Effects of YM175, a New-Generation Bisphosphonate, on Hypercalcemia Induced by Tumor-Derived Bone Resorbing Factors in Rats

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ABSTRACT—YM175 (disodium cycloheptylaminomethylenebisphosphonate monohydrate) is a new-generation bisphosphonate with stronger inhibitory activity on bone resorption than first-generation bisphosphonates. In the present study, the effect of YM175 on hypercalcemia induced in rats by single administration of either parathyroid hormone-related protein (PTHrP) or concomitant administration of PTHrP and interleukin 1β (IL-1β) was investigated. YM175 (0.01–1 mg/kg, i.v.) inhibited the increase in serum free calcium concentration induced by continuous administration of PTHrP alone (3 μg/rat/day, s.c., 7 days) dose-dependently. The inhibitory effect of YM175 appeared the day after administration and remained 3 days after administration. The effect of YM175 reached a maximum 2 days after administration, at which time the ED50 value of YM175 was calculated to be 0.041 mg/kg, i.v., revealing a potency approximately 50- and 10-fold stronger than those of either pamidronate or alendronate, respectively. In contrast, etcloxatin (1–10 units/kg, s.c.) only transiently inhibited PTHrP-induced free calcium increase. YM175 (0.1–3 mg/kg, i.v.) also inhibited the increase in the serum free calcium concentration induced by continuous concomitant administration of both PTHrP and IL-1β in a dose-dependent manner. These results indicated that YM175 is expected to be a useful drug for hypercalcemia associated with malignant tumors due to its efficacy and range of effect.

Keywords: Bisphosphonate, Hypercalcemia, Parathyrin hormone-related protein, Interleukin 1β, Bone resorbing factor

Blood calcium is regulated by balance between bone resorption, intestinal absorption and renal tubular reabsorption. These responses are controlled by various factors, mainly parathyroid hormone, calcitonin and 1,25-dihydroxyvitamin D3. Collectively, these are called the calcium-regulating hormones. To maintain homeostasis, blood calcium concentration is kept within a very narrow range. Hypercalcemia is frequently observed in patients with various types of malignant tumors (1). Malignant tumor-associated hypercalcemia can be roughly divided into the following 2 categories: humoral hypercalcemia of malignancy, where hypercalcemia results from an increase in systemic bone resorption by bone resorbing factors produced from tumor cells, and local osteolytic hypercalcemia, where hypercalcemia results from regional osteolysis induced by bone resorbing factors secreted from a hematologic malignant tumor or a metastatic solid cancer of bone. In this case, tumor necrosis factor, parathyroid hormone-related protein (PTHrP), interleukin 1β (IL-1β), interleukin 6, prostaglandin E2 and transforming growth factor are thought to serve as hypercalcemia-inducing factors (2).

Bone resorption inhibitors have been used to treat hypercalcemia, along with treatment of the primary disease tumors. Calcitonin reduces the blood calcium concentration through inhibition of osteoclastic bone resorption or stimulation of calcium excretion into urine, and its action appears within 2 hr after administration (3). Since it was reported that bisphosphonates inhibit bone resorption in vitro and in vivo in experimental animal models (4), many bisphosphonates, such as pamidronate, clodronate and etidronate, have also been shown to be clinically effective for hypercalcemia associated with

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malignant tumors (5–8). Bisphosphonates are analogues of pyrophosphoric acid, which is thought to exist in the body and have an important role in metabolism of hard tissue such as inhibition of calcification. They are synthesized as compounds that have in vivo stability because of their resistance to enzymes such as alkaline phosphatase. These compounds have been confirmed to have an inhibitory effect on the formation and lysis of calcium phosphates (physicochemical property) and also to have biological effects such as inhibition of ectopic calcification and inhibition of bone resorption (4). In recent years, compounds with no correlation between in vitro physicochemical properties, in vivo inhibition of calcification, and in vivo inhibition of bone resorption have been discovered. It has also been revealed that the inhibitory effect of bisphosphonates on bone resorption is markedly strengthened in molecules that have a nitrogen atom in the side-chain terminal, such as pamidronate and alendronate, compared with those that have a straight-chain hydrocarbon substituent in the side chain, such as etidronate and clodronate, indicating enhancement of the inhibitory effect of compounds with the former side chain on bone resorption (9).

