Oral Administration of Calcium Hydroxide Stimulates Bone Metabolism in the Femoral Diaphysis of Rats with Skeletal Unloading

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The preventive effect of calcium hydroxide on the disorder of bone metabolism caused by skeletal unloading was investigated. Skeletal unloading was designed using the model of hindlimb hang in rats. Skeletal unloading for 7d caused a significant decrease of inorganic phosphorus concentration in the serum and of alkaline phosphatase activity and deoxribonucleic acid (DNA) content in the femoral diaphysis of rats. Oral administration of calcium hydroxide (16 and 24 mg Ca/kg) caused a significant increase in serum inorganic phosphorus concentration and femoral-diaphyseal calcium content and alkaline phosphatase activity of rats with skeletal unloading. Bone DNA content was significantly increased by the dose of 24 mg Ca/kg. These results clearly indicate that skeletal unloading-induced disorder of bone metabolism is partly prevented by oral administration of calcium hydroxide. Calcium ingestion may be useful as a therapeutic tool in the disorder of bone metabolism caused by skeletal unloading.

Keywords calcium hydroxide; bone metabolism; skeletal unloading; rat femur

It is well known that calcium ion plays an important role in cellular functions. The requirement of calcium in a living body is greatest in the growing and aging stages, however, calcium deficiency is often seen. Calcium supply, therefore, is of pharmacological and nutritional importance. Among the many calcium compounds, calcium lactate and calcium gluconate are most used as nutrients. Meanwhile, calcium hydroxide may exist in non-ionic form in an aqueous solution in the gastrointestinal tract, because the dissociation constant of the compound is so small. Since the non-ionized form of a substance penetrates membranes more easily than the ionized form, one could expect a considerable amount of absorption of this substance when it is orally administered. This, however, has not been fully clarified. More recently, the bioavailability of calcium hydroxide has been examined in growing rats; calcium hydroxide orally administered caused increases in intestinal calcium absorption and bone calcium content more effectively than did calcium gluconate. Therefore, the present study was undertaken to clarify the preventive effect of calcium hydroxide on the disorder of bone metabolism caused by skeletal unloading (immobilization). Findings suggest a therapeutic role of calcium hydroxide in the disorder of bone metabolism.

Materials and Methods

Weanling male Wistar rats (conventional), weighing 75—80g (4 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The animals were fed commercial laboratory chow (solid) containing 37.5% carbohydrate, 1.1% Ca and 1.1% P at a room temperature of 25°C, and were given distilled water freely.

The method of skeletal unloading comprised a number of steps. Rats were placed in a circular cage with a steel-wire floor and fed chow and distilled water freely. The hindlimbs were allowed to hang freely but were immobilized with filament tape. The forelimbs enabled the rat to move about in the cage. Hindlimbs were hung for up to 7 d. The animals were divided into two groups; one group of 5 normal rats was fed and allowed to move around the cage. The other group of 5 rats with hindlimb hang (skeletal unloading) were fed in the circular cage. Initiation of hindlimb hang was designed for each group so that all animals were the same age at the end of the experiment.

Rats with skeletal unloading received oral administration of a calcium hydroxide solution by a stomach tube for 7 d (twice a day). Calcium hydroxide was supplied by Tachikawa Penicillin Co., Ltd. (Tokyo, Japan). The solution was given to rats at 9:00 and 17:00 in individual doses of 8.0, 16, and 24 mg Ca per kg body weight. Rats were killed 17 h after the final administration of calcium hydroxide. Control rats with skeletal unloading received vehicle solution (distilled water) by mouth.

Rats were bled by cardiac puncture under light anesthesia with ether, and the blood and femur were removed immediately. Blood samples were centrifuged 30 min after collection. The serum was separated and analyzed immediately. Serum calcium was determined by the method of Wills. Serum inorganic phosphorus was measured by the method of Tauskys and Shon.

The femur was removed after bleeding and soaked in ice-cold 0.25 M sucrose solution. The femur was cleaned of soft tissue and marrow, and the diaphysis and epiphysis (containing metaphyseal tissue) were separated and weighed. The diaphyseal tissues were ashed for 3 h at 60°C, weighed, and then dissolved in 6.0 M HCl solution. Calcium was determined by atomic absorption spectrophotometry. The calcium content in bone was expressed as mg per g bone ash.

