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Rat model of the hypercalcaemia induced by parathyroid hormone-related protein: characteristics of three bisphosphonates

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Abstract

In our preliminary experiment, we found that a constant infusion of a high dose of parathyroid hormone-related protein induced both hyperphosphataemia and hypocalcaemia, secondary to renal dysfunction. Therefore, in this study, we developed two types of parathyroid hormone-related protein-induced hypercalcaemia models. One is the hypercalcaemia model, which did not show renal-dysfunction-induced hypocalcaemia. This model might be suitable for estimating hypocalcaemic activities of drugs, especially of those that act on bone resorption. The other is the model for estimating histological changes, which is associated with renal dysfunction. We then used these models to investigate the effects of three different bisphosphonates. Since the hypercalcaemic effect of parathyroid hormone-related protein infusion plateaued at 20 pmol/h, and higher doses of parathyroid hormone-related protein caused an elevation of blood urea nitrogen, the parathyroid hormone-related protein infusion rate was fixed at 20 pmol/h to avoid renal dysfunction and at 40 pmol/h to elicit renal dysfunction. The hypocalcaemic efficiencies of clodronate and etidronate were almost the same but pamidronate was 17.9 times more potent than clodronate. Additionally, both clodronate and pamidronate decreased the plasma concentrations of blood urea nitrogen and the Ca^{2+} times inorganic P product, whereas etidronate lacked these effects. Clodronate suppressed renal calcification and tubular dilatation in the renal-dysfunction model. These data indicated that clodronate and pamidronate not only decrease the plasma Ca^{2+} concentration but also improve the renal dysfunction induced by hypercalcaemia.

Keywords: Bisphosphonate; Hypercalcaemia; Renal dysfunction

1. Introduction

Malignant tumours are frequently accompanied by hypercalcaemia. This hypercalcaemia can be roughly divided two types based on the underlying mechanisms. One is the humoral hypercalcaemia of malignancy, which is caused by an increase in systemic bone resorption induced by bone-resorbing factors secreted from tumour cells. The other is local osteolytic hypercalcaemia, which is caused by local osteolysis induced by tumour-secreted bone-resorbing factors.

Parathyroid hormone-related protein has been identified within a wide range of solid tumours, as well as in fetal and normal adult tissues (Danks et al., 1989; Kramer et al., 1991; Moseley et al., 1991). Increased concentrations of plasma parathyroid hormone-related protein in the hypercalcaemia of malignancy have been found in up to 88% of patients and there is overwhelming evidence that tumour-derived parathyroid hormone-related protein is the major hypercalcaemic factor in this paraneoplastic syndrome. Indeed, parathyroid hormone-related protein plays an integral role in mediating the syndrome via parathyroid hormone-like actions (Broadus et al., 1988; Mundy, 1988).

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The parathyroid hormone-like activity of the parathyroid hormone-related protein molecule is contained within its amino-terminal portion (Horiuchi et al., 1987; Kemp et al., 1987) and is exerted via the parathyroid hormone/parathyroid hormone-related protein receptor (Abou-Samra et al., 1992; Orloff et al., 1992).

A constant infusion of parathyroid hormone in thyroparathyroidectomized or parathyroidectomized rats has been employed to develop animal models of primary hyperparathyroidism, featuring parathyroid hormone-induced hypercalcaemia and nephrocalcinosis (Jaeger et al., 1987). A constant infusion of parathyroid hormone-related protein in rats has also been used as an animal model of the hypercalcaemia of malignancy (Endo et al., 2000; Horiuchi et al., 1990; Kitazawa et al., 1991; Takahashi et al., 1998; Rizzoli et al., 1989, 1992). In our preliminary experiment on normal rats, a constant infusion of parathyroid hormone-related protein (95 pmol/h) induced an increase in blood urea nitrogen, an index of renal function, together with an increase in the plasma inorganic phosphorus concentration and a decrease in the plasma calcium concentration (Fig. 1). That being so, we thought the hypocalcaemic activity of a given drug might be impossible to estimate with any accuracy under those conditions. We therefore used a lower dose of parathyroid hormone-related protein to estimate hypocalcaemic activity of drugs in the present study. In addition, we used a higher dose of parathyroid hormone-related protein to elicit renal histological changes, which is associated with the renal dysfunction.

Bisphosphonates are pyrophosphate derivatives, with an oxygen molecule being replaced by a carbon. This substitution makes bisphosphonates resistant to biological degradation. They have high affinities for hydroxyapatite and inhibit osteoclast-mediated bone resorption (Fleisch, 1987). They also inhibit the calcification in the aorta and kidney induced by vitamin D_3 (Fleisch et al., 1970). For some years, they have been used clinically against several metabolic bone diseases; namely, Paget's disease (Adami et al., 1986; Meunier et al., 1979), the hypercalcaemia of malignancy (Paterson et al., 1983; Ralston et al., 1985), osteolytic lesions produced by bone metastases (Attardo-Parrinello et al., 1987; Paterson et al., 1993), and osteoporosis (Filipponi et al., 1995; Rossini et al., 1994).

