Pharmacokinetic and Pharmacodynamic Evaluation of Intermittent Versus Continuous Alendronate Administration in Rats

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ABSTRACT: We studied the differences in pharmacokinetics and pharmacodynamics of the same dose of alendronate administered subcutaneously as intermittent bolus injection or continuous infusion in rats. Two rat models of bone disease were applied. Bone cancer was produced by intrathelial inoculation of Walker carcinosarcoma cells, and a model of augmented bone resorption was produced by vitamin D3 treatment of rats that had undergone thyroidparathyroidectomy. Higher amounts of alendronate were found in bones and in internal organs after bolus drug administration as compared with continuous infusion. Drug effects on plasma calcium levels and on urine calcium excretion were similar in both modes of alendronate administration. Results of the study indicate that the pharmacokinetics (disposition) of alendronate is administration-dependent. The total amount found in bone does not directly represent the amount of alendronate that is pharmacologically active at the site of action in the bone and that affects bone remodeling. The findings suggest that there is no pharmacodynamic advantage for continuous infusion of alendronate. It is concluded that the preferred mode of administration should be selected according to secondary clinical criteria (like incidence of adverse effects and convenience of administration). © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 91:508–516, 2002

Keywords: alendronate; bisphosphonates; pharmacokinetics; pharmacodynamics; mode of administration dependency

INTRODUCTION

Bisphosphonates are drugs used in treating various metabolic bone disorders, including postmenopausal osteoporosis, hypercalcemia of malignancy, tumor osteolysis, and Paget's disease. The pharmacokinetic (PK) behavior of bisphosphonates is unique in that they have very low oral bioavailability, typically less than 1%. Most of the clinically used bisphosphonates (aminobisphosphonates) are not metabolized in the body, and are eliminated from systemic circulation by kidney excretion or following entrapment and disposition in bone.

Because of difficulties in quantifying bisphosphonate concentration at the site of action and an unknown concentration—response relationship, the optimal protocol of bisphosphonate treatment has not yet been established. Different treatment protocols have been applied clinically and include intravenous bolus administration, continuous infusion over 4 h, 24 h, or longer periods in hypercalcemia of malignancy. For osteoporosis treatment, the studied modes of administration included daily treatment with immediate or sustained release oral dosage forms, weekly, or cyclical therapy.

Despite wide clinical use and established efficacy of bisphosphonates for these indications,
one of the principal questions concerning bisphosphonate treatment remains unsolved: Which mode of drug input is required to produce the best clinical outcomes? Most of the studies that have compared different administration schedules of the same dose of a given bisphosphonate failed to demonstrate differences in the magnitude of drug effects. For instance, short-term single infusions of bisphosphonates over 4 or 24 h produced similar outcomes in hypercalcemia of malignancy.\textsuperscript{11,12} Similar magnitude of drug effects in osteoporosis was found for daily-versus-weekly and cyclic-versus-continuous administration.\textsuperscript{10,13} However, clinical investigations pose practical limitations in studying different input regimens. Long-term continuous infusion of bisphosphonates is not applicable in clinical settings, and in “continuous” (daily) regimens of oral tablets, continuous input of the drug to the body is not provided.\textsuperscript{14,15} Thus, the question whether continuous or intermittent treatment is clinically preferable has not yet been clarified.

The aim of this investigation was to study whether continuous or intermittent input function of the same dose of bisphosphonate leads to differences in the drug’s PK and pharmacodynamics (PD) in rats. For this purpose, we applied contrasting subcutaneous regimens of continuous-versus-intermittent alendronate administration over 5- and 14-day treatment periods in two models of bone disease in rats [Walker carcinosaoma-implanted rats and thyroidparathyroidectomy (TPTX) rats, respectively]. The alendronate selected for this study is a third-generation aminobisphosphonate that is used clinically for various metabolic bone disorders, including postmenopausal osteoporosis and cancer-induced hypercalcemia.\textsuperscript{2,7}

