Intranasal Delivery of the Bisphosphonate Alendronate in the Rat and Dog

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INTRODUCTION

Alendronate (4-amino-1-hydroxy-butylidene-1,1-bisphosphonic acid), a bisphosphonate, has been investigated for the management of a broad range of skeletal disorders characterized by increased bone turnover, such as Paget's disease and hypercalcemia of malignancy. Bisphosphonates in general (1) have variable and low (≈1.0%) peroral bioavailability in humans. Intranasal administration was examined as an alternative to the peroral route. This paper reports the nasal bioavailability of alendronate in rats and dogs following its administration as a solution.

METHODS

Chemicals

^{14}C-Alendronate (12.34 μCi/mg) and unlabeled alendronate were received from Merck & Co., Inc. (Rahway, NJ). All other chemicals were of reagent grade.

Animal Experiments

Rats. Fasted male Sprague–Dawley rats (250–300 g) were anesthetized by ether inhalation. Intravenous administration was achieved via an exposed exterior jugular vein. Following administration of the i.v. dose (1 mg/kg alendronate with tracer quantities of ^{14}C-alendronate dissolved in 100 μL water, pH adjusted to 7 with 0.5 *N* NaOH), the skin wound was sutured and the animal was returned to its cage. The nasal dose was administered to anesthetized, supine rats, via a Silastic-tipped adjustable air-displacement pipette (Rainin Instrument Co., Inc., Woburn, MA) in a total pH-adjusted volume of 10 μL. Animals were anesthetized for an additional 2–5 min. It is felt that since the nasal absorption of most components is rapid (T_{max}, <5 min) (2), absorption would be essentially complete before animals regained consciousness. The animals were allowed to recover and returned to their cages. Twenty-four hours following administration, the animals were euthanized with CO_2 and the femur and tibia ^{14}C-alendronate content was determined.

Dogs. Three fasted beagle dogs (15–20 kg) were intravenously administered alendronate (2.5 mg in 1 mL saline, pH ≈7) via the jugular vein and returned to metabolism cages for 72-hr urine collection. Similarly, nasal administration of alendronate (6 mg) to these same dogs was achieved by the direct instillation of 100 μL of a 30 mg/mL dosing solution (saline, pH ≈7) into each nostril.

Analytical

In the rat studies, the tibia and femur were removed from animals 24 hr following ^{14}C-alendronate administration, cleaned of muscle and connective tissue, combusted to $^{14}CO_2$ in a Packard Tricarb sample oxidizer (Model 306, Packard, Downes Grove, IL) equipped with a platinum burning coil, and counted in a liquid scintillation counter (Model LS6800, Beckman Instruments, Irvine, CA) for total ^{14}C content. The background bone ^{14}C content was also determined in four sham-treated (no-^{14}C-alendronate) rats. Since alendronate is not metabolized in the rat (3), the total radioactivity in a specific bone, following administration by nasal or intravenous routes, has been used to calculate alendronate bioavailability (see below and Ref. 3).

Exploratory dog studies were completed with unlabeled material. Total urinary excretion of alendronate following i.v. and nasal administration in the dog was determined by an HPLC assay (4) and used to calculate alendronate bioavailability.

Pharmacokinetic Calculations and Statistical Tests

Bioavailability (F) was individually calculated (5) for each animal by Eq. (1):

$$F = \frac{D_{iv}}{D_{nasal}} \cdot \frac{A_{nasal}}{A_{iv}} \cdot 100 \quad (1)$$

where D_{iv} and D_{nasal} and A_{iv} and A_{nasal} are dose and amounts for i.v. and nasal absorption studies, respectively. For the rat ^{14}C-alendronate studies, F in Eq. (1) was calculated substituting the total femur or tibia ^{14}C counts (tcpm) following i.v. and nasal administration for A_{iv} and A_{nasal}, respectively. The D_{iv} and D_{nasal} values were estimated by counting the amount of ^{14}C-alendronate actually delivered by the respective pipette. Similarly, for the dog alendronate studies, F in Eq. (1) was calculated by using the total milligrams alendronate excreted in the 24-hr urine following i.v. and nasal administration as A_{iv} and A_{nasal}, respectively. Comparisons were made with Student's t test or ANOVA and differences were considered significant at $P < 0.05$ (6). All data are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

^{14}C-Alendronate Assay

Alendronate is not metabolized in the rat or dog, and 24 hr following an oral dose, approximately half of the absorbed drug was excreted in the urine, the remainder being bound to

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bone (3). Therefore, the amount of total radioactivity in a specific bone (e.g., femur or tibia) can be directly related, following separate administration of ^{14}C-alendronate by different routes, to bioavailability. The total ^{14}C counts for an entire femur or tibia (tcpm) were corrected for background radioactivity by the mean data from four sham-treated animals. No corrections were made for counting efficiency or sample size, but similarly sized samples and sample preparation were used throughout. All results reported here reflect tibia data only; femur data were essentially identical.