In this study, we set out to develop two types of parathyroid hormone-related protein-induced hypercalcaemia models. One is the suitable model for estimating hypocalcaemic activity of drugs, especially of those that act on bone resorption, which would not entail a renal-dysfunction-induced decrease in the plasma Ca^{2+} concentration. The other is the model for estimating renal histological changes, which is associated with renal dysfunction. Having done so, we used these models to estimate the effects of three bisphosphonates (clodronate, etidronate, and pamidronate) on parathyroid hormone-related protein-induced hypercalcaemia. Our results demonstrate a marked difference among these bisphosphonates in their effects on hypercalcaemia-induced renal dysfunction.

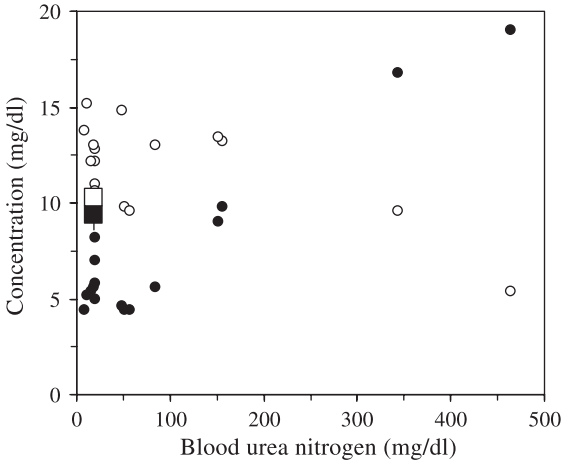

Fig. 1. Correlation between blood urea nitrogen and plasma ion concentrations in parathyroid hormone-related protein-infused (95 pmol/h) normal rats. Open symbols and closed symbols indicate Ca^{2+} and inorganic P concentrations, respectively. Circle symbol represents data for an individual parathyroid hormone-related protein-infused (95 pmol/h) normal rat. Square symbol represents mean±S.E.M. (n=12) for normal rats. The r-values are calculated using the data, which showed the blood urea nitrogen values over 50 mg/dl. Ca^{2+} vs. blood urea nitrogen, r=0.6541; inorganic P vs. blood urea nitrogen, r=0.9886.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, 5 weeks old, were purchased from Japan SLC (Shizuoka, Japan). They were housed in group cages under a 12-h light–dark cycle and given free access to commercial chow (CE-2; Japan Clea, Tokyo, Japan) and water. The protocols were approved by the local ethics committee.

2.2. Model preparation

Thyroparathyroidectomy was performed under pentobarbital anaesthesia (Nembutal; 50 mg/kg, i.p.; Abbott, North Chicago, IL). Thyroparathyroidectomy adequacy was checked by determining the plasma Ca^{2+} concentration in a blood sample obtained 4 or 5 days after the operation. The thyroparathyroidectomy was considered successful if the plasma Ca^{2+} concentration was below 7 mg/dl (normal rats, 10 to 11 mg/dl). After this check, all rats were supplemented with 4 μg thyroxin subcutaneously three times a week. On the first day after the adequacy check, rats were subcutaneously implanted (under ether anaesthesia) with an osmotic minipump (Alzet miniosmotic pump, model 2001; Alza, Palo Alto, CA) filled with the amino-terminal fragment of parathyroid hormone-related

protein (Human, 1–34 Amide; Peptide Institute, Osaka, Japan), dissolved in physiological saline containing 2% L-cysteine and 10 mM HCl (pH 1.5). The parathyroid hormone-related protein was chronically infused at rates of 10 to 40 pmol/h.

2.3. Blood sampling

Blood (0.4 ml) was always taken, after an overnight fast, from a tail artery under ether anaesthesia, except in the case of the parathyroid hormone-related protein dose–response experiment, in which it was taken under nonfasted conditions. The blood was transferred to a heparinized tube and the separated plasma was stored at −40 °C until biochemical assay.

2.4. Effects of subcutaneous chronic infusion of parathyroid hormone-related protein on plasma Ca^{2+} concentration and renal function

Rats were divided into 5 groups (n=3–5). One was thyroparathyroidectomized-control, the animals in which received no parathyroid hormone-related protein infusion. Rats in other groups were infused with parathyroid hormone-related protein at doses of 10, 20, 30, or 40 pmol/h. The rats in each group were anaesthetized with ether and bled at 1, 3, 5, and 7 days after osmotic minipump implantation.

2.5. Effects of bisphosphonates on parathyroid hormone-related protein-induced hypercalcaemia

The weights of etidronate (disodium etidronate tetrahydrate; Lion, Tokyo, Japan; extracted by Kissei Pharmaceutial), clodronate (disodium clodronate tetrahydrate; Leiras Oy, Turku, Finland), and pamidronate (disodium pamidronate dihydrate; synthesized by Eiweiss Chemical, Yokohama, Japan) were calculated using the respective anhydrous molecular weights (clodronate, 1 mg=3.44 μmol; etidronate, 1 mg=4.00 μmol; pamidronate, 1 mg=3.58 μmol). They were dissolved in sufficient saline to make the injected volume 1 ml/kg, and adjusted to pH 7.0 using NaOH. Thyroparathyroidectomized rats were chronically infused with parathyroid hormone-related protein (20 pmol/h). The day after osmotic-minipump implantation, one of the bisphosphonates was injected intravenously. Blood was collected 2 days after the bisphosphonate injection, except in the time-course experiment, in which it was collected at 0, 2, and 6 days after bisphosphonate injection.

