molecules that could regulate tubulin assembly and function in cells. It is important to elucidate the conformation and structure of these binding sites to comprehend the cellular control mechanisms for microtubules. In addition, such information may prove essential for understanding the chemotherapeutic properties of taxol.

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Pharmacokinetics of Pamidronate in Patients With Bone Metastases

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Background: Pamidronate is a second-generation bisphosphonate used in the treatment of tumor-induced hypercalcemia and in the management of bone metastases from breast cancer, myeloma, or prostate cancer. The pharmacokinetics of pamidronate is unknown in cancer patients. **Purpose:** To determine the influence of the rate of administration and of bone metabolism, we studied the pharmacokinetics of pamidronate at three different infusion rates in 37 patients with bone metastases. **Methods:** Three groups of 11-14 patients were given 60 mg pamidronate as an intravenous infusion over a period of 1, 4, or 24 hours. Urine samples were collected in the three groups of patients. Plasma samples were obtained only in the 1-hour infusion group. The assay of pamidronate in plasma and urine was performed by high-performance liquid chromatography with fluorescence detection after the derivatization of pamidronate with fluorescamine. **Results:** The body retention (BR) at 0-24 hours of pamidronate represented 60%-70% of the administered dose and was not significantly modified by the infusion rate. In particular, the BR at 0-24 hours was not reduced at the fastest infusion rate. Among patients, a threefold variability in BR at 0-24 hours occurred, which was related directly to the number of bone metastases and, to some extent, to creatinine clearance. At 60 mg/hour, the plasma kinetics followed a multiexponential course characterized by a short distribution phase. The mean (± SD) half-life of the distribution phase was 0.8 hour (±0.3), the mean (±SD) of the area under the curve for drug concentration in plasma × time at 0-24 hours was 22.0 ± 8.8 μmol/L × hours, and the mean (±SD) of the maximum plasma concentration was 9.7 μmol/L (±3.2). Pharmacokinetic variables remained unchanged after repeated infusions applied to four patients. Clinically, the three infusion rates were equally well tolerated without significant toxicity. **Conclusions:** The 1-hour infusion rate could be proposed as kinetically appropriate for the administration of pamidronate to patients with metastatic bone diseases. [J Natl Cancer Inst 84:788–792, 1992]

Bisphosphonates are structural analogues of pyrophosphate, a natural regulator of bone mineral precipitation and dissolution. Pamidronate is a second-generation bisphosphonate that strongly inhibits bone resorption without interfering with bone mineralization (1,2). Its activity, measured by inhibition of bone resorption, is more potent when compared with the activity of etidronate and clodronate, probably because of a direct action on osteoclast precursors (1-4).

Pamidronate has been used widely in benign clinical conditions such as Paget's disease (5) and osteoporosis (6). In malignancy, it has been administered to treat tumor-induced hypercalcemia (7-9) and to reduce morbidity caused by bone metastases (10,11). The intravenous route of administration is generally preferred for the treatment of malignant hypercalcemia or bone meta-

stases, because of poor absorption by the gut (12) and gastric irritation caused by oral administration. The optimal schedule and infusion rate have not yet been defined, however, and might be clarified by pharmacokinetic information.

The pharmacokinetic properties of bisphosphonates have been determined in laboratory animals (13,14) in which half of the dose was accumulated in the skeleton and half excreted unchanged in the urine. Accumulation of bisphosphonates in the reticuloendothelial system of animals varies between species (15) and between individual bisphosphonates (16), suggesting different distribution kinetics—factors that mandate cautious interpretation when extrapolating data from animals to humans.

Pamidronate pharmacokinetics were studied in rats following oral and intravenous administration of ^{14}C-labeled compound (17). Bone accumulation was constant at 25%-35% of dose. The uptake of labeled pamidronate by the reticuloendothelial system was, however, dose dependent, with less than 3% in the liver at 0.01 mg/kg and 30% in the liver at 10 mg/kg. Because the liver is an important storage compartment with a short half-life ($t_{1/2}$) of retention, further increase of bone accumulation occurred with time. Release of pamidronate from the skeleton was extremely slow with an estimated $t_{1/2}$ of at least 300 days.

