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RESEARCH ARTICLE

A simple pharmacokinetic model of alendronate developed using plasma concentration and urine excretion data from healthy men

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Abstract

The study of pharmacokinetics of alendronate has been hampered by difficulties in accurately and reproducibly determining their concentrations in serum and urine. Thus, pharmacokinetic characteristics of alendronate have been described in many reports based on urinary excretion data; and plasma pharmacokinetics and the simultaneous pharmacokinetic models of alendronate in plasma and urine are not available. The aims of this study were to measure alendronate concentration in plasma and excretion in urine concurrently and to develop compartmental pharmacokinetic model using urine data. In open-label, single-dose pharmacokinetic study, 10 healthy male volunteers received oral dose of alendronate (70 mg tablet). Blood and urine alendronate concentrations were determined using validated high-performance liquid chromatography method. Non-compartmental analysis was performed using WinNonlin program (Pharsight Inc., Apex, NC). A one-compartment pharmacokinetic model was applied to describe pharmacokinetics of alendronate. A peak plasma alendronate concentration of 33.10 ± 14.32 ng/mL was attained after 1.00 ± 0.16 h. The cumulative amount of alendronate excreted in urine and peak excretion rate were 731.28 ± 654.57 μg and 314.68 ± 395.43 μg/h, respectively. The model, which included first-order absorption rate for oral dosing, showed good fit to alendronate data obtained from plasma and urine. The absorption rate constant was $2.68 \pm 0.95\ h^{-1}$. The elimination rate constants K_{urine} and $K_{non\text{-}ur}$ were $0.005 \pm 0.004\ h^{-1}$ and $0.42 \pm 0.08\ h^{-1}$, respectively. The pharmacokinetics of alendronate in plasma and urine of healthy men can be predicted using one-compartment model, and thus the behavior of drug in plasma can be estimated from urinary excretion data.

Keywords

Alendronate, modeling, men, pharmacokinetics, plasma, urine

History

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Introduction

The rates of fracture-related morbidity and mortality are consistently higher in men than in women. Thus, the rates of osteoporosis and fragility fractures are higher than previously thought in men[1]. In the history of osteoporosis treatment, the use of bisphosphonates has been considered as a milestone[2]. Alendronate (CAS 121268-17-5, 4-amino-1-hydroxybutylidine-1,1-bisphosphonic acid) is the first amino bisphosphonate to reach the market for the treatment of osteoporosis[3,4]. It was first used clinically to treat Paget's disease[3,4]. Alendronate administration is suggested primarily for the management of idiopathic, glucocorticoid-induced osteoporosis in men[1]. The pharmacokinetics of alendronate in the fasted state in humans are unique in which the drug shows very low and highly variable oral bioavailability when measured in plasma[4–7]. The oral bioavailability of alendronate in the fasted state is about 0.7% and is not significantly different between men and women. Absorption and disposition appear to be independent of dose[4,7].

The pharmacokinetics of alendronate in humans have been characterized only to a limited extent, because it is difficult to measure this compound in biological fluids, and its disposition characteristics make it difficult to examine its pharmacokinetic behavior in plasma[4–6]. The concentrations of the drug in plasma following therapeutic doses generally fall below the limit of sensitivity of the assay[4,5]. Although sensitive method for quantification of alendronate has been developed with fluorescence detection (FD), the concentrations in plasma following oral administration do not rise sufficiently to allow an examination of plasma kinetics with therapeutically relevant doses (10 mg daily), even after 3 years of daily administration[4–6]. Consequently, the pharmacokinetic characteristics of alendronate in humans have been derived mostly from urinary excretion data[8,9]. For the direct determination of bisphosphonate in human plasma[5,6], chromatographic methods have been developed for the accurate determination of alendronate in plasma; only then it becomes feasible to establish plasma pharmacokinetics and simultaneous pharmacokinetic models of alendronate in plasma and urine.

In this study, we examined the correlation between the concurrently measured plasma concentration of alendronate and cumulative urinary excretion and developed a pharmacokinetic model that includes the urine compartment. Monte Carlo simulation (5th and 95th percentiles of prediction interval) was

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used to evaluate the quality of the final model[10]. This model should enable prediction of the time course of changes in alendronate plasma concentration using cumulative urinary excretion data in men.