Nasal Absorption in Rats

Compared to the tcpm following i.v. administration of 1 mg/kg alendronate, the tcpm following nasal administration of a 27 mg/mL (1 mg/kg) solution of alendronate indicated a bioavailability (F) of 22.0 ± 6.2%. The bioavailability of alendronate following the administration of a less concentrated dosing solution (3.3 mg/mL; total dose, 0.11 mg/kg) was significantly ($P < 0.001$) less: 7.3 ± 1.6%. While alendronate uptake in the bone following i.v. administration is linear in the concentration range studied and alendronate is not metabolized (3), the present study suggested that the bioavailability of this compound was dose dependent. This apparent dependence could be due to nonspecific binding and/or generalized effects on the nasal mucosal barrier. Alendronate, like EDTA, can chelate calcium and readily binds to foodstuffs and tissues (7). If, at low doses, much of the alendronate is bound to mucosal tissue, very little may be available for absorption. At higher doses, a larger fraction would be available for absorption and interaction with the nasal mucosal barrier. Alterations in the mucosal cell barrier, as shown for bile acids (8), could alter permeability, resulting in a greater bioavailability.

The possibility that alendronate alters the absorptive mucosa was examined *in vitro* in a side-by-side diffusion cell (9). The permeability of ^{14}C-alendronate across rat colon mucosa, in the presence of 1, 10, or 100 mM unlabeled alendronate (made equiosmotic with mannitol), was determined. As shown in Table I, ^{14}C-alendronate permeability increased 16-fold as unlabeled alendronate concentrations increased from 1 to 100 mM. Also shown in Table I are the corresponding transepithelial electrical resistance (TEER) measurements. The alterations in TEER and drug permeation are similar to that seen with the absorption enhancer palmitoylcarnitine chloride (PCC), which is thought to exert its effects at the tight junction. Alendronate decreased TEER as the permeability of a poorly absorbed compound (in this case, ^{14}C-alendronate) increased. Upon the removal of alendronate, a partial recovery of the TEER was observed (data not shown), suggesting that the effect is at least partially reversible. Although the design of this experiment did not allow a determination of mechanism, the data are consistent with the hypothesis that alendronate [like EDTA (10)] loosens tight junctions between the absorptive cells. The data are also consistent with a study by vanHoogdalem *et al.* (11), where a structurally similar bisphosphonate was shown to enhance rectal cefoxitin absorption in rats.

Table I. Concentration Dependence of Alendronate *in Vitro* Transport Across Rat Stripped Colon Mucosa

Alendronate (mM)[a]	Permeability (cm/min) × 10^6	TEER ratio (%)[b]
1	8.3 ± 20	78.8 ± 19.1[c]
10	38 ± 12	82.2 ± 12.2
100	130 ± 58	41.7 ± 22.7

[a] Concentration of unlabeled alendronate in the donor compartment. Solutions were made approximately equiosomolar with mannitol.

[b] An estimate of the TEER lowering effect of the treatment relative to the baseline TEER: ($TEER_{30\ min}/TEER_{0\ min}$) · 100.

[c] Mean ± SD; n = 3–6.

Alendronate Absorption in the Dog

The nasal bioavailability of a 200-μL (100 μL per nostril) concentrated (30 μg/mL) saline solution of alendronate in the dog was 2.87 ± 0.61%. No overt signs of discomfort were observed in dogs nasally administered alendronate. Nasal absorption of alendronate in the dog was greater than peroral absorption (3), but the differences compared to the rat model were remarkable. Alendronate nasal bioavailability in the rat (using the 30 mg/mL solution) was ≈8 times greater than in the dog (30 mg/mL solution). Possible explanations for these findings include (1) incomplete nasal absorption in the dog, due to post nasal drainage; (2) enhanced nasal absorption in the rat, following exposure of the nasal mucosa to ether anesthesia; and (3) incomplete absorption of the nasally administered dose in dogs due to nonspecific binding with the comparatively larger surface area in the dog's nasal cavity. It is unlikely that a fraction of the dose administered to dogs drained from the nose into the throat since, in other experiments (data not shown) using propranolol as a model nasally absorbed compound, it was shown that 100 μL of dosing solution per nostril resulted in the complete bioavailability of propranolol. While ether was employed as the anesthesia in the rat model when both a low and a high dose of alendronate was administered, F was apparently enhanced only at the higher dose. Therefore, while ether anesthesia may induce some nasal membrane changes, the contribution of these changes is difficult to assess in light of the apparent dose-dependent F. Although nasal mucociliary clearance can play a significant role in limiting the residence time of drug in contact with absorptive epithelium (12), these forces are probably minimal for strongly bound compounds. If the relatively low bioavailability of alendronate following nasal administration in dogs was due to nonspecific binding of the administered dose to nasal mucosa, improved availability may be possible by increasing the nasal dose or by some other formulation modification.

In summary, the nasal route of alendronate administration appears to be about 25 times as effective as the peroral route [$F \simeq 0.9\%$ (3)] in rats. Although the rat has been shown to be an excellent predictor for optimum nasal bioavailability in humans (13), it remains to be proven whether nasal alendronate bioavailability could be substantially improved with the proper formulation in the dog (and possibly human). With a substantial improvement of bioavailability, together with the long half-life of alendronate (≥1 year), nasal administration could be a weekly or even monthly alternative to daily peroral administration.

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