2.6. Histopathological analysis of the kidney

Thyroparathyroidectomized rats were chronically infused with parathyroid hormone-related protein at a rate of 40 pmol/h. Clodronate was injected intravenously the day after osmotic-minipump implantation at a dose of 30, 50, 70, or 90 mg/kg. Kidneys were collected 6 days after the clodronate injection, then fixed in 10% formalin neutral-buffer solution. For histopathological analysis, sections were stained with hematoxylin–eosin. The severity of both calcification and tubular dilatation were rated using four grades.

2.7. Biochemical analysis

The plasma Ca^{2+}, inorganic P and blood urea nitrogen concentrations were measured using an autoanalyzer (Technicon RA-1000 SSR; Nippon Technicon, Tokyo, Japan).

2.8. Statistical analysis

All data were analyzed using a one-way analysis of variance, employing the factors parathyroid hormone-related protein administration (vehicle and parathyroid hormone-related protein) and treatment (parathyroid hor-

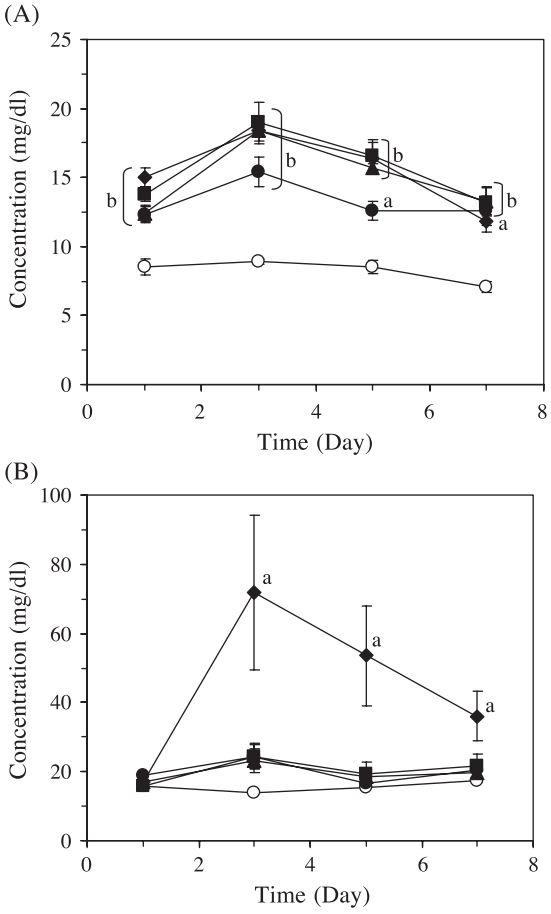

Fig. 2. Changes in plasma Ca^{2+} (A) and blood urea nitrogen (B) concentrations in parathyroid hormone-related protein-infused thyroparathyroidectomized rats. Parathyroid hormone-related protein was infused at a rate of 0 (○), 10 (●), 20 (▲), 30 (■), or 40 pmol/h (◆). Mean±S.E.M. (n=3–5). a, P<0.05; b, P<0.01 vs. 0 group at same time.

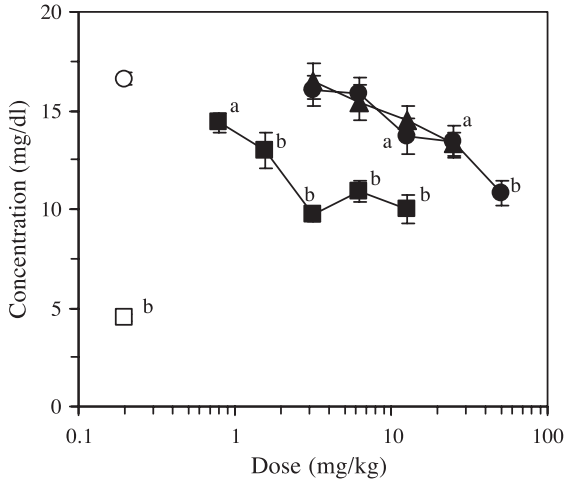

Fig. 3. Dose–response curves for the effects of three different bisphosphonates on the plasma Ca^{2+} concentration at 2 days after drug administration. Thyroparathyroidectomized rats (□) were infused with parathyroid hormone-related protein at a rate of 20 pmol/h (○; control). Clodronate (●), etidronate (▲), or pamidronate (■) was injected 1 day after osmotic-minipump implantation. Mean±S.E.M. (n=9–10). a, P<0.05; b, P<0.01 vs. parathyroid hormone-related protein-infused control.

mone-related protein-control and bisphosphonate treatment). If significant differences were detected between groups, comparisons versus the parathyroid hormone-related protein-control were carried out using Dunnett's multiple comparison test. In the histopathological analysis, comparisons versus the parathyroid hormone-related protein-control were carried out using the Williams–Wilcoxon test. The potency ratio was calculated using a two-by-three assay after a parallel-line assay by means of a validity test.