Methods

Chemicals

Tablets containing 70 mg alendronate sodium were obtained commercially (Fosamax®; Merck Sharp & Dohme, Whitehouse Station, NJ). Alendronate sodium and pamidronate disodium (Panorin®, internal standard) were obtained from a local pharmaceutical company (Hanlim Pharm. Co., Ltd., Seoul, Korea). Methanol [high-performance liquid chromatography (HPLC) grade] and potassium dihydrogen phosphate (analytical grade) were purchased from Merck (Darmstadt, Germany). Acetonitrile (HPLC grade) was obtained from Duksan (Seoul, Korea). 9-Fluorenylmethyl chloroformate (Purris p.a.) and other chemicals were Aldrich products (Seoul, Korea).

Apparatus

HPLC analysis was performed using a Shimadzu Class-VP system (Shimadzu Scientific Instruments, Inc., Columbia, MD), which comprised a SIL-10ADvp autosampler, an LC-10ADvp pump, a DGE-14 A degasser, a SCL-10AVP controller and a RF-10AXL fluorescence detector. Diethyl amino solid-phase extraction (SPE) cartridges were purchased from Varian (Bond Elut-DEA, 100 mg/mL; Seoul, Korea). Liquid chromatography was performed on a Capcell Pak C18 stationary-phase column (150 × 4.6 mm i.d., 5 μm particles; Shiseido Co., Ltd., Tokyo, Japan).

Subjects and study design

The study was performed in accordance with the ethical standard formulated in the Helsinki Declaration of 1964, and the protocol was approved by the local institutional review board. All subjects provided written informed consent prior to study participation. Ten male Korean volunteers were recruited. The subjects were all apparently healthy, non-smoking adults within 85–115% of ideal body weight and with no medical abnormality. All subjects underwent a pre-study screening evaluation that included a medical history, physical examination, clinical chemistry, hematology, urinalysis, drug screening and vital signs. The examination results are illustrated in Table 1. The creatinine clearance was calculated with the Cockroft–Gault formula[11]. Subjects were confined to the study site overnight prior to dosing and for 12 h after dosing, and they were not allowed to consume alcohol or caffeine or take any medication during the study period. Informed written consent was obtained from all volunteers before participation in the study.

The volunteers fasted overnight prior to and for 4 h after administration of a tablet containing 70 mg alendronate. The tablet was administered with 240 mL water, and additional water was given 2 h after administration. No other beverage, including coffee, milk or diet drink, was permitted during the study period. Following the 4-h period, the subjects consumed a specified controlled diet. Blood samples were collected into vacuum tubes containing heparin sodium prior to and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6 and 7 h after administration. Plasma was separated by centrifugation and stored at −70 °C for analysis. Urine was collected into plastic tubes in eight fractions (0–0.25, 0.25–1, 1–2, 2–3.5, 3.5–5, 5–7, 7–9 and 9–12 h). At the end of each collection period, the total volume of urine was recorded, and aliquots were frozen at −70 °C for analysis[5–9].

Table 1. Characteristics of 10 Korean healthy men included in the pharmacokinetic analysis.

Characteristics (unit)	Mean ± SD	Range
Age (years)	23 ± 1.92	19–25
Weight (kg)	67.10 ± 7.51	58–79
Body Mass Index (kg/m^2)	21.81 ± 2.03	19.44–24.93
Creatinine (mg/dL)	1.04 ± 0.05	1–1.22
Blood urea nitrogen (mg/dL)	12.47 ± 2.61	11–18
Total cholesterol (mg/dL)	175.02 ± 19.3	142–195
Alkaline phosphatase (IU/L)	63.89 ± 16.51	48–87
Alanine transaminase (IU/L)	24.37 ± 5.41	14–30
Aspartate transaminase (IU/L)	28.16 ± 3.92	21–33
Total bilirubin (mg/dL)	0.97 ± 0.31	0.52–1.31
Albumin (g/dL)	4.83 ± 0.28	4.51–5.32
Calculated creatinine clearance (mL/min)	148.42 ± 22.13	126.35–170.52

Analytical methods

Analysis of alendronate concentrations in plasma and urine was performed by HPLC-FD, as described previously[5,6,8]. In previous studies, the plasma and urine samples also belonged to male volunteers. All samples for HPLC analysis were spiked with an internal standard (pamidronate disodium). Sample preparation for HPLC-FD determination of alendronate concentrations in plasma and urine involved manual coprecipitation of the bisphosphonate with calcium phosphate, followed by automated SPE on anion-exchange columns and derivatization with 9-fluorenylmethyl chloroformate in sodium carbonate buffer (pH 11.9). The mobile phase was a series of steps in a gradient comprising a mixed organic solution (solvent A: acetonitrile:methanol, 1:1) and buffer (solvent B: 10 mM citric acid and 10 mM sodium pyrophosphate tetrabasic, adjusted to pH 4.8). The total run time was 22 min, with a flow rate of 1.5 mL/min. FD was performed at 260 nm for excitation and 310 nm for emission. The calibration curves of alendronate concentrations in plasma ($r^2 = 0.99$) and urine ($r^2 = 0.99$) were similar to the results of Yun et al.[5] and Kang et al.[8]. In this study, the lower limits of alendronate quantification were 2 ng/mL in plasma and 5 ng/mL in urine.

