Effect of Long-Term Administration of Sodium Fluoride on Plasma Calcium Level in Relation to Intestinal Absorption and Urinary Excretion in Rabbits

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The present study was undertaken to determine the effect of chronic fluoride toxicity on calcium metabolism. Rabbits (n = 10) were orally administered an aqueous solution of 10 mg NaF/kg body wt daily for 18 months. Equal numbers (n = 10) of age-, sex- and weight-matched rabbits kept under identical laboratory conditions, but not treated, with NaF solution, served as control. The fasting plasma calcium concentrations of fluoride-treated rabbits were significantly lower (P < 0.001) than those of control animals. In contrast to the decrease in plasma calcium level, an increase in intestinal radioactive calcium (45Ca) absorption was observed (P < 0.001) in all fluoride-treated animals. However, urinary calcium excretion levels were found to be reduced (P = not significant) in fluoride-treated rabbits. It is therefore concluded that long-term fluoride poisoning alters the calcium homeostatic mechanism, thereby affecting calcium metabolism.

INTRODUCTION

Pathogenesis of calcified tissue in chronic fluoride toxicity is mainly due to a direct effect of fluoride on bone, resulting in abnormal bone formation, mineralization, and resorption (Farley et al., 1983; Hicks and Ramp, 1975; Johnson, 1965). However, some reports suggest a disturbed calcium metabolism in chronic fluoride toxicity as the primary cause of the hard tissue lesion (Fejerskov et al., 1979; Yates et al., 1964). But results from some laboratories are not in full agreement with this hypothesis (Andersen et al., Rosenquist et al., 1983).

In view of the conflicting reports on calcium metabolism, the objective of this investigation was to determine the effect of long-term administration of fluoride on plasma calcium level, intestinal calcium absorption, and urinary calcium excretion and to assess the effect of these factor(s) on calcium metabolism.

MATERIALS AND METHODS

Twenty healthy male rabbits weighing 1700–2000 g each were selected and were kept in two groups (10 each) under identical laboratory conditions. The rabbits were given a standard laboratory diet (1.2% calcium and 0.6% phosphate) and water ad libitum. One group of animals (n = 10) was orally administered an aqueous solution of NaF (10 mg/kg body wt/day) for 18 months. The other group (n = 10) served as control. Twenty-four-hour urine was collected for three consecutive days on the 28th, 29th, and 30th day of the 18th month of fluoride treatment and blood was drawn early on the 4th day (9:00 AM) after the animals had been fasted overnight. Similarly, urine and blood samples were also collected from the control group. Intestinal calcium absorption study was performed immediately after blood sample collection.
Intestinal Calcium Absorption

Intestinal calcium absorption was studied by two methods, viz, (a) in vitro everted gut technique (EGT) and (b) in vivo ligated segment technique (LST).

In vitro study. Five animals of the 18-months-treated group with an equal number of matched controls were taken for the in vitro study. Intestinal calcium uptake was evaluated by a modification of the everted gut technique (Martin and Deluca, 1969). The animals were sacrificed and the proximal duodenal segments (8–10 cm) were quickly removed, everted, and washed in cold 0.9% NaCl. The duodenal sacs were filled with 1 ml of oxygenated prewarmed (37°C) test solution containing 0.1 mCi $^{45}$Ca/ml (as CaCl$_2$ from Amersham International PLC, U.K.) in 125 mM NaCl$_2$, 30 mM Tris–HCL, 10 mM fructose, and 0.25 mM CaCl$_2$, adjusted to pH 7.4. The sacs were placed in a beaker containing 20 ml of the same test solution. The sacs were incubated at 37°C with gentle agitation by bubbling 95% O$_2$ + CO$_2$ from the bottom of the beaker. After the incubation period of 90 min, 0.5 ml each of the test solution from mucosal (outer) and serosal (inner) regions were removed for the measurement of radioactivity in a Packard liquid scintillation counter (Model 3320). The results are calculated as the ratio of $^{45}$Ca in serosal fluid to that in mucosal fluid (S/M ratio).

In vivo study. The intestinal calcium absorption was measured with the in vivo ligated segment technique (Zikos et al., 1986) in five animals treated with fluoride for 18 months and in five control animals.

After intraperitoneal (ip) sodium pentobarbital anesthesia (30 mg/kg body wt), the rabbits were placed on warm plate to keep their body temperature around 37°C. A small abdominal incision was made, the proximal 6 cm of duodenum was identified, and the distal end was ligated. The proximal end was loosely tied around the pyloric region of the stomach. Next, 1.5 μCi of $^{45}$Ca (as CaCl$_2$ from Amersham International PLC, U.K.) was introduced into the duodenum in 1 ml of NaCl (150 mM) containing 0.5 mM CaCl$_2$ (pH adjusted to 7.4) with the help of a 28-gauge needle inserted through the stomach. An aliquot of the injected material was saved for subsequent counting.

The proximal suture was quickly tied. The abdomen was closed and the rabbit was allowed to recover from anesthesia. Approximately 30 min was required for the recovery. Rectal temperature was maintained between 36.5 and 37.5°C. Two hours after injection of isotopic calcium, animals were sacrificed and the ligated duodenal sac was removed, weighed, and digested in 10 ml of perchloric acid and nitric acid mixture at a ratio of 1:1. A total of 100 μl of the digested gut sac solution and 100 μl of the injectant were counted for radioactivity in the Packard liquid scintillation counter (Model 3320). $^{45}$Ca uptake of duodenum is calculated by subtracting the $^{45}$Ca (total counts/min) remaining in the segment solution 2 hr after luminal injection from the total calcium count of the solution injected. This difference is expressed as a percentage of the total.

