Effects of Strontium on Calcium Metabolism in Rats  
I. A Distinction between the Pharmacological and Toxic Doses

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ABSTRACT—Strontium at low doses has been used to treat osteoporosis. However, excessive doses can disturb calcium metabolism. The aim of the present study was to determine a dose that does not have any significant toxic effects on calcium contents in bone and calcium metabolism, and, consequently, to distinguish between pharmacological and toxic doses in rats. The rats were divided into a control, 0.05%-Sr, 0.10%-Sr and 0.50%-Sr groups (strontium intake approx. 0, 87.5, 175 and 875 pmol/day, respectively). All of the rats were pair-fed their respective diets containing various doses of strontium in single metabolic cages from when they were 36 to 63 days old. When the rats were 60 days old, bone formation, bone resorption, calcium balance and intestinal calcium absorption were calculated as calcium metabolic parameters over a 3-day period using calcium balance and kinetic studies. At the age of 64 days, the rats were sacrificed under anesthesia, and the femur and blood were collected. Calcium and strontium levels in the bone and serum were then measured. In the strontium groups that received less than 175 pmol/day, none of the calcium metabolic parameters were significantly affected. However, the calcium contents in the bone were significantly increased in the group that received 87.5 pmol/day group. On the other hand, in the group that received the highest dose of strontium (875 pmol/day), all of the calcium metabolic parameters measured were markedly suppressed. A decrease in calcium level in both the bone and serum was also observed. These results suggest that strontium at doses of less than 175 pmol per day does not have a toxic effect on calcium contents in bone or calcium metabolism in rats, and a dose of 87.5 pmol/day may be adopted for future evaluations of the efficacy of strontium in various experimental skeletal diseases.

Keywords: Calcium, Strontium, Kinetics

Administration of excessive doses of strontium to animals has been shown to reduce intestinal calcium absorption (1–5) and to induce hypocalcaemia and/or hypocalcified bone (2, 4–7). These changes in calcium metabolism are thought to be induced indirectly by depression of 1α,25-dihydroxyvitamin D₃ synthesis (2, 8, 9). It has recently been reported that strontium accumulated in bone directly reduces bone resorption and/or formation at toxic dose levels in vivo (4) and in vitro (5). In addition to these toxic effects of strontium, clinical beneficial effects have been reported by Skoryna and Fuskova in that low doses of strontium remineralize skeletal lesions and increase bone mass in patients with metastatic bone cancer. These authors have drawn a distinction between pharmacological and toxic levels in strontium therapy (10). In addition, it has also been reported that patients with osteoporosis show a favorable response to treatment with strontium (11). These beneficial effects (10, 11) are mainly assessed by radiographic findings, and the mechanism of these effects is still unclear. A few animals studies support the beneficial effects of strontium. Marie et al. (12) reported that an augmentation of trabecular bone volume is induced by low doses of strontium without any changes in serum 1α,25-dihydroxyvitamin D₃ in rats. They suggested that strontium directly suppresses bone resorption, and that strontium may have beneficial effects on skeletal lesions.

The general procedure for a calcium balance and kinetics study was originally described by Aubert and Milhaud (13). The method is based on the measurement of the intake and excretion of stable calcium and of the kinetics of injected ⁴⁴Ca during a 72-hr period. Using this method, many parameters of calcium metabolism, such as intake, intestinal absorption, intestinal and urinary excretion, and deposition and resorption of bone, can be determined or estimated. The highly integrated and complex
calcitropic hormones and cytokines regulate the flow of minerals into and out of the extracellular fluid compartment at each organ. Bone mineral metabolism may be affected not only by drugs but also by changes in mineral flow in various organs. In most of the previous studies, food was available ad libitum, and strontium was administered in either food or drinking water, so that the amount of strontium and calcium intake were not controlled. We assessed the effects of a low dose of strontium in rats that were fed diets that were identical, except with regard to the amount of strontium (pair-feeding). The aims of this experiment were to estimate the minimum toxic dose of strontium and to investigate the effects of strontium at levels less than this toxic dose in young growing rats.