3. Results

3.1. Effects of parathyroid hormone-related protein on plasma Ca^{2+} and blood urea nitrogen concentrations

A constant infusion of parathyroid hormone-related protein (10–40 pmol/h) increased the plasma Ca^{2+} concentration from day 1 to day 7 after osmotic-minipump implantation (i.e., the entire study period; Fig. 2A). The incremental effect of this agent reached a plateau at an infusion rate of 20 pmol/h. The blood urea nitrogen concentration, an index of renal function, was markedly elevated by parathyroid hormone-related protein infusion at a rate of 40 pmol/h but not at lower rates (Fig. 2B). In the histopathological examination, renal calcification and tubular dilatation were found in parathyroid hormone-related protein-infused (40 pmol/h) rats (Table 2). For those reasons, we fixed the parathyroid hormone-related protein infusion rate at 20 pmol/h to avoid renal-dysfunction-induced hypocalcaemia and 40 pmol/h to elicit renal histological changes, which is associated with renal dysfunction.

3.2. Dose–response effects of bisphosphonates on parathyroid hormone-related protein-induced hypercalcaemia

We assessed the effects of three bisphosphonates (clodronate, etidronate, and pamidronate) on parathyroid hormone-related protein-induced hypercalcaemia in thyroparathyroidectomized rats (Fig. 3 and Table 1). All three bisphosphonates decreased the plasma Ca^{2+} concentration in a dose-dependent manner (at 2 days after drug injection). The efficiencies of clodronate and etidronate were almost

Table 1
Effects of bisphosphonates on plasma biochemicals in parathyroid hormone-related protein-infused thyroparathyroidectomized rats

	Dose (mg/kg)	Ca^{2+} (mg/dl)	Inorganic P (mg/dl)	Ca^{2+} ×Inorganic P (mg^2/dl^2)	Blood urea nitrogen (mg/dl)
Thyroparathyroidectomy		4.5±0.2[a]	13.8±0.6[a]	62.3±4.3[a]	17.8±1.2[b]
Control		16.6±0.3	5.3±0.2	88.1±4.3	30.0±4.2
Clodronate	3.13	16.0±0.8	4.1±0.2[b]	66.1±4.7[a]	21.2±1.8
	6.25	15.9±0.8	4.0±0.2[a]	62.9±3.3[a]	17.0±1.5[a]
	12.5	13.7±0.9[b]	4.4±0.3	59.1±3.9[a]	21.0±2.9[b]
	25.0	13.4±0.8[b]	4.0±0.1[a]	52.7±2.9[a]	15.2±1.7[a]
	50.0	10.8±0.6[a]	4.8±0.4	50.6±3.8[a]	13.2±0.8[a]
Etidronate	3.13	16.5±0.9	4.6±0.3	76.9±7.1	26.2±3.0
	6.25	15.4±0.9	5.4±0.3	83.2±7.4	17.2±2.4
	12.5	14.5±0.7	5.7±0.5	80.7±5.3	26.7±5.2
	25.0	13.3±0.6[a]	7.0±0.5[a]	91.8±5.6	49.0±8.5[b]
Pamidronate	0.78	14.4±0.5[b]	4.5±0.4	64.8±6.8[b]	16.8±1.7[a]
	1.56	13.0±0.9[a]	4.7±0.3	62.3±6.4[b]	20.4±2.5[b]
	3.13	9.7±0.3[a]	4.9±0.3	47.5±3.5[a]	15.4±1.1[a]
	6.25	10.9±0.5[a]	4.1±0.2	44.6±2.4[a]	17.0±1.8[a]
	12.5	10.0±0.7[a]	5.8±0.6	59.2±9.1[a]	17.6±1.8[a]

Data represent the mean±S.E.M. (n=9, 10). Bisphosphonate was injected intravenously the next day of osmotic-minipump implantation. In addition, blood was collected 2 days after the bisphosphonate injection.

[a] P<0.01 vs. parathyroid hormone-related protein-infused control.

[b] P<0.05 vs. parathyroid hormone-related protein-infused control.

the same, while that of pamidronate was 17.9 (95% confidence limits, 11.5–28.8) times greater than that of clodronate. Clodronate and pamidronate, but not etidronate, also decreased the Ca^{2+} times inorganic P product (Ca^{2+}×inorganic P) and blood urea nitrogen concentration (Table 1).

3.3. Duration of the effects of clodronate and pamidronate on parathyroid hormone-related protein -induced hypercalcaemia

As mentioned above, pamidronate was 17.9 times more powerful than clodronate in its Ca^{2+}-lowering effect. Therefore, we compared 25 mg/kg of clodronate and 1.39 mg/kg of pamidronate (which were doses of almost equal efficiency at 2 days after drug-injection). At these doses, both drugs decreased the plasma Ca^{2+} concentration at 2 days after drug injection but it had returned to a level not significantly different from the control parathyroid hormone-related protein-infused level at 6 days after drug-injection (Fig. 4A). This duration of action was the same when a two times higher dose of either drug was injected. The plasma inorganic P concentration was not increased by parathyroid hormone-related protein infusion during the experimental period (Fig. 4B).