In humans, pharmacokinetic data on bisphosphonates are limited. In healthy subjects, means of 73% and 81% of the administered dose of clodronate were recovered unchanged in the urine within 24-48 hours, with no difference between the three doses tested (18,19). In six patients with metastatic breast cancer, total urinary excretion was similar at 75% (20). However, in patients with Paget's disease, mean urine excretion was lower at 58%. Because clodronate was administered as a slow infusion over a 5-day period, it was concluded that slow infusion increases accumulation in the body and decreases urine excretion (21). It is not known by how much the pharmacokinetics of bisphosphonates could be affected by the rate of administration or by bone metabolism.

In initial clinical studies with pamidronate, multidose regimens have been used for a period of 6-9 days, but, recently, single-day infusions have been shown to be equally effective and are currently recommended (7). We report

the pharmacokinetic study of pamidronate given to cancer patients with bone metastases. Pamidronate was administered as a single infusion, at constant dose, but at three different infusion rates, to test the influence of infusion rate on pharmacokinetic variables.

Subjects and Methods

Study Subjects

The study was conducted at the Centre Pluridisciplinaire d'Oncologie, University Hospital, Lausanne, and at the Kantonsspital, St. Gallen, Switzerland. It was approved by the ethical committee of both institutions. Informed consent was obtained from every patient. Entry criteria included an age limit greater than or equal to 18 years, a histological diagnosis of cancer with radiological evidence of bone metastases, serum creatinine level less than 150 $\mu mol/L$ or bilirubin level less than 25 $\mu mol/L$, and normal levels of liver enzymes. Except for one patient with a serum calcium level at 2.91 mmol/L, the serum calcium level was not above 2.75 mmol/L. The patients were not treated previously with bisphosphonates. No new medications and no chemotherapy were started for at least 72 hours prior to entry or at any time during the study. Patients on hormone therapy were eligible, provided they did not have a change in treatment schedule for at least 2 weeks. A total of 37 patients were enrolled in the study; their characteristics are summarized in Table 1.

Treatments

The study was performed in two phases over a 15-month period. During the first phase, 23 patients were randomly assigned to receive 60 mg pamidronate, diluted in 500 mL of 0.9% saline, as an intravenous infusion over a period of 4 or 24 hours. To ensure adequate urine flow, a total of 2 L of intravenous fluid was given during the day of pamidronate administration and the following day. Blood samples were not drawn during this phase of the study. When analysis disclosed that the amount of drug excreted in urine was similar at both infusion rates, we started the second phase of the study.

During the second phase, an additional group of 14 patients was given 60 mg pamidronate, diluted in 250 mL of 0.9% saline, over a period of an hour without hydration. Blood and urine samples were collected in this group of patients.

Among this group of patients, four were selected in whom the 1-hour infusion was repeated at 4- to 5-week intervals at a maximum of four infusions.

Urine Collection and Blood Sampling

During the first part of the study, urine collections were performed at 4-hour intervals for 12 hours (three collections) and then at 12-hour intervals for a further 36 hours (three collections). During the second part of the study, collection of blank samples and two collections of urine were performed from 0 to 4 hours and from 4 to 24 hours.

Table 1. Patient characteristics

	Pamidronate infusion rates		
	60 mg/4 h	60 mg/24 h	60 mg/ 1 h
No. of patients	12	11	14
Male/female	2/10	0/11	3/11
Mean age, y (range)	67 (56–80)	63 (28–78)	62 (42–73)
Mean height, cm (range)	162 (135–175)	157 (148–165)	162 (139–178)
Mean weight, kg (range)	62 (34–85)	60 (52–71)	65 (52–75)
Median creatinine clearance, mL/min (range)	65 (37–98)	66 (48–102)	68 (35–110)
No. of breast carcinomas	10	11	11
No. of prostatic carcinomas	1	—	2
No. of bronchial carcinomas	1	— ·	—
No. of hypernephromas	—	—	1
No. of bone metastases*			
<5	3	2	1
5–15	7	7	9
>15	2	2	4

*Evaluated on standard x rays.