YM175 (disodium cycloheptylaminomethylenediphosphonate monohydrate) is one of new-generation bisphosphonates, in which a portion of the amino group in the side chain is substituted by a heptacyclic side chain, and it has been reported that the inhibitory effect of this compound on bone resorption is stronger than those of pamidronate and etidronate without causing inhibition of mineralization (10–14). PTHrP and IL-1β are the main causative substances of hypercalcemia associated with malignant tumors. Administration of these substances can be used to make animal models of hypercalcemia, in which increased bone resorption is deeply involved in pathogenesis. In the present study, the effect of YM175 in a rat hypercalcemia model induced by PTHrP and IL-1β was compared with those of pamidronate and alendronate and also with that of elcatonin, an eel calcitonin derivative.

MATERIALS AND METHODS

Experimental animals
Male Wistar rats (5-week-old, body weight of 65–141 g; Japan SLC, Shizuoka) were used. Animals were housed in group cages under a 12 hr light-dark cycle and given free access to laboratory chow and water.

Effects in a model of hypercalcemia induced by single administration of PTHrP

Model preparation

Vehicle continuous administration group: Rats were divided into 5 groups of 5 animals each. On the first day of experiments, rats in one of the 5 groups were anesthetized, and blood from each was withdrawn from the abdominal aorta under laparotomy using vacuum blood sampling tubes (Venoject vacuum blood sampling tube, VT079B; Terumo, Tokyo). Blood was taken using the same procedure in the following experiments. After that, rats in the remaining 4 groups were anesthetized with ether, and an osmotic pump (Alzet miniosmotic pump, model 2001; Alza Co., Palo Alto, CA, USA) filled with physiological saline containing 2% L-cysteine (vehicle of PTHrP, pH 1.5) was subcutaneously implanted in the back of each animal. Each osmotic pump was loaded with the vehicle so as to achieve subcutaneous administration at 24 µl/rat/day for 7 continuous days. Rats in each group were respectively anesthetized and bled at 1, 2, 4 and 7 days after osmotic pump implantation.

PTHrP continuous administration group: Rats were divided into 24 groups of 5 animals each. On the first experimental day, rats in the 24 groups were anesthetized with ether, and an osmotic pump filled with PTHrP (dissolved in physiological saline containing 2% L-cysteine) was implanted subcutaneously so as to achieve subcutaneous administration at 1, 3, 5, 10, 20 or 30 µg/24 µl/rat/day for 7 continuous days. This was done for each rat in the 4 experimental groups for each dose. Rats in each group were anesthetized and bled for each dose at 1, 2, 4 and 7 days after osmotic pump implantation.

Effects of YM175, pamidronate and alendronate on hypercalcemia

Vehicle continuous administration group: Rats were divided into 8 groups of 5 to 10 animals each. On the first experimental day, rats in one of the 8 groups were anesthetized with ether, and blood was taken from the abdominal aorta under laparotomy using vacuum blood sampling tubes. Blood was withdrawn using the same procedure in the following experiments. After that, rats in the remaining 7 groups were anesthetized with ether, and an osmotic pump filled with physiological saline containing 2% L-cysteine was subcutaneously implanted in the back of each rat. Each osmotic pump was loaded with the vehicle so as to achieve subcutaneous administration at 24 µl/rat/day for 7 continuous days. Rats in each group were anesthetized and bled at 1, 2, 3, 4, 5, 6 and 7 days after osmotic pump implantation.