MATERIALS AND METHODS

Animals and feeding plan

Twenty-seven female Wistar rats (88–109 g b.wt.) were divided into four groups: a control group (n=6), a 0.05%-Sr group (n=6), a 0.10%-Sr group (n=7) and a 0.50%-Sr group (n=8). The rats in the control group were fed a semi-synthetic vitamin D-deficient diet containing 0.5 w/w% calcium (125 mmol calcium/kg of dry diet) and 0.35 w/w% phosphorus (4). The strontium-fed groups were fed diets similar to those of the control group, but to which strontium carbonate (SrCO₃) had been added. All of the rats were given distilled water ad libitum, and 10 IU of vitamin D₃ dissolved in 0.1 ml of cottonseed oil was placed on each diet every day just before feeding.

The diets described above were given to the rats in the respective groups from when they were 36 to 63 days old. All of the rats were pair-fed each diet in single metabolic cages. When the rats were 36 to 45 days old, the amount of diet given was increased each day to match the growth of the rats in each group. All of the rats were given 0.92 mmol calcium/day at the age of 36 days and 1.73 mmol calcium/day at the age of 45 days. Thereafter, the calcium supply was constant (1.75 mmol calcium/day). The treatment of the experimental animals was approved by the Experimental Animal Committee of Showa University.

Calcium and strontium metabolic studies

Calcium balance and kinetic studies were performed to examine the effect of strontium on calcium metabolism, using the method described by Aubert and Milhaud (13). Briefly, this method involves the examination of the intake and excretion of calcium and the measurement of the kinetics of injected ⁴⁵Ca over a 3-day period. The decay of the specific activity of ⁴⁵Ca in plasma was fitted to a double exponential function and analyzed according to the two-compartment model for calcium metabolism. In the present investigation, a strontium balance study was also performed to measure the intake and excretion of strontium. The parameters in the metabolic studies are described in Table 1.

When the rats were 60 days old, 1.11 MBq of ⁴⁵CaCl₂ solution (radioactivity of approximately 37 MBq/ml) in 0.3 ml of 0.15 M NaCl was injected into the tail vein of each rat. Blood samples were obtained from the tail at 2, 4, 6, 25, 49 and 73 hr after the injection. The amount of ⁴⁵Ca in these samples was determined by using a liquid scintillation counter (Model 3255; Packard Instrument Company, Inc., Meriden, CT, USA). The amount of diet consumed by each rat was noted. Urine and feces were harvested on sheets of filter paper over the 3-day period. The feces and filter papers that contained urine were ashed and then dissolved in 2.0 N HCl. Aliquots of these urine and feces sample solutions were counted for ⁴⁵Ca.

The amount of calcium and strontium in the feces, and the amount of strontium in the urine were also determined by an atomic absorption spectrophotometer (Model 603; Perkin Elmer Co., Ltd., Norwalk, CT, USA) or by EGTA titration (Corning calcium analyzer 940; Corning Co., Ltd., Corning, NY, USA). The concentrations of calcium and strontium in the serum were measured at the end of the experiment.

Calcium and strontium contents in bone and histological studies

When the rats were 64 days old, they were sacrificed un-

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Table 1. Metabolic study parameters (mmol/day, except for β and β*)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
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<tbody>
<tr>
<td>Vi (sVi)</td>
<td>Calcium (strontium) intake</td>
</tr>
<tr>
<td>VF (sVF)</td>
<td>Total fecal calcium (strontium)</td>
</tr>
<tr>
<td>VNa⁺ (sVNa⁺)</td>
<td>Net calcium (strontium) absorption in intestine: Vi−VF (sVi−sVF)</td>
</tr>
<tr>
<td>Vu (sVu)</td>
<td>Calcium (strontium) excretion into urine</td>
</tr>
<tr>
<td>V₄⁺ (sV₄⁺)</td>
<td>Calcium (strontium) retention in the body: Vi−VF−Vu (sVi−sVF−sVu)</td>
</tr>
<tr>
<td>Vo⁺</td>
<td>Bone formation</td>
</tr>
<tr>
<td>Vo⁻</td>
<td>Bone resorption</td>
</tr>
<tr>
<td>β⁺</td>
<td>Calcium (strontium) absorption ratio: Vna/Vi (sVna/sVi)</td>
</tr>
</tbody>
</table>

*: These parameters can be calculated directly from the respective measurements of diet consumption (Vi), calcium and strontium in feces (VF and sVF), ⁴⁵Ca in urine (Vu) or strontium in urine (sVu).