Blood samples were obtained only during the second part of the study, before and then every 15 minutes for 2 hours after the start of the infusion and then at 2.5, 3, 5, and 24 hours.

Assay of Pamidronate in Urine and Plasma

The assay of pamidronate in plasma and urine was performed by high-performance liquid chromatography with fluorescence detection, after derivatization of pamidronate with fluorescamine (22,23). The limits of quantitation were 1.4 μmol/L in plasma and 1.8 μmol/L in urine.

Clinical Assessment

In the first part of the study, serum calcium, phosphate, creatinine, and creatinine clearance levels were assessed before treatment.

Because of concerns about the possibility of increased toxicity of the more rapid infusion rate used during the second part of the study, the following parameters were measured in serum before infusion and at 24 and 48 hours after infusion: urea, creatinine, calcium, phosphate, albumin, bilirubin, alkaline phosphatase, and transaminases. In addition, the following parameters were measured in urine: calcium, phosphate, creatinine, sodium, protein, hydroxyproline, and lysozyme. Routine urinalyses were done for the presence of blood and protein.

In all patients, the number of bone metastases was evaluated on standard x rays and reported in three groups—those with fewer than five metastases, those with between five and 15 meta-

stases, and those with more than 15 metastases. In seven patients, a more accurate determination of the number of bone metastases was obtained by bone scan. Clinical examinations for toxicity were performed daily during the infusion period and then 2 to 4 weeks later.

Pharmacokinetic Calculations

Noncompartmental techniques were used in the pharmacokinetic analysis (24). The total urinary excretion (TUE) at 0-24 hours was the total amount of pamidronate excreted in urine over 24 hours. The body retention (BR) at 0-24 hours was estimated as follows: BR (% of dose) = dose (100%) − TUE (% of dose); fecal excretion was, in all probability, negligible as known from animal experiments (17).

The area under the curve (AUC) for drug concentration in plasma × time at 0-24 hours, expressed as (μmol/L) × hours, was determined by the linear trapezoidal rule from 0 to 24 hours. C_{max} (μmol/L) was the maximal plasma concentration, and T_{max} (hour) was the time at which C_{max} was reached. The apparent plasma distribution half-life ($t_{1/2}$ in hours) of pamidronate was calculated from the slope of the linear least-squares regression line through the concentration-time points in the time interval from 1 to about 2 hours. The total plasma clearance (CL_t [L/hour]) was calculated as the dose/AUC (0 − ∞), taking AUC (0-24 hours) as AUC (0 − ∞) because all 24-hour plasma concentrations were below the limit of detection and taken as zero. The renal clearance (CL_r [L/hour]) was calculated as the TUE (0-24 hour)/AUC (0-24 hour). The nonrenal clearance (CL_{nr} [L/hour]) was $CL_t − CL_r$.

No statistical comparison of the treatments was performed. This study being the first pharmacokinetic trial with pamidronate disodium, no information on the intersubject variability was available. The results of this trial showed a high interpatient variability in the urinary excretion, and, therefore, the number of patients included in each group of the study was too low to perform meaningful statistical tests with sufficient power.

Results

Pharmacokinetics in Urine

The TUE over 24 hours of each patient and the mean (±SD) values are shown graphically in Fig. 1, according to the three different infusion rates. The mean TUE (0-24 hours) (±SD) was not significantly different and was measured at 31% ± 15.2%, 34.9% ± 13.9%, and 41.0% ± 15.4% of the 60 mg of pamidronate administered over periods of 1 hour, 4 hours, and 24 hours, respectively. Considerable between-patient variability was noted.

