Pamidronate Treatment in Patients with Tumor-Associated Hypercalcemia: Pharmacological Effects and Pharmacokinetics

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Abstract. The purpose of this study was to investigate the effects of pamidronate, a second generation bisphosphonate, on the change in calcium homeostasis in patients with tumor-associated hypercalcemia. Eight patients with tumor-associated hypercalcemia received intravenous infusion of pamidronate (45 mg) and their high mean serum calcium concentration significantly decreased from 3.56 mmol/L, to 2.62 mmol/L 7 days after treatment. Serum intact PTH before treatment had been suppressed to below normal in all patients but returned to normal range in six patients within 7 days after treatment. Urinary PTH related peptide (PTHrP) excretion before treatment had been elevated in seven patients and then significantly increased further after pamidronate therapy. The serum bone Gla protein concentration was not apparently changed by the treatment. Pamidronate in serum was rapidly eliminated after the treatment and urinary excretion reached a plateau on the second day (13.8% of the administered dose), suggesting that the major portion of the infused dose had been distributed to the bone and other tissues. These findings suggest that pamidronate has a potent hypocalcemic effect and that PTHrP production in malignant tumors could be affected by pamidronate therapy.

Key words: Pamidronate, Hypercalcemia, PTH related peptide, Pharmacokinetics

PAMIDRONATE (disodium 3-amino-1-hydroxypropyldiene-1,1-bisphosphonate pentahydrate), a second generation bisphosphonate, has been used in the treatment of patients with tumor-associated hypercalcemia, and reduction in serum calcium in response to this drug has been reported [1–3]. It has generally been accepted that pamidronate reduces serum calcium through the suppression of osteoclast activity. Hypercalcemia in patients with malignancies almost always results from increased bone resorption and is often caused by humoral factors. Parathyroid hormone-related peptide (PTHrP) is considered to be a major factor involved in tumor-associated hypercalcemia [4, 5]. Recently, the measurement of PTHrP in urine has been reported to be a sensitive and useful method for evaluating PTHrP production in various states of a disease [6]. Although several biochemical responses to pamidronate therapy in the treatment of various bone disorders have been reported [1,
the precise effect of this drug on biochemical and endocrinological changes in tumor-associated hypercalcemic patients has not yet been completely clarified. In the present study, we investigated the effect of pamidronate therapy on the PTHrP concentration in urine as well as on other endocrinological and biochemical responses in eight patients with tumor-associated hypercalcemia. We also measured the pamidronate concentrations in serum and urine to examine its pharmacokinetics.

Subjects and Methods

Eight patients with tumor-associated hypercalcemia (Table 1) received a single intravenous infusion of 45 mg of pamidronate dissolved in 500 ml of normal saline over 4 h. This infusion had been preceded by hydration therapy of more than 2000 ml/day of normal saline for at least 2 days. Serum electrolytes, intact PTH and bone Gla protein (BGP), which is also called osteocalcin, were measured on days 0, 2, 4 and 7. Urine electrolytes, PTHrP and hydroxyproline were measured on days -1, 0, 1, 2, 3, 4, and 7 in fasting morning urine samples. Intact PTH (1-84) was measured by Allegro immunoradiometric assay (Nichols). PTHrP was measured by radioimmunoassay with an antibody directed against the C-terminal fragments of PTHrP [11, 12]. Anti-C-terminal PTHrP antibodies had been developed by immunizing a rabbit with [Tyr126]-PTHrP (127-141). PTHrP was excreted into urine as degraded fragments and a single immunoreactive peak of PTHrP in high-performance liquid chromatography was shown in the urine of patients with tumor-associated hypercalcemia [6]. The mean urinary PTHrP excretion corrected by urinary creatinine (Cr) was 24.88 pmol/mmol Cr in normal subjects (n=20) and the cutoff value was 45.24 pmol/mmol Cr (mean±2SD) [11]. Serum BGP was determined by immunoradiometric assay (BGP IRMA Kit, Mitsubishi Petrochemical Co., Ltd., Tokyo, Japan) [13]. Serum and urinary pamidronate were determined by pre-column derivatization with fluorescamine and high-performance liquid chromatography [14]. Serum and urinary concentrations of 0.85-21.25 µmol/L could be measured within 10% of the CV value. The limits of quantification were 0.85 µmol/L in both serum and urine. Values for serum calcium were adjusted with albumin (hereafter referred to as “corrected serum calcium”) [15]. Electrolyte and other biochemical tests in serum and urine were done in a Hitachi 736 autoanalyzer (Hitachi Ltd., Tokyo, Japan). Urinary hydroxyproline was determined by a spectrophotometric method. This trial was the first bisphosphonate therapy for all the patients and neither calcitonin nor steroid therapy had been performed for 4 weeks before the trial and during the observation period. The protocol of the study had been accepted by the Ministry of Health and Welfare of Japan and the Committee on Ethics of each university and hospital. All patients gave their informed consent regarding this trial.