Fluoride and Calcium Estimation

Fluoride concentration in plasma was measured by ION 85 ion analyzer (Radiometer, Copenhagen) (Hall et al., 1972). The calcium ion concentration in plasma and urine was determined by the method of Hulanicki and Trojanowicz (1974) using a calcium ion electrode in the ION 85 ion analyzer.
Statistics

The statistical significance of the data was evaluated by Student’s $t$ test.

RESULTS

It is evident from Table 1 that the plasma fluoride level increased from $0.097 \pm 0.014$ (control mean value) to $0.83 \pm 1.0$ ppm after 18 months of fluoride treatment ($P < 0.001$) and, contrary to the above observation, the plasma ionic calcium level decreased from $5.049 \pm 0.336$ (control value) to $3.724 \pm 0.338$ mg% in the treated group ($P < 0.001$). The urinary calcium excretion was also decreased in the fluoride-treated group ($54.9 \pm 7.6$ mg/kg/day). Although the differences was just shy of significance (Table 1).

A significant increase in calcium transport was observed in the fluoride-treated group ($n = 5$) using the everted gut technique. The mean ratio ($S/M$) of $^{45}$Ca in the serosal ($S$) and in the mucosal ($M$) fluid of the control group ($n = 5$) was $1.27 \pm 0.05$ and in the fluoride-treated group the $S/M$ ratio was $1.99 \pm 0.01$ ($P < 0.001$). The increase is about 56% (Table 2).

With the $in$ vivo ligated technique also a very significant increase in calcium absorption was observed in fluoride-treated group ($n = 5$), when compared to the control group ($38.11 \pm 2.04$ vs $25.68 \pm 4.45$%). The increase is about 48 percent ($P < 0.001$) (Table 2).

DISCUSSION

The possibility that fluoride induces some of the observed effects in calcified tissue by influencing calcium homeostasis is not a new concept (Fejerskov et al., 1979) but the available reports are of conflicting nature (Andersen et al., 1986).

The results of the present study clearly demonstrate disturbed calcium homeostasis in chronic fluoride toxicity. Despite the increase in intestinal calcium absorption and a drop in urinary calcium excretion, hypocalcemia was observed in fluoride-treated animals. These findings suggest increased calcium retention in chronic fluoride toxicity. As fluoride is known to induce mineralization (Hicks and Ramp, 1975) and inhibit bone resorption (Faccini, 1967) an increase in bone calcium uptake is expected. Previous studies have also reported increased calcium retention in chronic fluoride toxicity (Bernstein et al., 1963; Narasimha Rao et al., 1968). The increased intestinal calcium absorption, observed in the present study, is therefore an indication of increased demand for dietary calcium in order to restore normocalcemia. Jowsey et al. (1979) also reported increased demand for dietary calcium in osteoporosis patients on fluoride therapy and he stated further that if sufficient calcium is not supplemented, it may lead to osteomalacia. However, if a diet sufficiently high in calcium is provided to the experimental

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Plasma fluoride (ppm) Mean ± SD</th>
<th>Plasma calcium (mg %) Mean ± SD</th>
<th>Urine calcium (mg/kg/day) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ($n = 10$)</td>
<td>$0.097 \pm 0.014$</td>
<td>$5.049 \pm 0.336$</td>
<td>$60.8 \pm 3.0$</td>
</tr>
<tr>
<td>Fluoride treated ($n = 10$)</td>
<td>$0.830 \pm 0.100^*$</td>
<td>$3.724 \pm 0.338^*$</td>
<td>$54.9 \pm 7.6$</td>
</tr>
</tbody>
</table>

* Significant difference ($P < 0.001$).
TABLE 2

Intestinal Calcium Absorption in Experimental Rabbits Measured by in Vitro Everted Gut Technique (EGT) and in Vivo Ligated Segment Technique (LST)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Calcium absorption (EGT)</th>
<th>Calcium absorption (LST)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S/M ratio*</td>
<td>percentage</td>
</tr>
<tr>
<td></td>
<td>(Mean ± SD)</td>
<td>(Mean ± SD)</td>
</tr>
<tr>
<td>Normal</td>
<td>1.27 ± 0.05</td>
<td>25.68 ± 4.45</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Fluoride Treated</td>
<td>1.99 ± 0.01(^a)</td>
<td>38.11 ± 2.04*</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
</tbody>
</table>

* Significant difference (P < 0.001).
\(^a\) The ratio of \(^{45}\)Calcium in the serosal (S) and in the mucosal fluid (M).

animals, a normocalcemic condition may be found, as observed by Andersen et al. (1986) in his experimental animals given a high calcium and vitamin D diet. This normocalcemic condition is, however, not a true picture of calcium homeostasis as the calcium retention in these circumstances is much higher in fluoride-treated animals compared to normal control animals. This increased calcium retention is due to excessive calcification of bone (osteosclerosis) and ectopic calcification; both these calcification processes are known to be features of chronic fluoride toxicity (Huo, 1981). But if a diet sufficiently high in calcium is not provided, osteomalacia may prevail in chronic fluoride toxicity (Jowsey et al., 1979). Thus in osteofluorotic bone, both osteosclerosis and osteomalacia are known to coexist (Jolly, 1970).

It is therefore concluded that calcium homeostasis is disturbed in chronic fluoride toxicity as indicated by hypocalcemia, increased intestinal calcium absorption, and hypocalciuria. These indicators of disturbed calcium homeostasis can be biochemically identified only if dietary calcium intake is not sufficiently large as in the present study.

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REFERENCES