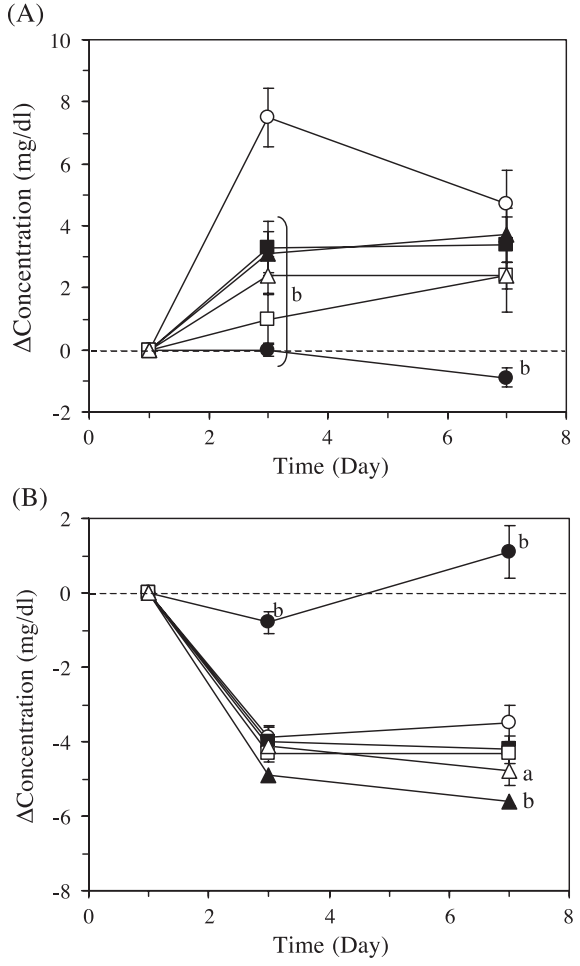

Fig. 4. Effects of bisphosphonates on changes in plasma Ca^{2+} (A) and inorganic P (B) concentrations in parathyroid hormone-related protein-infused rats. Thyroparathyroidectomized rats (●) were infused with parathyroid hormone-related protein at a rate of 20 pmol/h (○; control). Clodronate (25 (■) or 50 mg/kg (□)) or pamidronate (1.39 (▲) or 2.79 mg/kg (△)) was injected 1 day after osmotic-minipump implantation. Mean±S.E.M. (n=6–7). a, P<0.05; b, P<0.01 vs. parathyroid hormone-related protein-infused control at same time.

3.4. Effects of clodronate on renal histopathological changes

Both clodronate and pamidronate, but not etidronate, decreased Ca^{2+}×inorganic P and blood urea nitrogen (Table 1). These data indicated that the first two bisphosphonates might have protective effects against renal dysfunction. We therefore examined the effects of clodronate on renal histopathological changes in parathyroid hormone-related protein-infused (40 pmol/h) thyroparathyroidectomized rats. After 7 days' constant infusion, renal calcification and tubular dilatation were found in all parathyroid hormone-related protein-infused control rats, whereas no renal calcification or tubular dilatation were found in the parathyroid hormone-related protein non-infused group. In this model, clodronate inhibited both the renal calcification and the tubular dilatation dose-dependently, the effects being statistically significant at doses of 50 mg/kg or more.

4. Discussion

In this study, we developed two types of parathyroid hormone-related protein-induced hypercalcaemia models in thyroparathyroidectomized rats. One is the suitable model for estimating the hypocalcaemic activity of drugs, especially of those that act on bone resorption, which did not show renal-dysfunction-induced hyperphosphataemia or hypocalcaemia. The other is the model for estimating the renal histological changes, which is associated with hypercalcaemia-induced renal dysfunction. In these models, we then tested the effects of three bisphosphonates on the parathyroid hormone-related protein-induced hypercalcaemia and on renal function. Clodronate and pamidronate each decreased the plasma Ca^{2+} level in a dose-dependent manner. They also improved the renal dysfunction. Furthermore, clodronate also suppressed renal histopathological changes, calcification, and tubular dilatation. On the other hand, although etidronate decreased the plasma Ca^{2+} concentration, it did not improve renal dysfunction.

There have been reports of parathyroid hormone-related protein-induced hypercalcaemia models in thyroparathyroidectomized (Horiuchi et al., 1990; Rizzoli et al., 1989, 1992), parathyroidectomized (Endo et al., 2000; Kitazawa et al., 1991), and normal rats (Takahashi et al., 1998). In these reports, the parathyroid hormone-related protein infusion rate was within the range of 10.8–325 pmol/h,

with most of them using 90–160 pmol/h. In our preliminary experiment, in which 95 pmol/h was chosen (Fig. 1), the plasma Ca^{2+} concentration was decreased by the parathyroid hormone-related protein infusion when blood urea nitrogen, an index of renal function, was increased to above 100 mg/dl. On the other hand, the plasma inorganic P concentration was increased by the parathyroid hormone-related protein infusion when the blood urea nitrogen concentration was elevated to above 100 mg/dl. The decrease in the plasma Ca^{2+} concentration thus seemed to be linked to the increase in the plasma inorganic P concentration. It is well known from animal and clinical studies that the plasma inorganic P level is increased in chronic renal failure. Therefore, the above decrease in the plasma Ca^{2+} concentration might have been induced by the increment in the plasma inorganic P concentration that followed the induction of renal dysfunction. In our experiment, a parathyroid hormone-related protein-induced blood urea nitrogen elevation was apparent at a dose of 40 pmol/h (Fig. 2B). To judge from these data, most rats in which parathyroid hormone-related protein is infused at a rate of more than 40 pmol/h may suffer renal dysfunction, and that might in turn lead to a decrease in the plasma Ca^{2+} level.