*: Vo⁺ was defined as the amount of calcium that leaves the first compartment irreversibly over the time period measured, and which is not accounted for by calcium appearing in urine and feces.

*: Vo⁻ was calculated by the difference between the rates of Vo⁺ and V₄⁺.
under ether anesthesia, and their femurs were removed and fixed and defatted in 70% ethanol. Radiograms of the femurs in each group were taken with a soft X-ray apparatus (SOFRON type SRO-M50; Sofron Co., Ltd., Tokyo) to examine the degree of ossification and to note the bone length. The bones were then dried and ashed to measure calcium and strontium contents in the bone.

Statistical analyses
All of the results are expressed as the mean ± S.D., and the significance of the differences between the values of the control, 0.05%-Sr, 0.10%-Sr and 0.50%-Sr groups was evaluated by Student's t-test.

RESULTS

Animal growth
There were no significant differences in growth among the control and strontium-fed groups (Fig. 1). In addition, no adverse effects were found in the extremities, such as an inability to stand or paralysis (14).

Calcium metabolism
The results of the calcium balance and kinetic studies are shown in Fig. 2. Net intestinal calcium absorption (Vna) and the fractional calcium absorption (β) were markedly decreased in the 0.50%-Sr group (Fig. 2, b and c). The values of Vna and β in the 0.50%-Sr groups were about 80% of those in the control group. Furthermore, in the 0.50%-Sr group, marked decreases in the skeletal parameters, such as bone formation (Vo+), bone resorption (Vo−) and calcium retention in the body (Vd) were observed (Fig. 2; d, e and f). However, no significant changes were observed in either of the other strontium groups.

Strontium metabolism
The results of the strontium balance study are shown in Fig. 3. Strontium absorption in the intestine (sVna) was proportional to the strontium intake (sVi). However, as opposed to the intestinal calcium absorption ratio (β), there was no significant correlation in the strontium absorption ratio (sβ). Strontium was retained in the body (sVd) in proportion to the strontium intake (sVi).

Analysis of minerals in blood and femur, and radiographic findings
Hypocalcaemia was observed in the 0.50%-Sr group. However, normal serum calcium levels were observed in the other groups (Table 2). Serum strontium levels in the strontium-fed groups increased in a dose-dependent manner: i.e., approx. 68 μM, 175 μM and 550 μM, respectively (Fig. 3a). Calcium contents in ashed- and dried-femur increased moderately, but significantly, in the 0.05%-Sr group (P<0.05), remained unchanged in the 0.10%-Sr group, and decreased significantly in the 0.50%-Sr group. The total amounts of calcium and strontium (in terms of molarity) and the length of the femur in the strontium-fed groups were not significantly different than those in the control group (Table 2). As seen in Fig. 4, a large amount of trabecular bone was observed in the 0.50%-Sr group.

DISCUSSION
In the present study, the amount of calcium consumed in each group was almost identical (Fig. 2a), and the body weights of the rats in the strontium-fed groups matched those in the control group (Fig. 1).

In a previous study, when rats received a relatively low dose of strontium (0.0027 to 0.157% in the diet), strontium concentrations in bone reached a constant level after only 4 weeks of dietary treatment (15). These findings show that calcium and strontium metabolism in the rats had reached a steady state. Such a steady state condition is required for a calcium kinetics study. Thus, the duration of dietary treatment in the present study was considered adequate for investigating the effects of strontium.
Fig. 2. Effect of strontium on calcium metabolism in rats. Since one of the rats in the 0.50%-Sr group was not injected with $^{45}$Ca, it was excluded from the calculations for calcium balance and kinetics. Values are expressed as the mean ± S.D. Significant differences from the control group, if any, are indicated above the bars. The parameters are explained in Table 1.
Fig. 3. The correlations between strontium parameters. The parameters are explained in Table 1.
Table 2. Serum calcium (Ca) concentrations and an analysis of Ca and Sr in the femur