PTHrP continuous administration + physiological saline administration group: Rats were divided into 7 groups of 7–10 animals each. On the first experimental day, rats in the 7 groups were anesthetized with ether, and an osmotic pump filled with PTHrP was subcutaneously implanted in the back of each rat. Each osmotic pump was loaded with PTHrP so as to achieve subcutaneous administration of PTHrP at 3 µg/24 µl/rat/day for 7
continuous days. At 1 and 2 days after osmotic pump implantation, rats in one group each were anesthetized and bled. Moreover, at 2 days after osmotic pump implantation, physiological saline was intravenously administered to rats in the remaining 5 groups, and rats in one group each were respectively anesthetized and bled at 3, 4, 5, 6 and 7 days after osmotic pump implantation.

**PTHrP continuous administration + bisphosphonate administration group:** This experiment was performed according to the same experimental protocol as described above for the physiological saline administration group, excepting that YM175 (0.01 to 1 mg/kg), pamidronate (0.1 to 3 mg/kg) or alendronate (0.1 to 3 mg/kg) was intravenously administered, instead of physiological saline. Thus 5 groups were used for each dose of each bisphosphonate.

**Effect of elcatonin on hypercalcemia**

**Vehicle continuous administration group:** Rats were divided into 8 groups of 5 animals each. On the first experimental day, rats in one of the 8 groups were anesthetized with ether, and blood was taken from the abdominal aorta under laparotomy using vacuum blood sampling tubes. Blood was withdrawn using the same procedure in the following experiments. After that, rats in the remaining 7 groups were anesthetized with ether, and an osmotic pump filled with physiological saline containing 2% l-cysteine was subcutaneously implanted in the back of each rat. Each osmotic pump was loaded with the vehicle so as to achieve subcutaneous administration at 24 µl/rat/day for 7 continuous days. At 1 and 2 days after osmotic pump implantation, rats in one group were respectively anesthetized and bled. Moreover, at 2 days after osmotic pump implantation, physiological saline was intravenously administered to rats in the remaining 5 groups, and then rats in each group were anesthetized and bled after 1, 2, 4, 8 and 24 hr.

**PTHrP continuous administration + physiological saline administration group:** Rats were divided into 7 groups of 5 animals each. On the first experimental day, rats in the 7 groups were anesthetized with ether, and an osmotic pump filled with PTHrP was subcutaneously implanted in the back of each rat. Each osmotic pump was loaded with PTHrP so as to achieve subcutaneous administration of PTHrP at 3 µg/24 µl/rat/day for 7 continuous days. At 1 and 2 days after osmotic pump implantation, rats in one group each were anesthetized and bled. Moreover, at 2 days after osmotic pump implantation, physiological saline was intravenously administered to rats in the remaining 5 groups, and rats in one group each were anesthetized and bled at 1, 2, 4, 8 and 24 hr after dosing.

**PTHrP continuous administration + elcatonin administration group:** This experiment was performed according to the same experimental protocol as described above for the physiological saline administration group, excepting that elcatonin (1 to 10 units/kg) was subcutaneously administered, instead of physiological saline. Thus 5 groups were used for each dose.

**Measurement of serum free calcium concentration**

After serum separation, serum free calcium concentration was immediately measured by an electrolyte analyzer (SERA252; Horiba Seisakusho, Kyoto). Since free calcium concentration varies according to blood pH, measurement was performed under the anaerobic condition using vacuum blood sampling tubes. However, as pH varied during procedures, even though only slightly, the results obtained were adjusted to the free calcium concentrations at pH 7.4 according to the method of Schwartz et al. (15).