To assay alkaline phosphatase activity the diaphyseal tissues were immersed in 3.0 M ice-cold 6.5 M barbituric buffer (pH 7.4), cut into small pieces, homogenized with a Phycostron homogenizer, and disrupted for 60 s with an ultrasonic device. The supernatant centrifuged at 600 x g for 5 min was used for the measurement of enzyme activity. The enzyme assay described below was carried out under optimal conditions. Alkaline phosphatase activity was determined by the method of Lowry et al.

The enzyme activity was expressed as μmol of p-nitrophenol liberated per min per mg protein. Protein concentration was determined by the method of Lowry et al.

The diaphyseal tissues were shaken with 4.0 M ice-cold 0.1 M NaOH solution for 24 h after the homogenization of the bone tissue. After alkali extraction, the samples were centrifuged at 10000 x g for 5 min, and the supernatant was collected. Deoxribonucleic acid (DNA) content in the supernatant was determined by the method of Ceriotti and expressed as the amount of DNA (mg) per g wet weight of bone tissue.

Data are expressed as the mean±S.E.M. Statistical differences were analyzed using Student's t-test. p values of less than 0.05 were considered to indicate statistically significant differences.

Results

The effect of skeletal unloading with hindlimb hang on bone metabolism in rats was investigated. The rats with skeletal unloading for 7 d had no significant change of food ingestion in comparison with that of animals without the unloading (data not shown). Alterations in calcium and inorganic phosphorus concentrations in the serum of rats with skeletal unloading are shown in Table 1. Serum calcium concentration was not altered by skeletal unloading for 7 d, while serum inorganic phosphorus concentration was significantly decreased. This decrease was not seen when calcium hydroxide (16 and 24mg Ca/kg) was orally
Table 1. Effect of Calcium Hydroxide Administration on Calcium and Inorganic Phosphorus Concentrations in the Serum of Rats with Hindlimb Hang

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calcium (mg/100 ml)</th>
<th>Inorganic Phosphorus (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10.38 ± 0.12</td>
<td>9.81 ± 0.12</td>
</tr>
<tr>
<td>Skeletal unloading</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.11 ± 0.15</td>
<td>9.22 ± 0.15</td>
</tr>
<tr>
<td>8 mg Ca/kg</td>
<td>10.15 ± 0.09</td>
<td>9.39 ± 0.17</td>
</tr>
<tr>
<td>16 mg Ca/kg</td>
<td>10.22 ± 0.13</td>
<td>9.70 ± 0.20</td>
</tr>
<tr>
<td>24 mg Ca/kg</td>
<td>10.43 ± 0.11</td>
<td>9.83 ± 0.18</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E.M. of 5 rats. Rats were fed for 7 days with hindlimb hang (skeletal unloading). Calcium hydroxide was orally administered for 7 days (twice a day), and rats were killed 18 hours after the final administration. a) p < 0.05, as compared with the normal value. b) p < 0.01, as compared with the control value (skeletal unloading).

Fig. 1. Effect of Calcium Hydroxide on Calcium Content in the Femoral Diaphysis of Rats with Skeletal Unloading

Animals were fed for 7 days in the state of hindlimb hang (A). Calcium hydroxide (8.0, 16, and 24 mg Ca/kg) was orally administered twice a day to rats subjected to skeletal unloading for 7 days, and the animals were killed 17 hours after the final administration (B). Each value represents the mean ± S.E.M. for 5 rats. a) p < 0.05, as compared with the value of normal group or without calcium administration. □ normal; ■ skeletal unloading.

Fig. 2. Effect of Calcium Hydroxide on Alkaline Phosphatase Activity in the Femoral Diaphysis of Rats with Skeletal Unloading

Animals were fed for 7 days in hindlimb hang (A). Calcium hydroxide (8.0, 16, and 24 mg Ca/kg) was administered as described in Fig. 1. Each value represents the mean ± S.E.M. for 5 rats. a) p < 0.01, as compared with the value of normal group or without calcium administration. □ normal; ■ skeletal unloading.

Fig. 3. Effect of Calcium Hydroxide on DNA Content in the Femoral Diaphysis of Rats with Skeletal Unloading

Animals were fed for 7 days in hindlimb hang (A). Calcium hydroxide (8.0, 16, and 24 mg Ca/kg) was administered as described in Fig. 1. Each value represents the mean ± S.E.M. for 5 rats. a) p < 0.05, as compared with the value of normal. b) p < 0.05, as compared with the value without calcium administration. □ normal; ■ skeletal unloading.