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.05%-Sr</th>
<th>0.10%-Sr</th>
<th>0.50%-Sr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ca (mM)</td>
<td>2.56±0.08</td>
<td>2.48±0.05</td>
<td>2.43±0.08</td>
<td>2.23±0.11**</td>
</tr>
<tr>
<td>Dry weight (mg)</td>
<td>342.5±18.19</td>
<td>327.9±25.58</td>
<td>344.6±28.21</td>
<td>350.1±20.13</td>
</tr>
<tr>
<td>Ash weight (mg)</td>
<td>204.0±11.08</td>
<td>195.9±14.78</td>
<td>206.4±5.49</td>
<td>201.8±13.10</td>
</tr>
<tr>
<td>% Ca in dry weight</td>
<td>18.38±0.43</td>
<td>19.35±0.44**</td>
<td>18.67±0.52</td>
<td>15.77±0.64**</td>
</tr>
<tr>
<td>% Ca in ash weight</td>
<td>30.88±1.52</td>
<td>32.37±0.54*</td>
<td>31.18±0.97</td>
<td>27.37±0.70**</td>
</tr>
<tr>
<td>% Sr/Ca</td>
<td>0.015±0.003</td>
<td>1.008±0.053**</td>
<td>2.178±0.358**</td>
<td>10.82±0.782**</td>
</tr>
<tr>
<td>Ca + Sr</td>
<td>1.57±0.071</td>
<td>1.60±0.145</td>
<td>1.64±0.064</td>
<td>1.53±0.104</td>
</tr>
<tr>
<td>Bone length (mm)</td>
<td>32.02±0.56</td>
<td>31.46±0.46</td>
<td>31.49±0.76</td>
<td>31.49±0.89</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± S.D. *Significantly different from the value in the control group (P ≤ 0.05). **Significantly different from the value in the control group (P ≤ 0.01). a Molar ratio. b Sum of Ca and Sr in terms of molarity.

Fig. 4. Effect of dietary strontium on rat femoral radiography after 4 weeks on the control or strontium diets. The strontium-fed groups, 0.05, 0.10 and 0.50%-Sr groups, consumed strontium at the dose of 87.5, 175 and 875 pmol per day, respectively. Calcium intake in each group was constant (1.75 mmol per day). In the 0.5%-Sr group, a large amount of trabecular bone was found.
Hypocalcemia was observed in the 0.50%-Sr group (Table 2). This phenomenon has also been reported by several other investigators in strontium-fed rats (4, 5, 7, 12). When rats are subjected to calcium deprivation of various intensities, the decreased calcium intake results in decreased intestinal calcium absorption (Vna) (16). If only Vna is reduced, then the lack of calcium can be compensated for by increased bone resorption (Vo−) (17). However, in the present experiment, both Vna and Vo− were reduced in the 0.50%-Sr group. At this level of strontium, the reduction in Vo− may make it impossible to compensate for the reduced Vna, which may lead to hypocalcemia (Table 2).

Bone modeling and remodeling generally occurs by the following process: First, cartilage provides scaffolding for bone formation. The cartilage is then replaced by trabecular bone. Finally, the marrow cavity starts to increase in length as minerals are removed. If the final step is blocked, trabecular bone remains without the formation of a marrow cavity. In fact, a large amount of trabecular bone was observed radiographically in the 0.50%-Sr group (Fig. 4), which might be a consequence of the decreased Vo− (Fig. 2e). Since the decreased Vo− would result in both hypocalcemia and an increase in trabecular bone, strontium intake of 875 pM per day (0.50%-Sr group) may be considered the toxic level.