**Effects in a model of hypercalcemia induced by concomitant administration of PTHrP and IL-1β**

**Model preparation**

Rats were divided into 6 groups of 7 animals each. On the first experimental day, rats in all groups were anesthetized, and blood was withdrawn from the orbital venous plexus. Blood was taken by the same procedure in the following experiments. Thereafter, rats in 5 of the 6 groups were again anesthetized with ether, and 2 osmotic pumps, which were filled with PTHrP and IL-1β [dissolved in physiological saline containing 0.1% bovine serum albumin (BSA), respectively, so as to achieve subcutaneous administrations at 2 µg/24 µl/rat/day and 1 × 10^6 units/rat/day for 7 continuous days, respectively, were subcutaneously implanted in the back of each rat. These rats were included in the PTHrP and IL-1β continuous administration groups. In the remaining group, 2 osmotic pumps filled with 2% l-cysteine-containing physiological saline and 0.1% BSA-containing physiological saline instead of PTHrP and IL-1β, respectively, were subcutaneously implanted in the back of each rat, and these animals were included in the solvent continuous administration group.

**Effect of YM175 on hypercalcemia**

After blood was again sampled from rats in all groups on the day after osmotic pump implantation, physiological saline was intravenously administered to rats in the solvent continuous administration group and those in one of the PTHrP and IL-1β continuous administration groups. YM175 (0.1 to 3 mg/kg) was intravenously administered to the remaining 4 groups. Blood was therefore sampled at specified periods for 4 days after intravenous administration.

**Measurement of blood free calcium concentration**

In rats with hypercalcemia induced by concomitant administration of PTHrP and IL-1β, since the available amount
of IL-1β is limited, blood was taken from the orbital venous plexus under ether anesthesia, and blood free calcium concentration was measured over time using an automatic pH/Ca²⁺ analyzer (Model 634; Ciba Corning, Tokyo). Since blood pH slightly varied during the procedure, the results obtained were adjusted to the free calcium concentration at pH 7.4 according to the method of Schwartz et al. (15).

Fig. 1. Structures of YM175 and other bisphosphonates.

![Diagram of YM175 and bisphosphonates](image)

Fig. 2. Effect of continuous subcutaneous administration of PTHrP on the serum free calcium concentration in rats. ○: vehicle; ●: PTHrP, 1 µg/rat/day; ▲: PTHrP, 3 µg/rat/day; ■: PTHrP, 5 µg/rat/day; ●: PTHrP, 10 µg/rat/day; ▲: PTHrP, 20 µg/rat/day; ☆: PTHrP, 30 µg/rat/day. Each dot indicates the mean ± S.E. in 5 rats. * indicates a significant difference from the vehicle administration group (*P<0.05, **P<0.01, Dunnett’s multiple comparison).

![Graph of serum free calcium concentration over time](image)

Fig. 3. Effect of YM175 and other bisphosphonates on the increase in serum free calcium concentration induced by continuous subcutaneous administration of PTHrP. Drugs were intravenously administered at 2 days after osmotic pump implantation. □: vehicle, ○: saline, ★: 0.01 mg/kg, ▼: 0.03 mg/kg, ▲: 0.1 mg/kg, ×: 0.3 mg/kg, ■: 1 mg/kg, ●: 3 mg/kg. Each dot in the figure indicates the mean ± S.E. in 7–10 rats. * Indicates a significant difference from the vehicle (physiological saline containing 2% l-cysteine, pH 1.5) continuous subcutaneous administration group (*P<0.01, t-test). * Indicates a significant difference from the physiological saline administration group of rats continuously treated with PTHrP, s.c. (**P<0.05, ***P<0.01, Dunnett’s multiple comparison).
Table 1. The inhibitory effects of YM175 and other bisphosphonates on the serum free calcium concentration in rat models of hypercalcemia induced by single administration of PTHrP or by concomitant administration of PTHrP and IL-1β

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of rats</th>
<th>PTHrP single continuous s.c. administration</th>
<th>No. of rats</th>
<th>PTHrP + IL-1β concomitant continuous s.c. administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>YM175</td>
<td>5–10</td>
<td>0.041 (0.036–0.046) [I]</td>
<td>7</td>
<td>0.70 (0.57–0.87) [I]</td>
</tr>
<tr>
<td>Pamidronate</td>
<td>8–10</td>
<td>1.9 (1.4–2.5) [I/46]</td>
<td></td>
<td>Not tested</td>
</tr>
<tr>
<td>Alendronate</td>
<td>7–10</td>
<td>0.46 (0.30–0.71) [I/11]</td>
<td></td>
<td>Not tested</td>
</tr>
</tbody>
</table>