Discussion

It is known that skeletal unloading caused by immobilization, 10) spaceflight, 11) bed rest, 12) or hindlimb elevation 13) results in osteopenia. 14) Recently, it has been demonstrated that inhibition of bone formation is induced using hindlimb hang as the method of skeletal unloading. 3, 15) Inhibition of bone formation subsequent to skeletal unloading may be partly accompanied by falls in the serum level of 1,25-dihydroxyvitamin D3 16) and in the bone content of zinc, 7, 17) 18) which are known to stimulate bone formation and mineralization. However, the factors preventing skeletal unloading-induced disorder of bone metabolism are not fully resolved.

The present study was undertaken to clarify whether oral administration of calcium hydroxide can influence the administration twice a day for 7 days to rats with skeletal unloading.

The content of calcium in the femoral diaphysis of rats was not significantly reduced by skeletal unloading for 7 days, as compared with that of normal rats (Fig. 1A). Oral administration of calcium hydroxide (16 and 24 mg Ca/kg) produced a significant increase in calcium content in the femoral diaphysis of rats with skeletal unloading (Fig. 1B).

Also, oral administration of calcium hydroxide (16 and 24 mg Ca/kg) for 7 days to normal rats produced a significant increase in calcium content in the femoral diaphysis (data not shown). This increase was to the same extent as that of skeletal unloading.

The effect of calcium hydroxide administration on alkaline phosphatase activity in the femoral diaphysis of rats with skeletal unloading is shown in Fig. 2. Bone alkaline phosphatase activity was clearly decreased by skeletal unloading (Fig. 2A). This decrease was prevented to a significant extent by the administration of calcium hydroxide (16 and 24 mg Ca/kg) (Fig. 2B). The effect was not seen at the dose of 8.0 mg Ca/kg.

DNA content in the femoral diaphysis of rats was markedly reduced by skeletal unloading (Fig. 3A). This reduction was significantly blocked by oral administration of calcium hydroxide (24 mg Ca/kg), although the doses of 8.0 and 16 mg Ca/kg did not have an appreciable effect (Fig. 3B).
decrease in bone formation and calcification caused by skeletal unloading with hindlimb hang in rats. Oral administration of calcium hydroxide produces a temporal elevation of serum calcium concentration, indicating that calcium is absorbed form intestine. In the present study, oral administration of calcium hydroxide caused a significant increase of calcium content, alkaline phosphatase activity and DNA content in the femoral diaphysis of rats with skeletal unloading. These findings indicate that skeletal unloading-induced disorder of bone metabolism is prevented by oral administration of calcium hydroxide. This suggests that ingestion of a calcium hydroxide solution has preventive effect on the development of osteopenia during skeletal unloading.

Skeletal unloading also caused a significant decrease in serum inorganic phosphorus concentration; this decrease was completely blocked by oral administration of calcium hydroxide. Meanwhile, skeletal unloading with hindlimb hang did not have an appreciable effect on food ingestion. It appears that skeletal unloading-induced decrease in serum inorganic phosphorus concentration may be involved in calcitropic hormones. It is reported that serum 1,25-dihydroxyvitamin D₃ level is decreased by skeletal unloading, that the steroid produces an increase in serum inorganic phosphate concentration and that the production of steroid hormone is elevated by augmentation of extracellular calcium.

Oral administration of calcium hydroxide caused a significant increase of calcium content in the femoral diaphysis of rats with skeletal unloading, indicating that accumulation of calcium into the bone is stimulated by calcium administration. Furthermore, oral administration of calcium hydroxide produced a significant increase of alkaline phosphatase activity in the femoral diaphysis of rats with skeletal unloading. The enzyme is localized in osteoblastic cells related to bone mineralization. Presumably the increase in bone calcium content by calcium hydroxide administration may be involved in the rise of bone alkaline phosphatase activity. Administration of calcium hydroxide also produced a significant increase of DNA content in the femoral diaphysis of rats with skeletal unloading, although the effect required a comparatively high dose of calcium hydroxide. These findings suggest that calcium hydroxide administration has a stimulative effect on the proliferation of bone cells. Thus, oral administration of calcium hydroxide had an anabolic effect on bone metabolism in rats with skeletal unloading. A previous study showed that oral administration of calcium hydroxide (16 mg Ca/kg) could produce an appreciable increase of calcium content in the femoral diaphysis of normal rats. We do not yet know the mechanism by which oral administration of calcium hydroxide can produce an anabolic effect on bone metabolism in rats with skeletal unloading. It appears that calcium absorbed from intestine directly affects bone cells (osteoblastic cells), although the possibility of a calcitropic-hormonal effect cannot be excluded.

In conclusion, it has been demonstrated that oral administration of calcium hydroxide can prevent deterioration of bone metabolism in rats with skeletal unloading.

References