In our hypercalcaemia model which used for estimating hypocalcaemic activity (parathyroid hormone-related protein: 20 pmol/h), most rats had a blood urea nitrogen concentration of less than 50 mg/dl, indicating that a renal-dysfunction-induced decrease in the plasma Ca^{2+} concentration would not have occurred. In normal rats, a high concentration of parathyroid hormone-related protein induces a secondary elevation in calcitonin, which might lead to a decrease in the plasma Ca^{2+} concentration (Takahashi et al., 1998). However, since we used thyroparathyroidectomized rats, the action of calcitonin does not concern us here. As mentioned above, this hypercalcaemia model might be suitable for estimating the effects of drugs, especially of those that act on bone resorption.

The three bisphosphonates used in this study all decreased the plasma Ca^{2+} concentration, with pamidronate being about 18 times more effective than either clodronate or etidronate. In a previous clinical study, it was found that pamidronate was more powerful than clodronate against the hypercalcaemia of malignancy (Purohit et al., 1995). In that study, clodronate achieved normocalcaemia faster than pamidronate, but the normocalcaemic action of pamidronate lasted longer than that of clodronate. In the present study, no difference in effective duration was observed between clodronate and pamidronate.

In humans and other mammals, the extracellular concentrations of Ca^{2+} and inorganic P are regulated individually so as to maintain a constant level of $Ca^{2+}\times$inorganic P. It is well known that a decrease in $Ca^{2+}\times$inorganic P is a risk factor for osteomalacia, while an increment in $Ca^{2+}\times$inorganic P is a risk factor for ectopic calcification of soft tissues. In the present study, clodronate and pamidronate each decreased not only the plasma Ca^{2+} concentration but also blood urea nitrogen and $Ca^{2+}\times$inorganic P, which were both increased by parathyroid hormone-related protein infusion alone (Table 1). Additionally, in the histopathological experiment (parathyroid hormone-related protein: 40 pmol/h), one of the these bisphosphonates, clodronate, suppressed the parathyroid hormone-related protein-induced renal calcification and tubular dilatation (Table 2). Since neither clodronate nor pamidronate altered the plasma inorganic P concentration (Table 1), the decreases they induced in $Ca^{2+}\times$inorganic P predominantly reflect their hypocalcaemic effects. It is well known that bisphosphonates inhibit both the formation of Ca^{2+}–PO_4^{3-} crystals in vitro and ectopic calcification in vivo (Fleisch et al., 1970). For the reasons mentioned above, we speculate that the bisphosphonates suppressed renal dysfunction via an inhibition of the renal calcification induced by the hypercalcaemia. In the present study, we did not test the effects of pamidronate in the histopathological experiment; however, we consider that it might be as effective as clodronate.

Although etidronate decreased the plasma Ca^{2+} concentration by as much as clodronate, the former drug elevated the plasma inorganic P and blood urea nitrogen concentrations at the highest dose used. It has been reported that etidronate causes hyperphosphataemia by increasing the tubular reabsorption of phosphorus (Recker et al., 1973). A hyperphosphotaemic effect of etidronate was also observed in the present experiment (Table 1) and this hyperphosphataemic effect led to an increase in $Ca^{2+}\times$inorganic P despite the drug's hypocalcaemic effect. Such an elevated level of $Ca^{2+}\times$inorganic P might induce kidney calcification and renal dysfunction. In contrast to the findings, etidronate has been reported to be an effective inhibitor of kidney calcification in a vitamin-D_3-induced hypercalcaemic rat model (Fleisch et al., 1970). Until the explanation for this discrepancy has been clarified, it is difficult to know whether etidronate should be used for the treatment of hypercalcaemia.

Table 2
Effects of clodronate on parathyroid hormone-related protein-infusion-induced kidney toxicity in thyroparathyroidectomized rats

Clodronate	Calcification					Tubular dilatation				
(mg/kg)	–	+	++	+++		–	+	++	+++	
Nonparathyroid hormone-related protein										
0	3/3					3/3				
Parathyroid hormone-related protein, 40 pmol/h										
0		2/5	2/5	1/5			2/5	1/5	2/5	
30	1/4	2/4	1/4			1/4	3/4			
50	2/4	2/4			a	2/4	2/4			a
70	3/5	2/5			a	3/5	2/5			a
90	4/4				b	4/4				b

Effects were rated using four grades. Data represent the incidence/number. a, $P<0.05$; b, $P<0.01$ vs. parathyroid hormone-related protein-infused control.

In this study, pamidronate had the most potent hypocalcaemic effect among the three bisphosphonates tested. Structurally, pamidronate contains a nitrogen group; it is therefore classified as an amino-bisphosphonate. In vitro, the amino-bisphosphonates exhibit potent inhibitory actions against bone resorption, their potencies being up to 1000 times greater than those of clodronate and etidronate (Fleish, 1995). It has been reported that treatment with amino-bisphosphonates is associated with several adverse effects. In clinical studies, irrespective of the underlying disease, intravenous treatment with an amino-group-containing bisphosphonate (for example, pamidronate or alendronate) induced an acute-phase response manifested by falls in the circulating lymphocyte number and serum zinc concentration and a rise in C-reactive protein, together with fever. On the other hand, clodronate was devoid of these side effects (Adami et al., 1987; Harinck et al., 1987). Accordingly, among the drugs tested, clodronate would seem to be the best bisphosphonate to use for the treatment of the hypercalcaemia of malignancy, although its efficiency was weaker than that of pamidronate.