Statistical analysis

Results are expressed as the mean ± SEM. Comparisons between pre- and post-drug group means were done according to Dunnett’s test.

Results

Corrected serum calcium was sustained at high levels in all patients even after hydration therapy.
for at least 2 days (3.56 ± 0.12 mmol/L). Pamidronate administration significantly lowered this elevation almost to the normal range (2.62 ± 0.13 mmol/L) on the 7th day and the mean decrease in corrected serum calcium was 0.94 mmol/L (Fig. 1). Serum phosphorus significantly decreased on day 7 (0.72 ± 0.07 mmol/L vs. 1.05 ± 0.10 mmol/L on day 0, P<0.01). Serum alkaline phosphatase levels significantly increased on day 7 (607.9 ± 79.7 U/L vs. 514.0 ± 66.8 U/L on day 0, P<0.05). Serum magnesium, Na, K, Cl, blood urea nitrogen, creatinine and creatinine clearance were not significantly changed by pamidronate administration (data not shown).

Urinary calcium excretion had also significantly decreased 2 days after pamidronate treatment (10.25 ± 1.28 mmol/day on day −1 vs. 5.12 ± 1.22 mmol/day on day 2, P<0.01) and continued to decrease until the 7th day (2.11 ± 0.96 mmol/day). Urinary phosphorus excretion also significantly decreased on day 1 (8.61 ± 2.58 mmol/day vs. 17.86 ± 2.95 mmol/day on day −1, P<0.01) and the effect continued until the 7th day (7.75 ± 1.17 mmol/day). Urinary hydroxyproline excretion also significantly decreased one day after treatment (229.7 ± 31.7 µmol·d⁻¹·m⁻² at day −1 vs. 122.6 ± 20.6 µmol·d⁻¹·m⁻² at day 1, P<0.01) and remained at this level throughout the observation period.

Serum intact PTH in all patients before treatment (0.61 ± 0.30 pmol/L) was below the normal range (2.44–7.74 pmol/L). Intact PTH was gradually increased by pamidronate administration and had returned to normal in six of the eight patients between four and seven days later (Fig. 2). Intact PTH was still suppressed (0.53 pmol/L) and serum calcium was a little high in one patient, whereas a slight increase in intact PTH (7.84 pmol/L) with normalization of serum calcium at day 7 was seen in another patient. Urinary PTHrP excretion was higher than the normal upper limit (45.24 pmol/mmol Cr) in seven patients before the treatment and the mean value was 598.52 ± 148.37 pmol/mmol Cr. Only one patient with multiple myeloma (patient 7) showed normal urinary PTHrP excretion. Urinary PTHrP excretion in all patients

**Fig. 1.** Effect of pamidronate on corrected serum calcium concentration in patients with tumor-associated hypercalcemia. The patients were given a single intravenous infusion of 45 mg of pamidronate dissolved in normal saline on day 0. Each point represents the mean ± SEM. ***, P<0.01 vs. day 0.

**Fig. 2.** Effect of pamidronate on serum intact PTH (○), urinary PTHrP (●) and serum BGP (□) in patients with tumor-associated hypercalcemia. The patients were given a single intravenous infusion of 45 mg of pamidronate dissolved in normal saline on day 0. Each point represents the mean ± SEM. *, P<0.05 vs. day −1 or 0.
significantly increased after pamidronate therapy and the average on the seventh day was 1104.13 ± 304.84 pmol/mmol Cr (Fig. 2). Serum BGP before the therapy was distributed over a wide range and changed little after the therapy was instituted (Fig. 2).

The serum concentration of pamidronate was 4.29 ± 0.72 μmol/L at 4 h after the start of infusion and below 0.85 μmol/L at 24 h in all patients (Fig. 3). 13.8±4.2% of the administered dose of pamidronate was excreted in urine for 96 h after administration and the major portion was excreted during the first 24 h (Fig. 4).