In this experiment, serum strontium levels increased dose-dependently (Fig. 3a). There were no changes in serum calcium levels, Vo+, Vo− or in the amount of trabecular bone observed radiographically when the serum strontium concentration was less than about 175 pM, as was the case in the 0.05%- and 0.10%-Sr groups. In the 0.50%-Sr group, in which the serum strontium concentration was about 550 pM, hypocalcaemia and a large amount of trabecular bone were observed. In this experiment, the effects of strontium were not studied using serum strontium levels between 175 and 550 pM. Marie et al. (12) demonstrated an augmentation of trabecular bone volume without any changes in serum calcium concentration when rats were treated with strontium for 9 weeks. The dosage of strontium used in their study was between 0.1% and 0.5% in drinking water. They measured serum calcium and strontium at 4 and 9 weeks in the same rats. In their study, the serum strontium concentration dropped from approximately 350 pM at week 4 to 200 pM at week 9, and hypocalcaemia was observed at week 4. Although the serum strontium level at week 9 in their study was similar to the result observed in the present study for the 0.1%-Sr group, the level observed at week 4 in their study was at the toxic level. Therefore, the augmentation of trabecular bone volume in their study may have resulted from a decreased bone resorption at week 4, and the skeletal change persisted despite a recovery of serum calcium at week 9. Thus, a serum strontium level of 350 pM might inhibit calcium metabolism.

Strontium retention in bone increased in a dose-dependent manner (Fig. 3, b and f). In the 0.50%-Sr group, calcium retention in the body (Vd) decreased (Fig. 2f); however, the bone length did not change (Table 2). Accordingly, although the calcium contents in ashed-femur decreased by 20%, the sum of calcium and strontium, on a molar basis, did not change, as compared with the control group (Table 2). These results suggest that the decrease in calcium was due to an increased content of strontium in newly-formed bone in the 0.50%-Sr group. Rats in the 0.05%-Sr group, which consumed 87.5 pMol of strontium per day, showed slight but significant increases in calcium contents in dried- and ashed-bone. At this level of strontium, the strontium level in bone was about 1% of that of calcium, on a molar basis (Table 2); and the serum strontium concentration was about 68 pM. Since strontium intake greater than that in the 0.10%-Sr group does not affect serum parathyroid hormone, calcitonin (4) or 1α,25-dihydroxyvitamin D3 (12) concentrations, the increased calcium in bone was not believed to be due to hormonal changes. At a similar concentration of serum strontium, no effects were observed in histological (17) and morphometric (18) studies in rats and mice, respectively. Nevertheless, the increase in the calcium level in bone may be too small to be detected by metabolic or histological analyses. Thus the mechanism of increase in calcium in bone is unclear.

Low doses of strontium have been used for medicinal treatment. McCaslin and Janes (11) reported that marked subjective improvement was experienced by 80% of patients with osteoporosis who had been treated with strontium. In addition, serum strontium concentrations of approximately 60 pM decreased bone pain and increased mineralization, as observed radiographically, in patients with metastatic bone cancer (10). Furthermore, a similar beneficial effect of strontium on bone resorption was also found in our previous in vitro study (5). When newborn mice were injected with strontium for 5 days so that this element would accumulate in calvariae, the enhancement of bone resorption with parathyroid hormone in organ culture was suppressed. When the suppression was observed with a minimum dose of strontium, the strontium/calcium ratio, in terms of molarity, was about 1% (5). In the present experiment, the strontium levels in bone and serum in the 0.05%-Sr group were similar to those observed by Izumisawa et al. (5) and Skoryna and Fuskova (10), respectively. At low doses, the beneficial effects of strontium may appear more clearly in skeletal diseases or experimental models in cases where bone turnover is enhanced by ovariectomy, osteoporosis or metastatic bone cancer. In these cases, strontium may be
able to restore bone turnover to normal levels. In fact, both Vo+ and Vo− increased in ovariectomized rats, but high bone turnover was not observed in ovariectomized rats that had been fed 87.5 µmol/day of strontium (unpublished observation, T. Morohashi).

In conclusion, strontium did not alter calcium metabolic parameters in the 0.10%-Sr group; Therefore, strontium at doses of less than 175 µmol per day does not appear to have toxic effects. Since calcium contents in ashed- and dried-femur increased in the 0.05%-Sr group, 87.5 µmol/day of strontium may have some beneficial effects in skeletal diseases in experimental animals.

REFERENCES