To explain the wide disparity of TUE (0-24 hours) among patients, TUE was correlated with creatinine clearance and with tumor bone involvement. A weak relationship was found between creatinine clearance and TUE (0-24 hours) by linear least-squares regression analysis ($r = 0.42$), but a stronger association was demonstrated between the number of bone metastases and BR (0-24 hours). The mean (±SD) of the BR (0-24 hours) values in patients with fewer than five bone metastases was 50.6% ± 11.8% and increased with increasing number of bone metastases to 76.4% ± 12.0% in patients with more
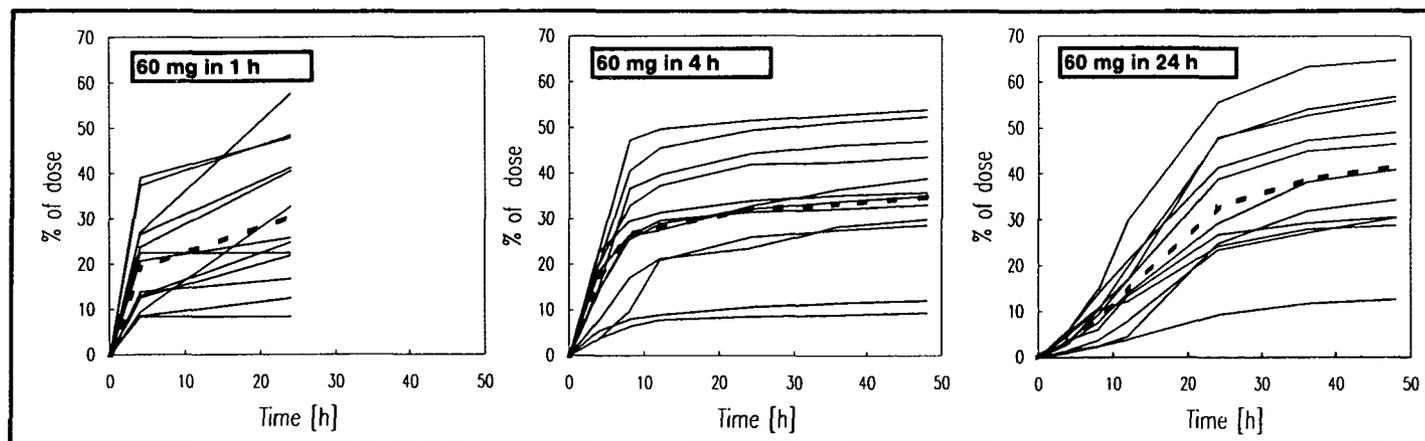
Fig. 1. Total urinary excretion [TUE (0-24 hours)] of each patient after an infusion of 60 mg of pamidronate disodium given over 24-, 4-, and 1-hour periods at a constant infusion rate. Mean values are presented as dash lines.

than 15 metastases (Fig. 2). These findings were confirmed in the subgroup of seven patients, where an accurate count of bone involvement could be made by bone scintigraphy and where the coefficient of correlation was 0.82 (linear least-squares regression). No correlation was found between serum and urine parameters such as calcium, phosphate, alkaline phosphatase, hydroxyproline, and TUE (0-24 hours).

Pharmacokinetics in Plasma

After the 1-hour infusion of 60 mg of pamidronate disodium, the mean ($\pm$SD) of the AUC for drug concentration in plasma $\times$ time at 0-24 hours was 22.0 $\pm$ 8.8 μmol/L $\times$ hours; the mean C_{max} ($\pm$SD) was 9.7 μmol/L $\pm$ 3.2 in 14 patients. The median value of T_{max} was 0.8 hour. The mean ($\pm$SD) plasma concentration times profile is shown in Fig. 3. The decline of pamidronate concentrations in plasma followed a multiexponential course. The distribution of pamidronate was rapid with a mean ($\pm$SD) apparent $t_{1/2}$ of 0.8 hour ($\pm$0.3). Plasma concentrations of pamidronate were near the limit of quantitation in most patients at 3 hours and below the limit at 5 and 24 hours. Therefore, no terminal $t_{1/2}$ could be estimated for the slow release of pamidronate from bone.