In conclusion, we have developed two types of parathyroid hormone-related protein-induced hypercalcaemia models by use different doses of parathyroid hormone-related protein for it purpose. One, which avoids renal-dysfunction-induced hypocalcaemia, is a suitable model for estimating the hypocalcaemic effect of drugs, especially of those that act on bone resorption. The other is a model for estimating the renal histological changes associated with hypercalcaemia-induced renal dysfunction. In our hypercalcaemia models (in thyroparathyroidectomized rats), three bisphosphonates (clodronate, etidronate and pamidronate) each decreased the plasma Ca^{2+} concentration in a dose-dependent manner, with the efficiency of pamidronate being the greatest. Clodronate and pamidronate also depressed renal dysfunction. These data suggest that clodronate and pamidronate may be effective at treating not only hypercalcaemia but also hypercalcaemia-associated renal dysfunction. In view of its relative lack of side effects, clodronate might be better than pamidronate for the treatment of the hypercalcaemia of malignancy.

References

Abou-Samra, A.B., Jüppner, H., Force, T., Freeman, M.W., Kong, X.F., Schipani, E., Urena, P., Richards, J., Bonventre, J.V., Potts Jr., J.T., Kronenberg, H.M., Segre, G.V., 1992. Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: a single receptor stimulates intracellular accumulation of both cAMP and inositol trisphosphates and increases intracellular free calcium. Proc. Natl. Acad. Sci. U. S. A. 89, 2732–2736.

Adami, S., Salvagno, G., Guarrera, G., Montesanti, F., Garavelli, S., Rosini, S., Lo Cascio, V., 1986. Treatment of Paget's disease of bone with intravenous 4-amino-1-hydroxybutylidene-1,1-bisphosphonate. Calcif. Tissue Int. 39, 226–229.

Adami, S., Bhalla, A.K., Dorizzi, R., Montesanti, F., Rosini, S., Salvagno, G., Lo Cascio, V., 1987. The acute-phase response after bisphosphonate administration. Calcif. Tissue Int. 41, 326–331.

Attardo-Parrinello, G., Merlini, G., Pavesi, F., Crema, F., Fiorentini, M.L., Ascari, E., 1987. Effects of a new aminodiphosphonate (aminohydroxybutylidene diphosphonate) in patients with osteolytic lesions from metastases and myelomatosis. Arch. Intern. Med. 147, 1629–1633.

Broadus, A.E., Mangin, M., Ikeda, K., Insogna, K.L., Weir, E.C., Burtis, W.J., Stewart, A.F., 1988. Humoral hypercalcemia of cancer: identification of a novel parathyroid hormone-like peptide. N. Engl. J. Med. 319, 556–563.

Danks, J.A., Ebeling, P.R., Hayman, J., Chou, S.T., Moseley, J.M., Dunlop, J., Kemp, B.E., Martin, T.J., 1989. Parathyroid hormone-related protein: immunohistochemical localization in cancers and in normal skin. J. Bone Miner. Res. 4, 273–278.

Endo, K., Katsumata, K., Hirota, M., Masaki, T., Kubodera, N., Nakamura, T., Ikeda, K., Ogata, E., 2000. 1,25-Dihydroxyvitamin D3 as well as its analogue OCT lower blood calcium through inhibition of bone resorption in hypercalcemic rats with continuous parathyroid hormone-related peptide infusion. J. Bone Miner. Res. 15, 175–181.

Filipponi, P., Pedetti, M., Fedeli, L., Cini, L., Palumbo, R., Boldrini, S., Massoni, C., Cristallini, S., 1995. Cyclical clodronate is effective in preventing postmenopausal bone loss: a comparative study with transcutaneous hormone replacement therapy. J. Bone Miner. Res. 10, 697–703.

Fleisch, H., 1987. Bisphosphonate—history and experimental basis. Bone 8 Suppl. 1, S23–S28.

Fleisch, H.A., Russell, R.G.G., Bisaz, S., Mühlbauer, R.C., Williams, D.A., 1970. The inhibitory effect of phosphonates on the formation of calcium phosphate crystals in vitro and on aortic and kidney calcification in vivo. Eur. J. Clin. Invest. 1, 12–18.

Fleish, H., 1995. Bisphosphonates in Bone Disease: From the Laboratory to the Patient, 2nd Rev. ed. The Parthenon Publishing Group, Lancs, UK.

Harinck, H.I.J., Papapoulos, S.E., Blanksma, H.J., Moolenaar, A.J., Vermeij, P., Bijvoet, O.L.M., 1987. Paget's disease of bone: early and late responses to three different modes of treatment with aminohydroxypropylidene bisphosphonate (APD). Br. Med. J. 295, 1301–1305.