Calculation of pharmacokinetic parameters and statistical analysis

Compartmental and non-compartmental pharmacokinetic parameters were calculated from the plasma concentration and accumulative urine level. Non-compartmental analysis to determine plasma and urine pharmacokinetic parameters was performed using the WinNonlin modeling and analysis software (version 2.1 a; Pharsight Inc., Apex, NC). The maximum concentration of alendronate in plasma (C_{max}) and the time to reach C_{max} (T_{max}) were calculated from the mean plasma concentration versus time profile. The area under the plasma concentration time curve up to the last measurable concentration ($AUC_{0-7\,h}$) and the area under the plasma concentration curve extrapolated to infinity (AUC_{inf}) were calculated using conventional trapezoidal summation and extrapolation. The elimination half-life ($t_{1/2}$), and apparent volume of distribution (V/F) in plasma were calculated. The amount of unchanged drug excreted into the urine (A_{et}) and maximum excretion rate into the urine (U_{max}) were calculated from the urine accumulation of alendronate versus time profile.

The experimental plasma concentration and urine accumulation versus time data were best described by a one-compartment open model with first-order absorption input and elimination from the central compartment using the ADAPT II computer program (Biomedical Simulation Resource, Los Angeles, CA) based on Akaike's information criterion and rule of parsimony[10]. In addition, natural log of plasma drug concentration versus

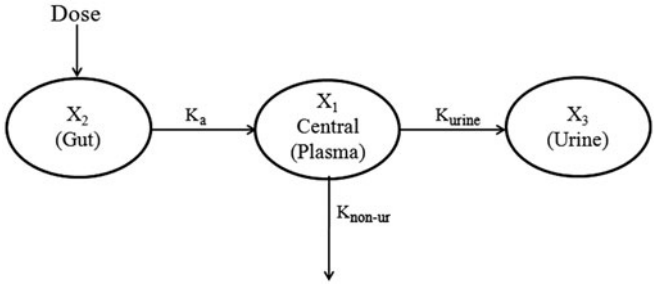

Figure 1. Compartmental model for the plasma concentration and urinary excretion of alendronate in healthy volunteers after oral administration of a single 70-mg dose of alendronate. X_1: central compartment; X_2: gut compartment; and X_3: urine accumulation compartment.

time was also straight line[12]. The compartmental model used in this study is shown in Figure 1. Fitting was performed by maximum likelihood estimation under the assumption that the SD of the measurement error was a linear function of the measured quantity. The differential equations used are shown below:

$$\frac{dx_1(t)}{dt} = -(K_{urine} + K_{non\text{-}ur}) \cdot x_1(t) + K_a \cdot x_2(t) \quad (1)$$

$$\frac{dx_2(t)}{dt} = -K_a \cdot x_2(t) \quad (2)$$

$$\frac{dx_3(t)}{dt} = K_{urine} \cdot x_1(t) \quad (3)$$

where x_1 represents central compartment, x_2 represents gut compartment and x_3 represents urinary accumulation compartment.

The following pharmacokinetic parameters were estimated using the one-compartment model: volume of distribution in the central compartment (V_c/F), elimination rate constants of non-renal ($K_{non\text{-}ur}$) and renal (K_{urine}) excretion and the apparent first-order absorption rate constant (K_a).

All data of the non-compartmental and compartmental analyses of alendronate concentrations in plasma and urine are expressed as means ± SDs.

Results

All 10 male volunteers completed the study. No severe adverse effect resulting in withdrawal from the study occurred. Minor adverse events included general muscle pain and fatigue ($n = 4$), chest tightness ($n = 4$), drug-related fever ($n = 1$) and headache ($n = 1$). The time course of changes in alendronate plasma concentration and urine accumulation after a single oral dose of 70 mg alendronate were evaluated (Figure 2). The solid line represents the best fit of the pharmacokinetic model to the measured concentrations based on the maximum likelihood criterion and visual inspection of the fit using ADAPT II. The estimated compartmental and non-compartmental pharmacokinetic parameters are summarized in Table 2.