Two hours after the start of the infusion, a slight increase in plasma concentration occurred in the majority of patients, possibly corresponding to a release of pamidronate from an unknown compartment. The mean ($\pm$SD) CL_t was 10.7 L/hour ($\pm$3.7), and the mean
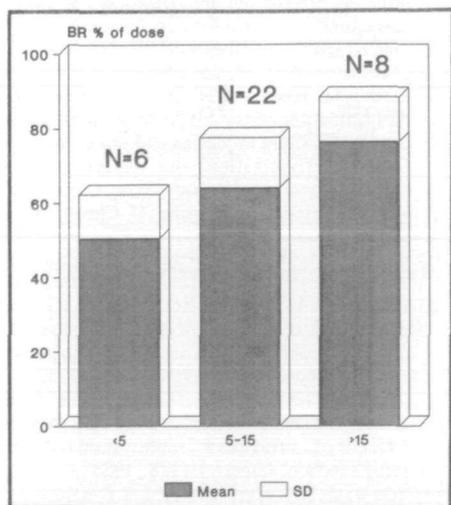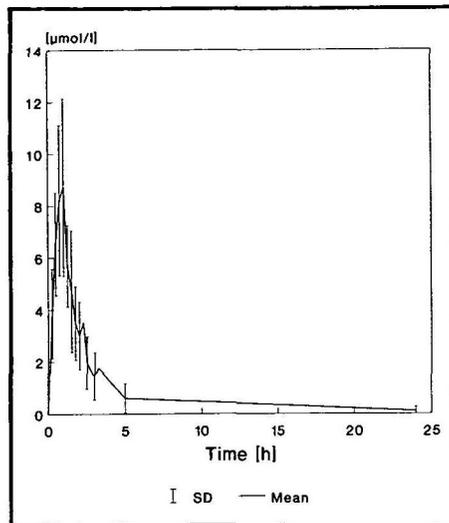
Fig. 3. Mean plasma concentration-time profiles ($\pm$SD) of pamidronate after an intravenous infusion of 60 mg pamidronate disodium over a 1-hour period (n = 14).

($\pm$SD) CL_r was 3.3 L/hour ($\pm$1.9). The mean ($\pm$SD) CL_{nr} amounted to 7.5 L/hour ($\pm$3.2) and constituted about 70% of the CL_t.

Infusions of pamidronate were repeated at 4- to 5-week intervals in four patients. BR (0-24 hours) was practically constant within each patient as shown in Table 2. These results suggest that bone compartment was not saturated after repeated dosing.

Tolerance

Except for one patient who developed a phlebitis at the infusion site, no local or systemic toxic effects were observed. In the group receiving the 1-hour infusion, the renal parameters remained within normal limits, including those in patients with multiple infusions. One

patient, who had baseline gamma glutamyltransferase levels at three times normal before infusion, had an elevation in the level of aspartate aminotransferase from 9 U/L to 89 U/L (normal level,<33 U/L). In all remaining patients, liver function tests did not show any significant modification.

Discussion

The pharmacokinetics of pamidronate were not influenced by the infusion rates as suggested with clodronate (21). The 24-hour TUE (0-24 hours) was not significantly modified when pamidronate was administered at 2.5, 15, or 60 mg/hour. The original hypothesis that the faster the infusion, the greater the loss of bisphosphonate in urine was not confirmed. On the contrary, the 24-hour infusion rate had a TUE (0-24 hours) at 41% of the dose, compared with 31% for the 1-hour infusion. BR (0-24 hours) represented 60%-70% of the administered dose and was higher than with other bisphosphonates (16-18). This result could contribute to the increased potency of pamidronate in vivo. The studies with clodronate conducted in healthy subjects demonstrated a BR (0-24 hours) of 19%-27% (18,19) and in metastatic breast cancer of 25% of the dose (20). In one study, however, the BR (0-24 hours) was 42% in patients with active Paget's disease and in one patient with prostatic cancer (21).

Bone metabolism and turnover might affect bisphosphonate pharmacokinetics (21,25). A direct correlation was suggested between the degree of bone involvement and BR (0-24 hours). Mean

Fig. 2. Body retention [BR (0-24 hours)] according to the number of bone metastases.