**MATERIALS AND METHODS**

**Materials**

Alendronate and [\textsuperscript{14}C]Alendronate were synthesized in the laboratory of Prof. E. Breuer at The Hebrew University of Jerusalem.\textsuperscript{16} The specific activity of the radiolabeled alendronate was 1 \( \mu \)Ci/mg. Walker carcinosarcoma cells (WCS) were obtained from Deutsches Krebsforschungszentrum, Heidelberg, Germany. 1,25 di-(OH) vitamin D\textsubscript{3} was purchased from Hoffman-LaRoche, Basel, Switzerland. Alzet osmotic pumps (models 1007D and 2002) were purchased from Alza Corporation, Mountain View, CA. All other reagents were of analytical grade.

**Experimental Protocols**

This investigation adhered to the principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985).

**TPTX Rats**

**Animals**

Male Wistar rats weighing approximately 120 g were kept individually in metabolic cages under a 12-h light/dark cycle for a period of 1 week before the investigation for acclimatization. The rats had free access to water and food (regular rat chow), with the exception of food deprivation for approximately 16 h before each blood sampling. TPTX was performed under ketamine/xylazine anesthesia [9% ketamine and 1% xylazine solution, intraperitoneally (ip), 1.0 mL/kg weight] 5 days before the beginning of alendronate administration. Rats with successful TPTX operation (plasma calcium levels < 1.88 mM) were randomly assigned into four experimental groups \((n = 5–6): 1. “positive” control—on days 1–15: daily subcutaneous saline injections, and daily 1,25 di-(OH) vitamin D\textsubscript{3} injections, 250 pmol/kg/day, ip; 2. “negative” control—on days 1–15: daily subcutaneous saline injections, and vehicle ip injections [no 1,25 di-(OH) vitamin D\textsubscript{3} treatment]; 3. continuous—alendronate administration on days 1–15 by subcutaneously implanted Alzet pump (2002) at the back of the animal, a total dose of 0.1 mg/kg; daily 1,25 di-(OH) vitamin D\textsubscript{3} injections, 250 pmol/kg/day, ip on days 1–15; and 4. intermittent—alendronate administration by subcutaneous injections at the back of the animal on days 1, 8, and 15, a total dose of 0.1 mg/kg; daily 1,25 di-(OH) vitamin D\textsubscript{3} injections, 250 pmol/kg/day, ip on days 1–15.

The vehicles for alendronate and 1,25 di-(OH) vitamin D\textsubscript{3} administration were saline, and a mixture of water/propylene glycol/ethanol 5:4:1 (v/v/v), respectively.

**Sample Collection**

The rats were weighed every other day, and blood samples (0.4 mL) were collected from the tail artery under ether anesthesia 5 days before (baseline) and at days 0 (TPTX validation), 8, and 16 after the beginning of the experiment.
Plasma was separated by immediate centrifugation (3000 rpm, 10 min) and frozen pending analysis of calcium levels. Urine samples were collected at 48-h intervals and were stored at −20°C pending analysis of calcium levels and alendronate concentrations. The rats were euthanized by overexposure to ether on day 16 after the beginning of the experiment, and the tibia, femur, kidney, liver, and spleen were obtained and frozen pending alendronate assay.

**Tumor-Implanted Rats**

**Animals**

Male Sprague-Dawley rats weighing approximately 100 g were kept individually in metabolic cages under a 12-h light/dark cycle for a period of 1 week before the investigation for acclimatization. Over the whole experimental period, the rats had free access to water and food (regular rat chow).

**Tumor Implantation**

For tumor-cell propagation and better graft of cells in the recipient animals, two subcutaneous passages of WCS tumor were performed in female Sprague-Dawley rats. Subcutaneous tumor tissue was carefully removed from the passage animal, and a suspension of the tumor cells was prepared by trypsinization. Tumor cells were implanted into right tibia of male Sprague-Dawley rats under ketamine/xylazine anesthesia (9% ketamine and 1% xylazine solution, ip, 1.0 mL/kg weight) with a similar technique described by Klenner et al. Briefly, the knee joint region of the right posterior limb was shaved and disinfected with a chlorhexidine solution. Tumor suspension (2·mln (2·10⁶) tumor cells) was injected into the bone marrow of right tibia using a 23G needle. No surgical wound dressing was necessary; occasional bleeding was stopped by light pressure.

