x 120. (Reproduced from Reference 27.)
Figure 1 shows the changes in AP during bone formation and osteocytic osteolysis. Osteoblasts, immature osteocytes and resorbing osteocytes all produce alkaline phosphatase and are, thus, metabolically very active. As such, they are highly susceptible to a toxic agent, fluoride in this case. Fluoride injures all of the cells in bone, alkaline phosphatase is released and SAP rises. This increase in SAP is consistent with the increased bone formation and decreased bone turnover that results from the death of activated, resorbing osteocyte.

It is a consistent finding that fluoride treatment in osteoporosis results in greater amounts of trabecular bone and a decrease in compact bone.\(^1\) This has been interpreted as evidence for enhanced bone formation, albeit abnormal bone.\(^2\) We offer a fundamentally different explanation. Remodeling of trabecular bone into compact bone requires bone resorption, mainly osteocytic osteolysis. In addition to stimulating pathological bone formation by injured osteoblasts, fluoride reduces osteolysis by injured osteocytes and greater amounts of trabecular bone persist. The amounts are significantly increased by the failure of remodeling.

Bone is a dynamic tissue in a state of turnover: it is formed and resorbed, it is always being renewed. Resorption is a physiological event aimed at maintaining normal serum calcium levels and it is required for normal remodeling. Causing pathological bone formation and reducing bone resorption is the wrong way to treat osteoporosis. The right way is to promote physiological bone formation by increasing dietary calcium and vitamin D and by increasing physical activity.

Acceptance of the documentation that alkaline phosphatase is an enzyme of both osteoblasts and resorbing osteocytes, reveals the true nature of fluoride's effect of bone cells. It is poisonous to bone cells and every osteoporosis patient who is being treated with fluoride should know that fluoride is toxic for all of the cells in their bones. It is unfortunate that many physicians who treat osteoporosis with fluoride do not realize that they are prescribing a drug that is toxic for all metabolically active bone cells.

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