Figures shown in the table indicate ED₅₀ values determined from the inhibitory effects at 2 days after administration. Figures shown in ( ) indicate 95% confidence intervals. Figures shown in [ ] indicate the relative potency to that of YM175, which is set at 1. ³PTHrP (3 µg/rat/day, s.c.) was continuously administered. ⁴PTHrP (2 µg/rat/day, s.c.) and IL-1β (1 × 10⁵ units/rat/day, s.c.) were continuously administered.

Drugs

YM175 (Lot H-9), pamidronate (Lot H-1) and alendronate synthesized by Yamanouchi Pharmaceutical Co., Ltd. were used (structures of these bisphosphonates are shown in Fig. 1). These compounds were dissolved and diluted with physiological saline and then used for experiments. Human IL-1β (2.2 × 10⁹ units/ml) produced by Yamanouchi Pharmaceutical Co., Ltd. was used after being dissolved and diluted with physiological saline containing 0.1% BSA. PTHrP (human type, 1–34 amide; Peptide Institutes Co., Osaka) was dissolved and diluted with physiological saline containing 2% t-cysteine (pH 1.5). Eclacotin (40 units/ml; Asahi Chemical Industry Co., Osaka) was used after diluting with physiological saline.

Statistical analyses

Measurements are expressed as means±S.E. Comparison between 2 groups was performed by the t-test, and comparison among multiple groups was performed by Dunnett’s multiple comparison test. ED₅₀ values and 95% confidence intervals were calculated by the probit method.

RESULTS

Effects in a model of hypercalcemia induced by single administration of PTHrP

When subcutaneous administration of PTHrP (1–30 µg/rat/day) was continuously performed by an osmotic pump, the serum free calcium concentration increased dose-dependently up to a dose of 20 µg/rat/day, s.c., but showed no further increase at 30 µg/rat/day, s.c. Although the serum free calcium concentration continuously increased at 3 µg/rat/day, s.c. from the start of administration until day 7, the serum free calcium concentration transiently increased by administration at 5 µg/rat/day, s.c. or more, but showed no continuous increase (Fig. 2). Thus 3 µg/rat/day, s.c. was selected as the optimal dose of PTHrP in the following experiments.

When YM175 (0.01–1 mg/kg) was intravenously administered at 2 days after the start of continuous administration of PTHrP (3 µg/rat/day, s.c., 7 days), the increase in the free calcium serum concentration was inhibited at doses of 0.03 to 1 mg/kg, in a dose-dependent manner. The effect of YM175 began to appear on the day after administration, reached a maximum at 2 days after administration, and was continuously observed until 3 days after administration. Although pamidronate (0.1–3 mg/kg, i.v.) and alendronate (0.1–3 mg/kg, i.v.) also showed dose-dependent inhibitory effects at a dose of 3 mg/kg and doses of 1 to 3 mg/kg, respectively, the effect of pamidronate was observed only on day 2 after administration, and that of alendronate appeared at 2 days after administration until day 4 (Fig. 3). The inhibitory effects of these bisphosphonates all reached a maximum on day 2 after administration. The ED₅₀ value of YM175 was 0.041 (0.036–0.046) mg/kg, i.v., revealing approximately 50- and 10-fold stronger potency, compared with pamidronate [ED₅₀: 1.9 (1.4–2.5) mg/kg, i.v.] and alendronate [ED₅₀: 0.46 (0.30–0.71) mg/kg, i.v.], respectively (Table 1, Fig. 4). When eclacotin (1–10 units/kg) was subcutaneously administered, the increase in the serum free calcium concentration was inhibited at doses of 3 to 10 units/kg, in a dose-dependent manner, and this effect appeared at 1 hr after administration. However, the effect of eclacotin reached maximum at 2 hr after administration, rapidly decreased after that, and disappeared the following day (Fig. 5).
Effects in a model of hypercalcemia induced by concomitant administration of PTHrP and IL-1β