**Experimental Groups**

The four experimental groups were (n = 5–6): 1. “positive” control—tumor implantation on day 0, subcutaneous saline injections on days 2–7; 2. “negative” control—no tumor, subcutaneous saline injections on days 2–7; 3. continuous alendronate—tumor implantation on day 0, alendronate administration on days 2–7 delivered from subcutaneously implanted Alzet pump (1007D) at the back of the animal, a total dose of 0.75 mg/kg; and 4. intermittent alendronate—tumor implantation on day 0, alendronate administration by subcutaneous injections at the back of the animal on days 2, 4, and 6, a total dose of 0.75 mg/kg.

For the purpose of both continuous and intermittent administration, alendronate was dissolved in saline.

**Sample Collection**

The rats were weighed and blood samples (0.4 mL) were collected from tail artery under ether anesthesia on days 0, 5, 7, and 10 after tumor inoculation. Plasma was separated by immediate centrifugation (3000 rpm, 10 min) and frozen pending analysis of calcium levels. Urine samples were collected at 48-h intervals and were stored at −20°C pending analysis of calcium levels and alendronate concentrations. The rats were euthanized by overexposure to ether on day 10 after tumor implantation, and the left tibia, left femur, kidney, liver, and spleen were obtained and frozen pending alendronate assay.

**Measurements**

Total plasma and urine calcium levels were determined by means of atomic absorption spectroscopy. To determine alendronate disposition, specimens of the explanted organs (approximately 0.5 g), and urine samples (0.5 mL) were combusted in a Sample Oxidizer (model 307; Packard Instrument Co., Meriden, CT) using Carbo-Sorb carbon dioxide absorber and Permafluor E + scintillation cocktail (Packard Instrument Co.). Radioactivity then was determined in a Packard liquid scintillation counter (Packard Instrument Co.) and drug amounts were calculated by using an appropriate calibration curve. The calibration curves for alendronate in urine, bones, or soft tissues were linear for 0–2500 ng alendronate (r² > 0.998). The inter- and intra-day coefficients of variation were <1.0 and 0.5%, respectively.

**Statistical Analysis**

The Kruskal-Wallis ANOVA with subsequent Newman-Keuls multiple comparisons test were applied for analysis of plasma and urine calcium levels. Comparison between alendronate disposition after intermittent-versus-continuous drug administration was performed by the two-tailed nonparametric Mann-Whitney U test. A p value < 0.05 was termed significant.
RESULTS

TPTX Rats

In TPTX rats, lower amounts of alendronate were found in bone and liver after the administration of alendronate as a 2-week continuous input from a subcutaneously implanted osmotic pump as compared with intermittent bolus administration of the same dose (by approximately 30%; see Table 1). Amounts of alendronate found in urine and kidney were not different for continuous and intermittent treatment groups. The amounts that reached spleen were below the detection limit (see Table 1).

Plasma and urine calcium concentrations were used to assess alendronate’s effect on calcium homeostasis. As can be seen in Figure 1, TPTX operation led to a decrease in the baseline levels of plasma calcium that remained the same throughout the experimental period (“negative” control). Daily intraperitoneal injections of 1,25 di-(OH) vitamin D₃ led to an increase in plasma calcium levels producing marked hypercalcemia at the end of the 2-week treatment period (“positive” control group). Intermittent alendronate input decreased the vitamin D₃-induced increase in calcium levels to a somewhat greater extent compared with continuous drug administration. However, there was no significant difference between plasma calcium levels of continuous and intermittent alendronate administration modes during the experimental period.