Horiuchi, N., Caulfield, M.P., Fisher, J.E., Goldman, M.E., McKee, R.L., Reagan, J.E., Levy, J.J., Nutt, R.F., Rodan, S.B., Schofield, T.L., Clemens, T.L., Rosenblatt, M., 1987. Similarity of synthetic peptide from human tumor to parathyroid hormone in vivo and in vitro. Science 238, 1566–1568.

Horiuchi, N., Hongo, T., Clemens, T.L., 1990. The synthetic human parathyroid hormone-related protein is inhibited by a parathyroid hormone antagonist in rats in vivo. J. Bone Miner. Res. 5, 541–545.

Jaeger, P., Jones, W., Kashgarian, M., Baron, R., Clemens, T.L., Segre, G.V., Hayslett, J.P., 1987. Animal model of primary hyperparathyroidism. Am. J. Physiol. 252, E790–E798.

Kemp, B.E., Moseley, J.M., Rodda, C.P., Ebeling, P.R., Wettenhall, R.E.H., Stapleton, D., Diefenbach-Jagger, H., Ure, F., Michelangeli, V.P., Simmons, H.A., Raisz, L.G., Martin, T.J., 1987. Parathyroid hormone-related protein of malignancy: active synthetic fragments. Science 238, 1568–1570.

Kitazawa, R., Imai, Y., Fukase, M., Fujita, T., 1991. Effects of continuous infusion of parathyroid hormone and parathyroid hormone-related peptide on rat bone in vivo: comparative study by histomorphometry. Bone Miner. 12, 157–166.

Kramer, S., Reynolds Jr., F.H., Castillo, M., Valenzuela, D.M., Thorikay, M., Sorvillo, J.M., 1991. Immunological identification and distribution of parathyroid hormone-like protein polypeptides in normal and malignant tissues. Endocrinology 128, 1927–1937.

Meunier, P.J., Chapuy, M.C., Alexandre, C., Bressot, C., Edouard, C., Vignon, E., Mathieu, L., Trechsel, U., 1979. Effects of disodium dichloromethylene diphosphonate on Paget's disease of bone. Lancet 2, 489–492.

Moseley, J.M., Hayman, J.A., Danks, J.A., Alcorn, D., Grill, V., Southby, J., Horton, M.A., 1991. Immunohistochemical detection of parathyroid

hormone-related protein in human fetal epithelia. J. Clin. Endocrinol. Metab. 73, 478–484.
Mundy, G.R., 1988. Hypercalcemia of malignancy revisited. J. Clin. Invest. 82, 1–6.
Orloff, J.J., Ribaudo, A.E., McKee, R.L., Rosenblatt, M., Stewart, A.F., 1992. A pharmacological comparison of parathyroid hormone receptors in human bone and kidney. Endocrinology 131, 1603–1611.
Paterson, A.D., Kanis, J.A., Cameron, E.C., Douglas, D.L., Beard, D.J., Preston, F.E., Russell, R.G.G., 1983. The use of dichloromethylene diphosphonate for the management of hypercalcaemia in multiple myeloma. Br. J. Haematol. 54, 121–132.
Paterson, A.H.G., Powles, T.J., Kanis, J.A., McCloskey, E., Hanson, J., Ashley, S., 1993. Double-blind controlled trial of oral clodronate in patients with bone metastases from breast cancer. J. Clin. Oncol. 11, 59–65.
Purohit, O.P., Radstone, C.R., Anthony, C., Kanis, J.A., Coleman, R.E., 1995. A randomised double-blind comparison of intravenous pamidronate and clodronate in the hypercalcaemia of malignancy. Br. J. Cancer 72, 1289–1293.
Ralston, S.H., Gardner, M.D., Dryburgh, F.J., Jenkins, A.S., Cowan, R.A., Boyle, I.T., 1985. Comparison of aminohydroxypropylidene diphosphonate, mithramycin, and corticosteroids/calcitonin in treatment of cancer-associated hypercalcaemia. Lancet 2, 907–910.
Recker, R.R., Hassing, G.S., Lau, J.R., Saville, P.D., 1973. The hyperphosphatemic effect of disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP™): renal handling of phosphorus and the renal response to parathyroid hormone. J. Lab. Clin. Med. 81, 258–266.
Rizzoli, R., Caverzasio, J., Chapuy, M.C., Martin, T.J., Bonjour, J.P., 1989. Role of bone and kidney in parathyroid hormone-related peptide-induced hypercalcemia in rats. J. Bone Miner. Res. 4, 759–765.
Rizzoli, R., Caverzasio, J., Bauss, F., Bonjour, J.-P., 1992. Inhibition of bone resorption by the bisphosphonate BM21.0955 is not associated with an alteration of the renal handling of calcium in rats infused with parathyroid hormone-related protein. Bone 13, 321–325.
Rossini, M., Gatti, D., Zamberlan, N., Braga, V., Dorizzi, R., Adami, S., 1994. Long-term effects of a treatment course with oral alendronate of postmenopausal osteoporosis. J. Bone Miner. Res. 9, 1833–1837.
Takahashi, K., Shirahata, A., Fukushima, S., Kokubo, S., Teramura, K., Usuda, S., 1998. Effects of YM175, a new-generation bisphosphonate, on hypercalcemia induced by tumor-derived bone resorbing factors in rats. Jpn. J. Pharmacol. 76, 155–163.