When YM175 (0.1–3 mg/kg) was intravenously administered on the day after the start of continuous administration of PTHrP (2 μg/rat/day, s.c.) and IL-1β (1 x 10^4 units/rat/day, s.c.), the increase in blood free calcium concentration was inhibited at doses of 1 to 3 mg/kg, in a dose-dependent manner. The effect of YM175 appeared on the day after administration, reached maximum on day 2 after administration, and was continuously observed until day 3 after administration. The ED₅₀ value obtained on day 2 after administration was 0.70 mg/kg, i.v. (Table 1, Fig. 6).

DISCUSSION

Among various tumor-associated syndromes observed in patients with malignant tumors, hypercalcemia has received particularly keen attention because this complication is both readily detected and often has a critical or fatal course. The incidence of hypercalcemia has been reported to be 3.5% in patients with cancer, but increases to 10% in patients with advanced cancer (16). It has been reported that various bone resorbing factors produced and secreted from malignant tumor cells, particularly PTHrP and IL-1β, are involved in the pathogenesis of hypercalcemia associated with malignant tumors (2, 17, 18). Although these factors can independently manifest their bone resorbing actions, it has been confirmed that a combination of these factors synergistically stimulates bone resorption (19). The present study was carried out to investigate the effect of YM175 on hypercalcemia induced by continuous subcutaneous administration of PTHrP, a main bone resorbing factor produced and secreted from malignant tumors, or by concomitant continuous subcutaneous administration of both PTHrP and IL-1β.

When PTHrP (1–30 μg/rat/day, s.c.) was continuously administered to rats, the serum free calcium concentration increased, at a dose of 3 μg/rat/day, s.c. or more, from the day after the start of administration. However, at a dose of 5 μg/rat/day, s.c. or more, the concentration decreased after day 4 of administration. This decreased serum free calcium concentration is thought to result

Fig. 4. Effects of YM175 and other bisphosphonates on the increase in the serum free calcium concentration induced in rats by continuous subcutaneous administration of PTHrP (the results obtained at 2 days after administration of YM175 or other bisphosphonates). Each column indicates the mean ± S.E. ** indicates a significant difference from the vehicle (physiological saline containing 2% l-cysteine, pH 1.5) continuous subcutaneous administration group (P<0.05, t-test). * indicates a significant difference from the physiological saline administration group of rats continuously treated with PTHrP, s.c. (P<0.05, **P<0.01, Dunnett’s multiple comparison).
from the secretion of calcitonin, an endogenous calcium-regulating hormone, as the serum free calcium concentration was rapidly increased by PTHrP. On the other hand, the hypercalcemic condition was maintained at a dose of 3 μg/rat/day, s.c. for 7 days after the start of administration. Thus, the minimum dose of PTHrP in rats that increases the serum free calcium concentration is 3 μg/rat/day. It might depend on the amounts of secreted calcitonin, which depends on the degree of stimulation of PTH/PTHrP receptors, whether the elevated serum free calcium concentration by PTHrP was maintained or decreased. Furthermore, the serum free calcium concentration does not increase again after it had decreased, in spite of continuous administration of PTHrP. This find-
ing may also result from the control by endogenous calcium-regulating hormones such as calcitonin, PTH and 1,25-dihydroxyvitamin D3. Thus the dose for the continuous subcutaneous administration of PTHrP alone was set at 3 µg/rat/day, s.c. This dose for PTHrP is almost equal to that reported by Rizzoli et al. (20). In this model, YM175 (0.01–1 mg/kg, i.v.) exhibited a dose-dependent inhibitory effect on hypercalcemia from 1 to 3 days after administration. Pamidronate (0.1–3 mg/kg, i.v.) and alendronate (0.1–3 mg/kg, i.v.) showed inhibitory effects on hypercalcemia at 2 days after administration and from 2 to 4 days after administration, respectively. The inhibitory effect of all bisphosphonates examined reached maximum at 2 days after administration. The ED50 value of YM175, determined on the basis of the inhibitory effect at 2 days after administration, was 0.041 (0.036–0.046) mg/kg, i.v., revealing the potency of YM175 to be approximately 50- and 10-fold stronger than those of pamidronate [ED50: 1.9 (1.4–2.5) mg/kg, i.v.] and alendronate [ED50: 0.46 (0.30–0.71) mg/kg, i.v.], respectively.

