STIMULATION OF BONE RESORPTION BY COMPARATIVELY HIGH DOSE OF ZINC IN RATS

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The changes of femoral calcium and acid phosphatase activity were examined in rats orally administered zinc sulfate (10 mg Zn/100 g body weight) for 3 d. Zinc administration to intact rats produced significant decreases of calcium levels in the serum, and femoral diaphysis and epiphysis, while it caused remarkable elevation of acid phosphatase activity in the femoral diaphysis and epiphysis. Thyroparathyroidectomy significantly prevented the alterations of the calcium content and the enzyme activity in the femoral diaphysis and epiphysis caused by zinc administration to intact rats. The present results suggest that comparatively high dose of zinc may stimulate bone resorption mainly mediated through the actions of parathyroid hormone, due to maintain calcium homeostasis.

Keywords — zinc; calcium; bone resorption; acid phosphatase; rat femur

INTRODUCTION
Zinc may play a physiological role in growth and calcification of bone tissue.1) High concentrations of zinc are shown histochemically at the sites of calcification in developing osteons of bone in rats.2) Zinc-deficient rats have decrease in calcium balance, and in rate of bone anabolism and resorption.3) Zinc is also required for the manifestation of vitamin D action on calcium metabolism in rats.4)

On the other hand, diets containing high zinc exert a porotic effect in bone of rats.5) Our previous report demonstrates that a single oral administration of high zinc causes a hypocalcemic effect mainly based on an increase in gastric calcium secretion.6) From these investigations, it is assumed that zinc administration may induce bone resorption related to the abnormality of calcium metabolism. The present study was therefore designed to investigate the effect of repeated administration of zinc sulfate on femoral calcium in rats. It was found that zinc administration stimulated bone resorption in rats.

MATERIALS AND METHODS
Male Wister rats, weighing approximately 100–120 g (5 weeks old), were used in the present experiments. The rats were kept at 25 ±1°C and fed laboratory chow containing 1.1% Ca and 1.1% P and tap water freely.

Zinc sulfate was dissolved in distilled water to a concentration of 10 mg Zn/ml. This solution (1 ml/100 g body weight) was given orally for 3 d in rats.

The thyroparathyroid gland complex of rats in other groups was removed with fine forceps under light ether anesthesia. Zinc (10 mg/100 g) was orally administered for 3 d immediately after thyroparathyroidectomy to deplete endogenous parathyroid hormone which stimulates bone resorption.

At 24 h after the last administration of zinc, the rats were bled by cardiac puncture. The serum was separated and analyzed immediately. The serum calcium was determined by atomic absorption spectrophotometry after precipitation with 10% trichloroacetic acid.7)

The femurs were immediately isolated after bleeding and soaked in cold 0.25M sucrose solution. The femur was cleaned of soft tissue and marrow, and the diaphysis and the epiphysis were separated, ashed for 24 h at 640°C,
weighed, and dissolved in 6 N HCl. Calcium was determined by atomic absorption spectrophotometry.

The femoral diaphysis and epiphysis tissues were immersed in 3 ml of 7.5 mM barbital buffer (pH 7.4) at 0°C, and were disrupted for 20 s with an ultrasonic device.8) The supernatant centrifuged at 6000 × g for 5 min was used for measurement of the enzyme activity. Acid phosphatase activity was determined by the method of Lindhardt and Walter.9) Enzyme activity was expressed as nmol of p-nitrophenol liberated per min per mg protein.

The data were subjected to analysis of variance, and the S.E. was calculated from the residual error term. Statistical significance is expressed as p values from Student's t-test.

RESULTS

The changes of calcium levels in the serum and femur of intact and thyroparathyroidectomized rats orally administered zinc sulfate (10 mg Zn/100 g body weight) for 3 d are shown in Table I. Zinc administration produced significant decreases of calcium concentrations in the serum of both intact and thyroparathyroidectomized rats. The calcium contents in the femoral diaphysis and epiphysis of intact rats were significantly decreased by zinc administration. Meanwhile, in thyroparathyroidectomized, the calcium content in the femoral diaphysis was not significantly decreased by zinc administration, while that in the femoral epiphysis was significantly reduced. However, the decrease in calcium content of the femoral epiphysis caused by zinc administration to thyroparathyroidectomized rats was less than that in intact rats.

The change of acid phosphatase activity in the femur of intact and thyroparathyroidectomized rats orally administered zinc sulfate (10 mg Zn/100 g) for 3 d is shown in Table II. Zinc administration to intact rats caused remarkable elevation of acid phosphatase activity in the femoral diaphysis and epiphysis. In thyroparathyroidectomized rats, the enzyme activity in the femoral diaphysis was not significantly altered by zinc administration, while that in the femoral epiphysis was significantly increased. However, the increase in acid phosphatase activity of the femoral epiphysis caused by zinc administration to thyroparathyroidectomized rats was less than that in intact rats.