Urine calcium excretion in TPTX rats is presented in Figure 2. Daily intraperitoneal injections of 1,25 di-(OH) vitamin D₃ (“positive”

Table 1. Disposition of Alendronate in TPTX Rats After Continuous (Subcutaneous Alzet Pump, Days 1–15) or Intermittent (Subcutaneous Injections on Days 1, 8, and 15) Administration, a Total Dose of 0.1 mg/kg (% of Administered Dose ± SD)

<table>
<thead>
<tr>
<th>Administration</th>
<th>Urine</th>
<th>Tibia</th>
<th>Femur</th>
<th>Kidney</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>22.3 ± 7.6</td>
<td>1.17 ± 0.15 a</td>
<td>1.68 ± 0.11 b</td>
<td>0.108 ± 0.090</td>
<td>0.175 ± 0.041 a</td>
<td>—</td>
</tr>
<tr>
<td>Intermittent</td>
<td>25.6 ± 6.9</td>
<td>1.55 ± 0.32</td>
<td>2.34 ± 0.16</td>
<td>0.040 ± 0.023</td>
<td>0.216 ± 0.054</td>
<td>—</td>
</tr>
</tbody>
</table>

aSignificant difference (p < 0.05).
bSignificant difference (p < 0.01).

— Not detected.

Figure 1. Rat plasma calcium levels before TPTX (day 5) and on days 0, 8, and 16 of the experiment (mean ± SD). During the treatment period (days 1–15), the “positive” control group received subcutaneous saline and ip 1,25 di-(OH) vitamin D₃; the “negative” control group received subcutaneous saline and ip vehicle; the continuous group received subcutaneous alendronate on days 1–15 by osmotic pump and ip 1,25 di-(OH) vitamin D₃ daily; the intermittent group received subcutaneous alendronate injections on days 1, 8, and 15, and ip 1,25 di-(OH) vitamin D₃ daily.

Figure 2. Daily urine calcium excretion in TPTX rats during the experimental period (mean ± SD). During the treatment period (days 1–15), the “positive” control group received subcutaneous saline and ip 1,25 di-(OH) vitamin D₃; the “negative” control group received subcutaneous saline and ip vehicle; the continuous group received subcutaneous alendronate on days 1–15 by osmotic pump and ip 1,25 di-(OH) vitamin D₃ daily; the intermittent group received subcutaneous alendronate injections on days 1, 8, and 15, and ip 1,25 di-(OH) vitamin D₃ daily.
control) led to an increase in urinary excretion of calcium compared with the “negative” control group. Continuous and intermittent alendronate administration partially decreased the vitamin D3-induced increase in urinary calcium excretion. A similar extent of effect on urinary calcium excretion was exerted by continuous and intermittent alendronate administration modes over the whole experimental period.

Tumor-Implanted Rats

Intratibial implantation of WCS led to the development of a tumor at the site of injection in all animals. The tumor became observable and palpable on day 5–6 after tumor injection and continued to grow throughout the experimental period. Tumor growth was associated with an up to 20% decrease of the rats’ body weight in comparison to the “negative” control group.

After alendronate administration over a 5-day treatment period to the tumor-implanted rats, significant differences in tissue disposition were found between the two studied modes of administration. Intermittent bolus administration resulted in higher disposition of alendronate in the femur and liver as compared with continuous input of the drug from the subcutaneously implanted osmotic pump (see Table 2). There was no significant difference in alendronate disposition in other tissues (including urine, tibia, kidney, and spleen; see Table 2)

As can be seen in Figure 3, tumor growth was not associated with an increase in plasma calcium levels during days 0–7 after tumor inoculation. At the end of the experiment (day 10), the WCS tumor caused a significant increase in plasma calcium levels (“positive” versus “negative” control, p < 0.01). Both continuous and intermittent alendronate treatment modes prevented WCS-induced hypercalcemia to a certain extent. There was no significant difference between plasma calcium levels of animals receiving the continuous and intermittent drug input.

There were no significant differences in urine calcium excretion between the experimental groups in each sample throughout the experimental period. The overall urinary calcium excretion during days 0–10 also did not differ significantly between the experimental groups (see Fig. 4).