YM175 also significantly lowered the free calcium concentration which was elevated by concomitant continuous infusion of both PTHrP and IL-1β. However, the ED50 value (0.041 mg/kg, i.v.) obtained in this model was 0.70 mg/kg, i.v., which was approximately 20 times higher than the ED50 value obtained in hypercalcemic rats induced PTHrP alone. One reason why the effect of YM175 on hypercalcemia induced by concomitant administration of PTHrP and IL-1β was weaker than that of PTHrP alone may be that hypercalcemia induced by concomitant administration of PTHrP and IL-1β is much more severe, because of the synergistic effect of both factors (19).

Since it has been reported that YM175 inhibits calcium resorption from the rat calvaria in vitro and pit formation by mature osteoclasts cultured on elephant ivory (11, 12), it is inferred that the effect of YM175 in reducing the blood free calcium concentration in the hypercalcemic models examined in the present study is expressed by inhibition of the osteoclast-related bone resorption process. Currently, bisphosphonates, including YM175, are thought to manifest their inhibitory action on bone resorption by killing or causing the degeneration of osteoclasts that ingest bisphosphonate bound to bone as the osteoclast resorbs bone (21, 22). On the other hand, calcitonin manifests its inhibitory action on bone resorption by directly acting on calcitonin receptors on osteoclasts and inhibiting osteoclast function. Because of this, the action of calcitonin appears and disappears rapidly, unlike that of YM175, and is strictly correlated with its blood level. In contrast, the actions of YM175 and other bisphosphonates appear more slowly and remain for a longer period of time. In fact, the highest activities of these bisphosphonates were observed at 2 days after administration. Since bisphosphonates are rapidly transported into bone, the slow manifestation of the inhibitory actions of these compounds on bone resorption as observed in the present study is thought to reflect the time lag between transportation to bone and the encounter with osteoclasts at the site of bone resorption. Sahni et al. have recently proposed the possibility that bisphosphonates inhibit bone resorption through osteoblasts, in addition to their osteoclast-mediated inhibitory actions (23). Thus further studies are necessary to clarify the mechanism of action of bisphosphonates, including YM175.

In conclusion, YM175 had a dose-dependent, slow-acting, and long-lasting inhibitory effect on hypercalcemia induced by continuous subcutaneous administration of either PTHrP alone, a bone resorbing factor produced and secreted from malignant tumors, or by concomitant continuous subcutaneous administration with IL-1β, another representative bone resorbing factor. The effect of YM175 was stronger than those of pamidronate and alendronate, and it was maintained for a longer period of time than that of calcitonin. Since the long-term administration of YM175 did not cause the inhibition of mineralization observed on treatment with conventional bisphosphonates (14), this compound is considered to have minimum adverse effects with a wide range of safety and is expected to have clinical usefulness not only for hypercalcemia, but also for Behçet’s disease, bone metastasis of cancer and bone resorption-disorder diseases such as osteoporosis.

REFERENCES

7 Conte N, Di Virgilio R, Roiter I and Caberlotto I: Hypercalcemia in malignancies: treatment with dichloromethylene
Effects of YM175 on Hypercalcemic Rats