Table 2. Pharmacokinetic parameters of pamidronate in plasma and urine*

Patient No.	Infusion No.	AUC (0–24 h), μmol/L $\times$ h	$t_{1/2}$, h	TUE (0–24 hs), % of dose	Cl_r, L/h	Cl_{nr}, L/h
2	1	31.5	0.8	48	3.3	3.6
	2	26.2	0.6	43	3.5	4.7
	3	25.4	1.8	62	5.3	3.2
	4	30.7	0.9	47	3.3	3.7
5	1	19.9	0.5	58	6.2	4.6
	2	23.1	0.7	39	3.6	5.7
	3	20.6	0.9	51	5.3	5.1
7	1	45.7	1.8	25	1.2	3.5
	2	24.3	1.0	26	2.3	6.5
	3	24.6	0.8	25	2.2	6.6
8	1	26.9	0.9	26	2.1	5.9
	2	27.2	0.7	31	2.5	5.5

*After repeated intravenous infusions of 60 mg pamidronate disodium over 1-hour period at intervals of 4–5 weeks in patients with bone metastases.

BR (0-24 hours) was 50% in patients with few bone metastases and 75% in those with numerous bone metastases. The relationship was stronger in a subset of patients in whom a more accurate determination of the number of metastatic sites was performed by counting lesions on bone scan.

The influence of renal function on urinary excretion of pamidronate is unknown. In the population studied which had either a normal or a slightly reduced creatinine clearance, the renal function had a weak effect on TUE (0-24 hours). However, in a study in which patients with osteodystrophy had a creatinine clearance of only 5-35 mL/minute, TUE (0-24 hours) of Tc-99m-etidronate was 11.4% of the administered dose (25). The disparity in skeletal invasion and in renal function could explain the almost threefold variability in BR (0-24 hours) among patients in our study.

The plasma pharmacokinetics of pamidronate at 60 mg/hour followed a multiexponential course and was characterized by a short distribution phase in plasma, followed by a rapid elimination of unchanged drug in urine. The distribution of pamidronate was rapid with a $t_{1/2}$ of 0.8 hour. Clodronate was also removed promptly from the blood, with a $t_{1/2}$ between 1.8 hours and 2.3 hours in healthy subjects (16) and in patients with bone disease (18,20). The total clearance of pamidronate was 10.7 L/hour with a CL_{nr} clearance of 7.5 L/hour. For clodronate, values varied between studies, with a total clearance of 6.4 and 11.5 L/hour, possibly due to different methods of measurement, a factor which prevents any direct comparison with our data. Plasma concentration increased slightly 2 hours after the start of the infusion. Pamidronate may have been released from the reticuloendothelial system, where it could have accumulated temporarily. A similar observation has been made in animals but not previously in humans. In mice, pamidronate accumulated in spleen and liver in contrast to clodronate and etidronate.

When pamidronate was infused at a dose of 60 mg over a 1-hour period at 4- to 5-week intervals, its pharmacokinetic values remained unchanged. With this schedule and dose, the bone compartment was not saturated. Similar conclusions were reached after five daily infusions of clodronate, where no signifi-

cant variations were detectable between daily infusions (20).

Clinically, the tolerance of patients for all three infusion rates was similar, with only minimal toxicity. In particular, no renal toxicity was observed after the 1-hour infusion rate. Previous studies have shown a transient increase in plasma creatinine (26) and rare cases of renal failure (27) after etidronate and clodronate administration. The plasma creatinine did not rise within 48 hours after the infusion of pamidronate. Proteinuria remained within normal range. The minimal elevation of aspartate aminotransferase seen in one patient had no clinical significance and could be related to a possible uptake of pamidronate into the reticuloendothelial system. A larger number of patients, however, should be studied for toxicity at the fastest rate of infusion before safety can be ensured.

On the basis of the pharmacokinetic data combined with the clinical findings, we conclude that BR (0-24 hours) of pamidronate was dependent on the amount of bone metastases and was not influenced by the rate of infusion. Thus, the 1-hour infusion rate can be proposed as a kinetically relevant treatment schedule for patients with malignant bone diseases.

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