DISCUSSION

Preclinical investigations provide a means to assess clinically relevant questions that are difficult to elucidate in human studies because of the complexities associated with the significant inter-subject variability as well as ethical and safety issues. A limited number of preclinical models can be used to study the influence of the mode of alendronate administration on tissue disposition and drug effects. Even fewer models are suitable to study the question of whether bisphosphonate

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**Table 2.** Disposition of Alendronate in Walker Carcinosarcoma-Implanted Rats After Continuous (Subcutaneous Alzet Pump on Days 2–7) or Intermittent (Subcutaneous Injections on Days 2, 4, and 6) Administration, a Total Dose of 0.75 mg/kg (% of Administered Dose ± SD)

<table>
<thead>
<tr>
<th>Administration</th>
<th>Urine</th>
<th>Tibia</th>
<th>Femur</th>
<th>Kidney</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>11.5 ± 1.5</td>
<td>0.45 ± 0.28</td>
<td>1.46 ± 0.24a</td>
<td>0.093 ± 0.014</td>
<td>0.149 ± 0.032b</td>
<td>0.013 ± 0.005</td>
</tr>
<tr>
<td>Intermittent</td>
<td>14.7 ± 4.7</td>
<td>0.61 ± 0.13</td>
<td>2.12 ± 0.26</td>
<td>0.136 ± 0.034</td>
<td>0.271 ± 0.046</td>
<td>0.021 ± 0.008</td>
</tr>
</tbody>
</table>

aSignificant difference (p < 0.05).
bSignificant difference (p < 0.01).
treatment should be given continuously or as an intermittent dose, because it should be sensitive enough to detect the difference in the magnitude of the pharmacological effect between the studied modes of administration.

To investigate the influence of mode of administration of alendronate on the magnitude of the pharmacological effect, we used two preclinical metabolic bone-disease models that are characterized by accelerated bone resorption that leads to hypercalcemia and augmented urinary calcium excretion.\textsuperscript{20–22} The enhanced bone resorption is achieved in the first model by vitamin D administration to the rats that had undergone TPTX, and in the second model by intra-tibial implantation of WCS. Bisphosphonate treatment decreases bone resorption and thus the magnitude of its effect may be quantified by comparing the plasma calcium levels and urine calcium excretion between the treatment groups and those of positive and negative control groups.\textsuperscript{23}

These two preclinical models allowed us to accentuate differences in drug administration. For both Walker carcinosarcoma and TPTX rats, intermittent administration consisted of three bolus injections, whereas continuous administration produced a constant input of alendronate during the whole treatment period. The length of the treatment period was 5 days and 14 days for Walker carcinosarcoma-implanted rats and TPTX rats, respectively, and it was chosen based on the time course of disease for each experimental condition. The dosage of alendronate for TPTX rats (0.1 mg/kg) and Walker carcinosarcoma-implanted rats (0.75 mg/kg) was selected based on preliminary experiments in which bone resorption was adequately inhibited. The selected doses lie in the linear portion of the alendronate dose–response curve.\textsuperscript{24,25} At smaller doses, differences in pharmacological effect for intermittent and continuous modes of alendronate administration are difficult to determine. However, higher doses are associated with saturation of dose–response curve (i.e., the maximum effect is observed irrespective of different amounts of alendronate that reached the site of action after intermittent and continuous modes of administration)\textsuperscript{26} and also are unsuitable for purposes of our study.

In the two preclinical models used in this work, relatively young rats had to be used because of both technical obstacles and to improve sensitivity of drug-action measurements. However, it should be noted that the bone-remodeling process in young rats is substantially different from that of older animals and humans, and is characterized by a high extent of bone formation compared with bone resorption. Thus, care should be taken in extrapolating results of this study to clinical settings.

