Effect of Calcitonin on the Development of Immobilization Osteoporosis in Rat

HAJIME ORIMO, TAKUO FUJITA AND MASAKI YOSHIKAWA

Department of Geriatrics, Faculty of Medicine, University of Tokyo

Synopsis

The inhibitory effect of porcine calcitonin on the development of immobilization osteoporosis was studied in rat through the analysis of calcium and collagen content of the femur and tibia and the measurement of cortical thickness of the femur. A marked decrease in the calcium content of the femur and collagen content of the tibia of the right hind limb immobilized through the application of a plaster cast was slightly but significantly inhibited by the simultaneous s.c. injection of either 50 MRCmU or 200 MRCmU of porcine calcitonin. However, calcitonin failed to show any effect against the decrease in the cortical thickness of the femur of the immobilized limbs of rats.

It is concluded that calcitonin diminishes the effect of immobilization on the development of osteoporosis possibly through the inhibition of bone resorption.

It has been shown that calcitonin exerts its hypocalcemic and hypophosphatemic effect through an action on bone (Munson and Hirsch 1966). The evidence indicates that it does so largely by inhibiting bone resorption (Johnston and Deiss, 1966; Milhaud et al. 1965; Martin et al. 1966; Friedman and Raisz, 1965; Aliapoulios et al. 1966), but stimulation of bone formation, in both intact and parathyroidectomized animals, has also been suggested (Wase et al. 1967; Foster et al. 1967; Gaillard, 1967; Kumar et al. 1968). Calcitonin ought therefore to be effective in the treatment of diseases characterized by pathologically increased bone resorption. We have previously demonstrated that calcitonin prevented the decrease in cortical thickness of the rat tibia caused by a combination of low calcium diet and prednisolone (Fujita et al. 1968). Using quantitative microradiography, Jowsey (1969) was able to prevent the development of low calcium diet osteoporosis in cat by the administration of porcine calcitonin.

Delling et al. (1970) and Singh and Jowsey (1970), on the other hand, were unable to show the inhibitory effect of calcitonin on the development of immobilization osteoporosis in rat and rabbit. We have therefore studied whether or not calcitonin is effective in preventing the development of immobilization osteoporosis induced by the application of a plaster cast in rats.

Materials and Methods

Twenty eight male Wistar-Imamichi rats weighing approximately 200 gm. were randomly divided into the following 4 groups. Group I: control rats, Group II: rats without any treatment, in which the right hind limbs were immobilized through application of a plaster cast, Group III: rats in which the right hind limbs were similarly immobilized and given daily s.c. injection of 50 MRCmU of porcine calcitonin, Group IV: rats in which the right hind limbs were immobilized and given daily s.c. injection of 200 MRCmU of porcine calcitonin. Calcitonin with the potency of 13 MRC U/mg was dissolved in 16% gelatin and injected daily for the period of 5 weeks. All
the rats were fed Oriental rat chow M. F. (Oriental Kobo Industry, Japan) with the Ca content of 1.5% and water *ad libitum* for the period of 5 weeks and casts were changed at weekly intervals, new cast being applied immediately after the old cast was removed while the animal was still anesthetized. After 5 weeks of immobilization and treatment, blood was drawn from the abdominal aorta and the right femur and tibia of each animal were removed and dissected free of soft tissue. Roentgenograms were taken with the use of a Shimazu 2-D-150-L-2-X apparatus under the conditions of 42 KUP, 50 mA, 0.1 sec. and FFD of 100 cm. Using these X ray picture, cortical thickness of the femur was measured at the middle of the bone length and expressed as % of the total width of the bone at the same level (Fujita *et al.* 1967). Serum calcium was determined by the colorimetric method of Webster (1962) and tissue calcium was determined by the same method after the tissue was ashed in a muffle furnace at 800°C overnight. Hydroxyproline content of the tibia was measured by the method of Miyada and Tappel (1956) and collagen content was calculated according to the formula, hydroxyproline × 13.2 = collagen. The significance of the difference was tested by using Student t-test (one-tailed).

**Results**

**Clinical course**

The final mean body weight of rats were: Group I, 254 ± 9.8 g, Group II, 237 ± 4.1 g, Group III, 238 ± 4.9, Group IV, 244 ± 6.4 g and were not significantly different from each other.

**Measurement of cortical thickness of the femurs**

Per cent cortical thickness of the femur of the immobilized rat was markedly smaller than that of the control rat. Administration of porcine calcitonin, however, failed to show any inhibitory effect on this decrease in the cortical thickness of the femur as shown in Table 1.