Non-compartmental plasma pharmacokinetics and urinary excretion were analyzed using the WinNonlin program. A C_{max} of 33.10 ± 14.32 ng/mL was attained after 1.00 ± 0.16 h (T_{max}). The AUC_{0-7h} and AUC_{inf} values were 88.36 ± 31.58 ng h/mL and 103.58 ± 31.58 ng·h/mL, respectively. The apparent volume of the central compartment (V/F) was 2472.18 ± 790.43 L. A_{et} and U_{max} were 731.28 ± 654.57 µg and 314.68 ± 395.43 µg/h, respectively, after 1.30 ± 0.38 h (T_{max}).

Compartmental pharmacokinetic parameters were calculated using the ADAPT II program. A one-compartment open model containing the urine compartment was used and showed a good fit to the data. K_{urine} and $K_{non\text{-}ur}$ were 0.005 ± 0.004 h^{-1} and 0.42 ± 0.08 h^{-1}, respectively. The V_c/F and K_a were 1821.86 ± 471.38 L and 2.68 ± 0.95 h^{-1}. To evaluate the quality of the final model, Monte Carlo simulation was performed. Time courses of the concentrations, accumulations in excreted urine and 90%

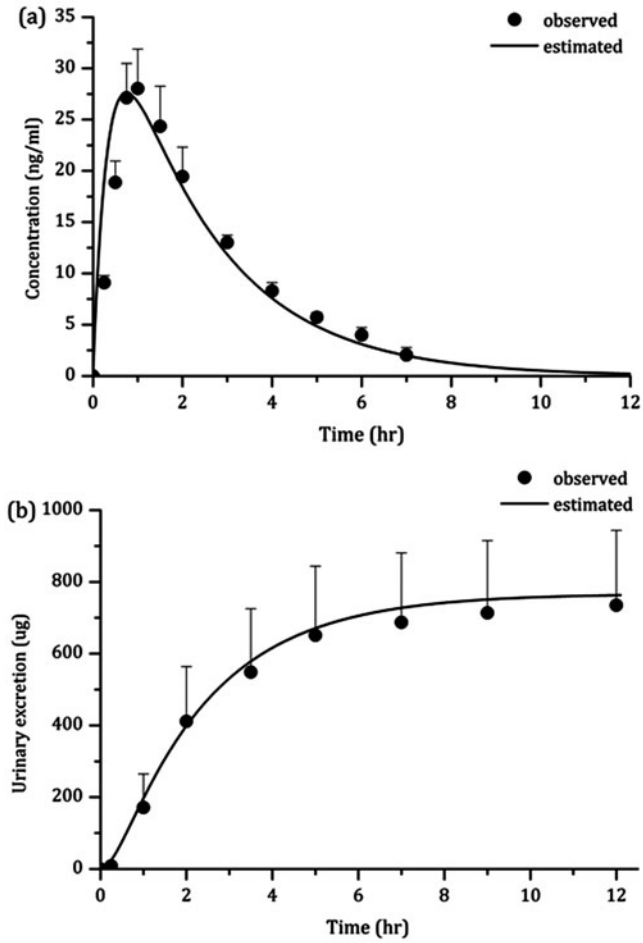

Figure 2. Time courses of changes in plasma concentration and cumulative urinary excretion in healthy subjects after a single oral administration of 70 mg of alendronate (mean ± SD, $n = 10$). Data points (•) are observed values, and the solid line shows the result of maximum likelihood fitting with the ADAPT II program.

Table 2. Non-compartmental and compartmental pharmacokinetic parameters of alendronate (Fosamax®) following administration of a single dose of 70 mg.

Parameter (unit)	Estimated value (mean ± SD)
Non-compartmental analysis in plasma	
$AUC_{0-7\,h}$ (ng·h/mL)	88.36 ± 31.53
AUC_{inf} (ng·h/mL)	103.58 ± 31.58
T_{max} (h)	1.00 ± 0.16
C_{max} (ng/mL)	33.10 ± 14.32
V/F (L)	2472.18 ± 790.43
Non-compartmental analysis in urine	
$A_{et0-12\,h}$ (µg)	731.28 ± 654.57
A_{etinf} (µg)	912.07 ± 981.52
T_{max} (h)	1.30 ± 0.38
U_{max} (µg/h)	314.68 ± 395.43
Compartmental analysis in plasma and urine	
K_{urine} (h^{-1})	0.005 ± 0.004
$K_{non\text{-}ur}$ (h^{-1})	0.42 ± 0.08
V_c/F (L)	1821.86 ± 471.38
K_a (h^{-1})	2.68 ± 0.95