**Tissue Disposition**

Alendronate’s bone and soft tissue disposition was found to be mode-of-administration-dependent. Greater alendronate amounts were found in bone in both tumor-implanted and TPTX rats after intermittent administration rather than in continuous infusion of the drug (Tables 1 and 2). Consequently, the ratio of bone/urine amounts of alendronate (the two major pathways of bisphosphonate elimination) was affected by the mode of administration, indicating the differences in the relative bone disposition of alendronate. The ratio of tibia/urine alendronate amounts for continuous and intermittent administration was 0.075 and 0.091, respectively, for TPTX rats, and 0.039 and 0.042, respectively, for tumor-implanted rats. This observation is consistent with outcomes of our previous PK studies in rats in which prolonged input of alendronate and pamidronate to the central circulation after per os (PO) administration resulted in lower bone disposition of the drug (normalized to AUC) compared with IV bolus administration. In that study, the ratio of tibia/
urine alendronate amounts was 0.084 and 0.268 for PO and IV administration, respectively, indicating higher relative bone disposition of alendronate after IV bolus administration. Similar results were found in our previous investigation of pamidronate in which the ratio of tibia/urine amounts after PO and IV bolus administration of pamidronate was 0.055 and 0.140, respectively, suggesting that mode of administration-dependency of bone disposition is a common feature for bisphosphonates.

Intermittent administration was associated with higher alendronate amounts found in liver in both tumor-implanted and TPTX rats (Tables 1 and 2). This finding is consistent with the results of our previous investigation that showed augmented bisphosphate disposition in reticuloendothelial tissue (liver and spleen) and kidney after IV bolus administration compared with slow drug input as PO administration or 4-h IV infusion. Bisphosphate that is disposed in these organs is gradually released over a period of several days to weeks. Thus, the significant differences in alendronate disposition that were observed in the liver in the present study, but not in the kidney or spleen, should be attributed to the longer duration of treatment period compared with the previous study (5 and 14 days versus 24 h).

**Drug Effects**

The PK findings show that higher amounts of alendronate were found in bone tissue in both tumor-implanted and TPTX rats after intermittent bolus administration as compared with the continuous drug input. Despite the fact that bone tissue is the site of the alendronate anti-resorptive effect, differences in drug bone disposition between the intermittent and continuous regimens were not associated with significant differences in the extent of drug effect. Alendronate effects on calcium homeostasis were similar for intermittent and continuous regimens in both models of bone disease.

This could be explained by the phenomenon that not all of the alendronate that is in bone tissue is prone to interaction with osteoclasts and to exerting its therapeutic effect at a given time point. Experimental findings indicate that most of the bisphosphonate that is in bone is enclosed inside the tissue and has no influence on osteoclast activity. Bisphosphonate may remain in the bone for exceptionally long periods of time that may reach months/years in humans, and it may become pharmacologically active if the remodeling process releases the drug from its disposition site.

Another explanation for the lack of difference in drug effect could be the unknown concentration-versus-time profile of bisphosphonates at the site of their action. Bisphosphonate concentration near or within the osteoclasts, that compose the final target of bisphosphonate action, cannot be measured directly in clinical studies, and may be estimated only indirectly in preclinical settings. Consequently, the concentration–response relationship of bisphosphonates at the site of their action is unknown. Results of dose-versus-response studies propose saturation of bisphophonate effects at high concentrations, but that is seen at higher doses than those applied in our investigation.

**CONCLUSIONS**

Preclinical rat models of bone disease enabled the investigation of contrasting treatment regimens of continuous input versus intermittent bolus alendronate administration over 5 and 14 days for Walker carcinosarcoma-implanted rats and TPTX rats, respectively. Because the rat bone remodeling process is more rapid, the results of this study may be generally extrapolated to treatment regimens of several weeks to months in humans.

The results of this investigation indicate that continuous alendronate administration exerts a similar effect on calcium homeostasis as compared with an intermittent treatment regimen. These findings are in accordance with the results of recent clinical studies that demonstrated that a once-weekly dosing regimen of alendronate is therapeutically equivalent to daily dosing, but is more clinically convenient and may be associated with a lower incidence of gastrointestinal adverse effects. Thus, it seems that continuous and intermittent bisphosphonate treatment regimens generally produce similar magnitude of drug effect and the preferred mode of administration should be selected according to secondary clinical criteria (such as incidence of adverse effects and convenience of administration). The clinical relevance of differences in overall bone and soft tissue bisphosphonate disposition that were observed between the continuous and intermittent regimens in this study is unknown.
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