IN VITRO ADSORBANCE OF FLUORIDE BY BONE

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SUMMARY: Adsorption of fluoride into normal bovine compact bone in vitro produced the brown mottling that is characteristic of fluoride. The data show that cell activity is not required to produce this sign of fluorotoxicosis. This suggests that fluoride induced cell injury in bone or dentin may result from the local release of fluoride that is incorporated into the apatite phase of hard tissues.

Key words: Bone; Brown mottling; Fluoride; In vitro adsorbance.

Introduction

The main mineral phase of hard tissues (bone, dentin and cementum) is calcium hydroxyapatite. When fluoride is available, it replaces the hydroxyl ions, producing calcium fluoroapatite. This causes "brown mottling" of hard tissues as described by Johnson.\(^1\) This brown mottling is used as a diagnostic indicator of the disease known as fluorosis.\(^2,3,4\) Histologically, the brown mottling is only evident in ground sections of mineralized tissues, not in demineralized sections. Chlubek et al.\(^5\) reported on fluoride accumulation in fossil human cranial bone and also presented a relevant literature review. In cranial bone fossils aged 3,700-6,000 years the fluoride concentration was up to 1900 ppm, while in cranial bone fossils aged less than 3,000 years the fluoride content was < 500 ppm, with a range of 80 to 500 ppm. These authors concluded that "... significant bone fluoride enrichment takes at least around 1000 years".

We recently reported on microscopic and chemical studies of matrix in tusks and bones of a Columbian mammoth skeleton aged ~11,200 years.\(^6\) This skeleton was excavated in 1988 from a saturated lake bed at ~9,000 feet elevation in the Wasatch mountains of central Utah, USA. Ash fluoride was up to 2,700 ppm in ribs and 1,260 ppm in tusks. Brown mottling was shown in ground sections of both bone and tusk, but there were no other lesions of fluorosis. Because of the absence of lesions of fluorosis, we did not include fluoride data at that time.\(^6\) Instead, we performed the experiments described in this report on in vitro adsorbance of fluoride by bone.

Materials and Methods

In a previous study, bones of cattle with severe fluorosis were found to have fluoride ash concentrations ranging up to 10,000 ppm.\(^2\) Therefore, in the present study, sections of normal bovine bone were soaked in a 10,000 ppm fluoride solution (22.105 g reagent grade NaF per litre of distilled water). Three 2 mm thick cross-sections were excised from the humerus distal to the deltoid crest of a clinically normal 3 year old cow. Two sections were soaked in the fluoride solution, one for 20 days and one for 60 days. The third section was used as an unexposed, 0 day, control. At the appropriate time the dorsomedial quadrant of each section was removed for histologic examination of ground sections. The remainder of each...
specimen was cut in half. One of these halves was washed for 1 min and the other for 24 hrs in water. The washed specimens were used for fluoride analysis. Washed bone sections were ashed overnight at 600°C in a muffle furnace. Ash fluoride was determined with an Orion Model 94-09 (Orion Research Inc) fluoride ion electrode as described previously.²

For preparation of ground sections, each specimen was fixed for 7 days in absolute ethanol that was changed twice. They were then embedded in methylmethacrylate, hardened, and cut on a bandsaw to ~1 mm. They were then ground to ~60 µm on a horizontal grinder as described by Ericsson.⁷ Thus, the ground sections were obtained from the deepest part of each bone specimen.

**Results and Discussion**

As shown in the Table, the fluoride content of bone ash increased 33 fold when soaked for 20 days, and ~ 60 fold when soaked for 60 days in a NaF solution. Since relatively little of this fluoride was washed out after the first minute of a water wash (see Table), the fluoride must have been adsorbed or incorporated into the mineral phase of the bone. Furthermore, at 20 days the bone fluoride concentration was still less than that in solution, but by 60 days the bone fluoride concentration exceeded that of the NaF solution by ~33% (see Table). This is consistent with our observed accumulation of fluoride in mammoth fossil bone aged 11,200 years,⁶ and the high bone fluoride in human skulls exposed to low environmental levels of fluoride for > 3,000 years.⁵

<table>
<thead>
<tr>
<th>F soak time</th>
<th>H₂O rinse time</th>
<th>Fluoride ppm</th>
</tr>
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<tbody>
<tr>
<td>0 d</td>
<td>1 m</td>
<td>243</td>
</tr>
<tr>
<td>20 d</td>
<td>1 m</td>
<td>8,409</td>
</tr>
<tr>
<td>20 d</td>
<td>24 h</td>
<td>8,271</td>
</tr>
<tr>
<td>60 d</td>
<td>1 m</td>
<td>14,802</td>
</tr>
<tr>
<td>60 d</td>
<td>24 h</td>
<td>13,272</td>
</tr>
</tbody>
</table>

The Figure shows photomicrographs of ground bone sections. The outer 2/3 of the cortex of the humerus consists of lamellar bone, and in the inner 1/3 has been modeled to osteonic bone. In cattle, modeling to osteonic bone is complete at age 4.5 to 5.0 years. As shown in panel B of the Figure, 20 days of soaking in fluoride solution resulted in intense brown mottling that was restricted to the lamellar bone. After 60 days of soaking in fluoride solution the brown mottling was more extensive, and it now also involved the osteonic bone (Panel C of Figure). Thus, prolonged soaking of bone in a fluoride solution produced a direct correlation between the increased fluoride content and increased brown mottling.

It has been known for many years that fluorosis produces brown mottling of bones and teeth in both man and domestic animals.¹⁻⁴ This brown mottling is recognized as a sign of degenerative change in the hard tissues. However, it was not clear whether brown mottling requires cells. The data reported here show that
Brown discoloration is more severe and it now involves both lamellar and osteonic bone. x 6.75

Panel A is from a section soaked 20 days in a 10,000 ppm thulium solution. There is severe brown discoloration of lamellar bone. Panel C is from a section soaked 60 days in the thulium solution. The discoloration of lamellar bone is severe. Panel C contains 1/2 of the cortex consists of lamellar bone and the inner 1/2 has been modeled into osteonic bone. The other bone is from the 0 day control bone that was not soaked in thulium solution. The other bone is from the 0 day control bone that was not soaked in thulium solution. The other bone is from the 0 day control bone that was not soaked in thulium solution.

In vitro adsorbance of thulium by bone.
the adsorption of ~8,000 ppm of fluoride into the mineral phase of bone will produce brown mottling in the absence of cell activity. Thus, brown mottling is not due, per se, to bone cell injury but is surely a sign that the fluoride concentrations causing the mottling will also cause cell injury resulting in impairment of both the formation and degradation of matrix in hard tissues.

Considerable data show that fluorotoxicosis injures both osteoclasts\(^8\) and resorbing osteocytes\(^2\). This cell injury may result from the release of fluoride ions during bone modeling or remodeling, since cells involved in these processes will be exposed to the highest concentrations of fluoride.

References