**Serum calcium**

Serum calcium value of the rats in which the right hind limb was immobilized was slightly but significantly lower than that of the control rats. The administration of 200 MRCmU of porcine calcitonin to these immobilized rats caused a slight decrease in serum Ca 24 hr after the last injection. (Table 2)

Table 1. Effect of calcitonin on the cortical thickness of the femur of immobilized rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rat</th>
<th>Cortical thickness, % of bone width</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>7</td>
<td>50.0 ± 1.0*</td>
</tr>
<tr>
<td>immobilized</td>
<td>7</td>
<td>35.0 ± 1.0**</td>
</tr>
<tr>
<td>immobilized + calcitonin 50mU</td>
<td>7</td>
<td>38.0 ± 2.0</td>
</tr>
<tr>
<td>immobilized + calcitonin 200mU</td>
<td>7</td>
<td>38.0 ± 1.0</td>
</tr>
</tbody>
</table>

** is significantly smaller than * with P < 0.01
Calcitonin and Experimental Osteoporosis

Table 2. Effect of calcitonin on the serum calcium in rats of immobilization osteoporosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rat</th>
<th>Serum calcium, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>7</td>
<td>9.44 ± 0.04</td>
</tr>
<tr>
<td>immobilized</td>
<td>7</td>
<td>9.12 ± 0.05*</td>
</tr>
<tr>
<td>immobilized + calcitonin 50mU</td>
<td>7</td>
<td>8.97 ± 0.08</td>
</tr>
<tr>
<td>immobilized + calcitonin 200mU</td>
<td>7</td>
<td>8.86 ± 0.10**</td>
</tr>
</tbody>
</table>

** is significantly smaller than * with P < 0.05.
Serum Ca was measured 24 hr after the last injection of calcitonin.

Calcium content of the femur

As shown in Figure 1, the calcium content of the right femur of the immobilized rats was markedly decreased as compared with that of the control rats. Administration of 50 MR-CmU of porcine calcitonin to these immobilized rats significantly prevented this decrease in calcium content of the femur. (P < 0.05) However, the effect of porcine calcitonin was not dose dependent.

Collagen content of the tibia

In rats, in which the hind limb was immobilized, the collagen content of the whole tibia was significantly decreased as compared with that of the control rats. This decrease in collagen content of the tibia of immobilized rats was significantly prevented by the administration of 200 MR-CmU of porcine calcitonin. (P < 0.05) (Fig. 2)

Discussion

According to the results of our present experiment, calcitonin appeared to prevent the loss of bone mass in immobilization osteoporosis in rat. The apparent failure of calcitonin to prevent the decrease in cortical thickness of the femur in immobilized rat might be due to the difference in the method of detecting the small change in the bone mass. It is generally thought that calcium and collagen content of the bone represent the total bone mass, while the cortical thickness only represent the periosteal ossification at the measured site. With regard to the immobilization osteoporosis in young growing rats, one must distinguish between osteoporosis and simple retardation in bone growth, since immobilization is known to retard the growth of the bone. According to our previous experiment (Orimo et al. 1971), the length of the femur of the casted side of the immobilized rat was not significantly different from that of the non-casted femur of the same rat, suggesting that the growth along the long axis of the bone is not retarded in these animals. It is therefore more likely that we are here dealing with immobilization osteoporosis rather than...
simple growth retardation. It has long been considered that immobilization leads to osteoporosis primarily due to diminished osteoblastic activity (Fourman and Royer 1968). However, the failure of the development of immobilization osteoporosis in dogs after the removal of the parathyroid gland as shown by Burkhart and Jowsey (1967) strongly suggests the importance of the parathyroid hormone-induced bone resorption as one of the etiologic factors in this type of osteoporosis. Since calcitonin has been shown to inhibit bone resorption, calcitonin might diminish the severity of immobilization osteoporosis through the inhibition of parathyroid hormone induced bone resorption. Of particular interest is the recent finding of Delling et al. (1970) and Ziegler and Delling (1969) that calcitonin failed to prevent the development of immobilization osteoporosis in rat. Using tetracycline labelling technique, they have shown that calcitonin stimulated the new bone formation around the fractured site rather than protecting against the development of immobilization osteoporosis. Using quantitative microradiography and tetracycline labeling, Jowsey's group were unable to show any inhibitory effect on the development of immobilization osteoporosis in rabbits (Singh and Jowsey, 1970) and dogs (Chiroff and Jowsey, 1970). Delling et al. (1970) used both intact and parathyroidectomized female Sprague Dawley rat in which the left tibiae were fractured and immobilized and gave s.c. injection of 100 MRCmU of calcitonin in 5% gelatin for the period of 42 days. Chiroff and Jowsey (1970), on the other hand, immobilized one hind limb of puppies through the application of a plaster of Paries and gave s.c. injection of 250 MRCmU per kilogram of porcine calcitonin in 16% gelatin for 8 weeks. Our present result was apparently contradictory to these data, but this difference might be explained by the difference in the animals used as well as the duration and the dose of calcitonin used. Although calcitonin was expected to show a marked inhibitory effect on the development of immobilization osteoporosis possibly by counteracting the PTH induced bone resorption, its effect, if any was found to be unexpectedly small. This might be explained by the secondary hyperfunction of the parathyroids due to calcitonin induced hypocalcemia. It is also possible that in young growing rats, the decrease in osteoblastic activity is more important than the increase in bone resorption in the loss of bone mass. If so, it is not surprising that the effect of calcitonin is small, since the major action of calcitonin is the inhibition of bone resorption. In conclusion, we have presented some evidence which suggests that calcitonin diminishes the loss of bone mass induced by immobilization in rat. These results in rats give some encouragement to the idea that calcitonin might have some beneficial effect in human osteoporosis.

Acknowledgments

The authors are indebted to Dr. K. Hayano and T. Sakurada for their cooperation and Dr. J. W. Bastian of the Armour Pharmaceutical company for the generous supply of calcitonin.

References

Endocrinol. Japon. 15, 8.