DISCUSSION

Recently it has been demonstrated that a single oral administration of high zinc causes a hypocalcemic effect mainly due to an increase in gastric calcium secretion.6) The hypocalcemia stimulates secretion of parathyroid hormone from parathyroid glands to maintain calcium homeostasis. Parathyroid hormone causes the mobilization of calcium into blood from bone by stimulating the release of lysosomal acid hydrolases from the bone cells.10)

The present study clearly indicated that oral administration of zinc (10 mg/100 g) for 3 d produced significant decreases of calcium levels in the serum and the femoral diaphysis and epiphysis. This finding suggests that comparatively high dose of zinc may induce bone resorption mainly resulted from the decrease of serum calcium levels caused by the metal administration.

On the other hand, zinc administration to thyroparathyroidectomized rats did not cause a significant alteration of calcium content in the femoral diaphysis, while that in the epiphysis was decreased significantly. However, net alteration of calcium content in the femoral epiphysis caused by zinc administration to thyroparathyroidectomized rats was less than the values obtained in the case of intact rats. Thus, thyroparathyroidectomy had a considerable effect to inhibit the decrease in the femoral calcium content observed by zinc administration to intact rats. From these results, it is suggested that the decrease in the femoral calcium by zinc administration may be due mainly to mobilization, induce by parathyroid hormone, of calcium into blood from bone to maintain calcium homeostasis.

Furthermore, comparatively high dose of zinc to intact rats produced a remarkable increase in
the activity of acid phosphatase, a lysosomal enzyme, of the femoral diaphysis and epiphysis. The increases in the enzyme activity observed by zinc administration were clearly prevented by thyroparathyroidectomy. These findings may also support the view that the decrease in the femoral calcium by zinc administration may be caused by the action of parathyroid hormone, since acid phosphatase activity in bone cells is increased in response to parathyroid hormone.\(^{10}\)

However, calcium content in the femoral epiphysis was significantly decreased by zinc administration to thyroparathyroidectomized rats, and acid phosphatase activity in that tissue was also increased significantly. Because of zinc concentration in the femoral epiphysis was higher

**TABLE I. Changes of Calcium Levels in the Serum and Femur of Intact and Thyroparathyroidectomized Rats orally administered Zinc Sulfate**

<table>
<thead>
<tr>
<th>Treatment(^{a}))</th>
<th>Serum calcium(^{b})) (mg/100 ml)</th>
<th>Bone calcium (mg/g ash)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diaphysis</td>
<td>Epiphysis</td>
</tr>
<tr>
<td>Intact rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.85 ± 0.27</td>
<td>371.0 ± 4.9</td>
</tr>
<tr>
<td>Zinc</td>
<td>9.10 ± 0.13(^{c})</td>
<td>325.0 ± 7.6(^{c})</td>
</tr>
<tr>
<td>Thyroparathyroidectomized rats</td>
<td>6.95 ± 0.23</td>
<td>368.9 ± 3.8</td>
</tr>
<tr>
<td>Zinc</td>
<td>5.79 ± 0.38(^{c})</td>
<td>360.0 ± 7.4</td>
</tr>
</tbody>
</table>

\(^{a})\) The rats received the oral administration of distilled water or zinc (10 mg/100 g) for 3 d after thyroparathyroidectomy, and they were sacrificed 24 h later.

\(^{b})\) Values are mean ± S.E. of six rats.

\(^{c}\) \(p<0.01\) as compared with control.

\(^{d}\) \(p<0.01\) as compared with intact rats treated with zinc.

**TABLE II. Change of Acid Phosphatase Activity in the Femur of Intact and Thyroparathyroidectomized Rats orally administered Zinc Sulfate**

<table>
<thead>
<tr>
<th>Treatment(^{a}))</th>
<th>Bone acid phosphatase activity(^{b})) (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diaphysis</td>
</tr>
<tr>
<td>Intact rats</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>77.2 ± 5.9</td>
</tr>
<tr>
<td>Zinc</td>
<td>113.9 ± 6.9(^{c})</td>
</tr>
<tr>
<td>Thyroparathyroidectomized rats</td>
<td>75.2 ± 1.7</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>81.8 ± 3.8</td>
</tr>
</tbody>
</table>

\(^{a})\) The rats received the oral administration of distilled water or zinc (10 mg/100 g) for 3 d after thyroparathyroidectomy, and they were sacrificed 24 h later.

\(^{b})\) Values are mean ± S.E. of six rats.

\(^{c}\) \(p<0.01\) as compared with control.

\(^{d}\) \(p<0.01\) as compared with intact rats treated with zinc.
than that in the diaphysis (data not shown), it is possible that the metal may, at least partly, affect the bone cells. The action of zinc on bone cells, however, remains to be elucidated.

In conclusion, the present study suggests that comparatively high dose of zinc stimulates bone resorption mainly mediated through the actions of parathyroid hormone to maintain calcium homeostasis, although low zinc may play a physiological role in the growth and calcification of bone tissues.1)

REFERENCES