Discussion

Pamidronate significantly improved clinical parameters in patients with tumor-associated hypercalcemia. Even an abnormally high serum calcium concentration was readily suppressed by a single intravenous administration of this drug. Serum intact PTH before treatment had been noticeably suppressed by the continued high serum calcium in all patients. Pamidronate significantly reversed this suppression by decreasing serum calcium, and rapid normalization of intact PTH was seen in almost all patients. In all the patients except one, pre-treatment urinary excretion of PTHrP had been high. In these patients, PTHrP might have played crucial role in the manifestation of hypercalcemia. Interestingly, pamidronate therapy increased intact PTH as well as the urinary excretion of PTHrP without much change in renal function. It is known that PTH is physiologically regulated by serum calcium levels and the progressive increase in PTH after treatment could be caused by this control mechanism. On the other hand, the release of PTHrP has been reported to be independent of the serum calcium concentration in hypercalcemic patients with several malignant tumors [10, 16]. There is some evidence that PTHrP plays roles in calcium metabolism such as that of a regulatory factor in maintaining physiological hypercalcemia in the fetus [17, 18]. PTHrP is reported to exist in human milk and PTHrP mRNA is expressed in the normal lactating mammary tissue, suggesting that it may act as a hormone for the mobilization of calcium from the bone or for transferring calcium to the milk [19, 20]. These findings indicate the possibility that PTHrP has limited but physiological actions even in normal humans including the fetus. Although the precise regulatory mechanism of PTHrP release in both physiological and pathological conditions is not clear, it is reported that an increase in calcium in the medium inhibits PTHrP release from cultured cytotrophoblast cells [21]. Moreover, low-

![Fig. 3. Serum concentration of pamidronate after an intravenous administration of 45 mg of pamidronate disodium given over 4 h. Each point represents the mean ± SEM.](image)

![Fig. 4. Cumulative urinary excretion of pamidronate after an intravenous administration of 45 mg of pamidronate disodium. Each point represents the mean ± SEM.](image)
erating the extracellular calcium concentration resulted in an increase in PTHrP production in cultured human squamous carcinoma cells [22]. These findings seem to shed some light on our present results. In contrast to our observation of a progressive increase in urinary PTHrP after pamidronate therapy, a few reports showed that pamidronate did not affect the plasma PTHrP concentration in patients with tumor-associated hypercalcemia [1, 10]. Although the reason for this discordance is unclear, the following possibility has been considered. PTHrP is reported to exist at least as two molecular forms in the plasma of patients with tumor-associated hypercalcemia: one is an N-terminal peptide and the other is a C-terminal peptide [16, 23]. C-terminal fragments of PTHrP metabolized from intact PTHrP are readily excreted into urine and PTHrP excretion in urine is much higher than that in plasma [6], suggesting that urinary PTHrP might be a sensitive index for the evaluation of PTHrP synthesis. The measurement of PTHrP levels in the urine may therefore be a more sensitive way of evaluating such a small change as seen in the present investigation than the measurement of the PTHrP concentration in the plasma. Body et al. reported not significant but slight increase in plasma PTHrP after pamidronate therapy in some patients with tumor-associated hypercalcemia [10], suggesting that further investigations with the measurement of urinary PTHrP will be required to clarify the precise effect of pamidronate.

An increase in serum alkaline phosphatase activity may be consistent with the increase in PTH and/or PTH-like activity. The effects of pamidronate on bone metabolism were also evaluated by measuring urinary excretion of hydroxyproline and serum BGP. Because urinary hydroxyproline could be used as a marker for bone resorption, a significant decrease in urinary excretion of hydroxyproline by pamidronate therapy confirms that bone resorption caused by PTHrP was strongly suppressed by this drug. In contrast to the increase in serum alkaline phosphatase activity, serum BGP did not show a significant change following the use of this drug. It is possible that serum BGP might not reflect osteoblastic activity which is linked to acute suppression of the function of osteoclasts within such a short term.

When 14C-labeled pamidronate was administered to rats and mice, pamidronate was mainly distributed in bone [24] and the elimination half-life of pamidronate from bone was estimated to be approximately 300 days [25]. Furthermore pamidronate was mainly excreted in urine, in which its metabolites were not detected [25]. In the present study, we demonstrated that pamidronate in serum was rapidly eliminated after administration. Urinary excretion of pamidronate almost reached a plateau on the second day and total urinary excretion was approximately 14% of the dose administered. Most of the infused dose may therefore be distributed to the bone and other tissues in patients with tumor-associated hypercalcemia. The amount of pamidronate retained by the body, which was estimated in terms of its excretion into urine, was apparently high compared with a previous report dealing with normocalcemic patients with bone metastases [26]. It is unclear why the retention estimates for the hypercalcemic patients in our study were greater than those for normocalcemic patients with bone metastases. However, this fact would still indicate the suitability of pamidronate treatment for patients with tumor-associated hypercalcemia.

In conclusion, pamidronate is an effective and potent drug for tumor-associated hypercalcemia. Increases in urinary PTHrP excretion along with a rapid reverse decrease in serum calcium following pamidronate treatment suggested that PTHrP secretion from a tumor could be partially regulated by the serum calcium concentration.

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References