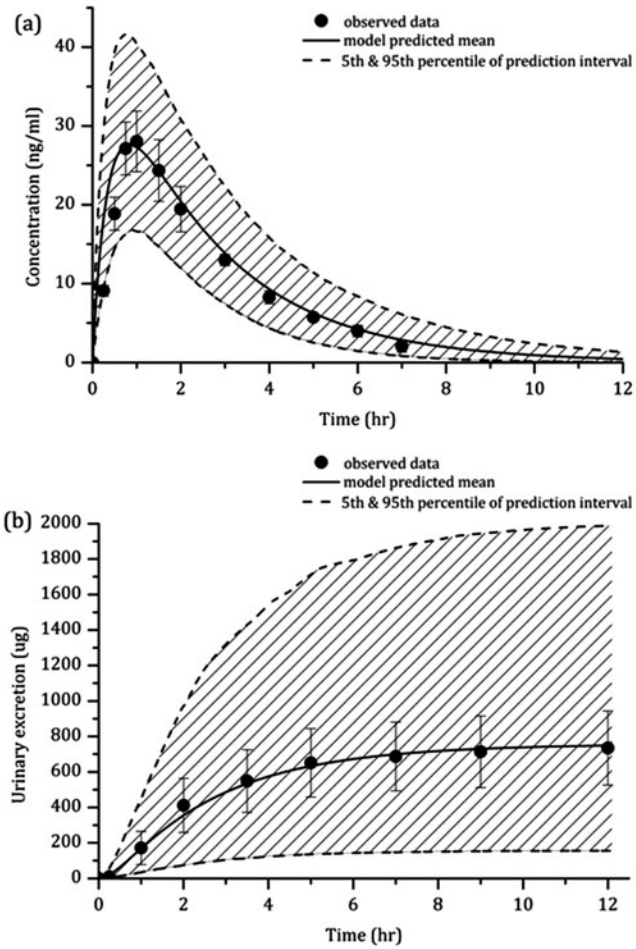

Figure 3. Model-predicted plasma concentration and cumulative urinary excretion versus time profiles of a single oral administration of 70 mg of alendronate from Monte Carlo simulation (mean ± SD, $n = 1000$). The solid line shows the model predicted means, and the dashed lines indicate the 90% population prediction limits.

population prediction limits of alendronate following 70 mg oral administration predicted by Monte Carlo simulation are shown in Figure 3. The model-predicted means of alendronate concentration and accumulation in excreted urine described the experimental data well between the 5th and 95th percentiles of the prediction interval.

Discussion

Olszynski and Davison[1] suggested that 70 mg alendronate administration once weekly is very safe and tolerable, with an exceedingly low adverse-event profile, in men. Thus, this study employed an oral administration of 70 mg alendronate in male volunteers. The pharmacokinetic behavior of alendronate was well defined by the final model, and the non-compartmental and compartmental parameters obtained were comparable to published data[5,6,8,9]. There was broad standard deviation in parameters of non compartmental analysis of plasma and urine, which were comparable to previous study[5,8,9]. This suggests that there may be presence of interindividual variability through plasma and urine analysis. Future studies will be conducted to find the influence of covariates in interindividual variability using population approach of pharmacokinetics. The amount of alendronate absorption measured through plasma was very poor in the present and previous studies[5,6]. This poor absorption probably results from the drug's polarity, as it is negatively charged at physiological pH[2,13,14]. The pharmacokinetic effects of alendronate are quite similar in men and women, and the oral bioavailability is about 0.7%[1]. In this study, the oral bioavailability could not be determined because this value is estimated by comparing the plasma concentrations of the drug after oral and intravenous administration. Future studies should be conducted to examine different routes of drug administration.

In this study, the average amount of alendronate excreted in the urine during the 12 h after oral administration was about 1.04% of the dose administered. This value is twice that observed in a previous study, which reported that the average 24-h urinary excretion of alendronate was 0.5–0.6% of the dose administered in patients with Crohn's disease[13]. The apparent wide variation in urinary excretion may be due to differences between patients with Crohn's disease and healthy male subjects.

This study used a simplified version of the alendronate pharmacokinetic model, a one-compartment open model. DiPiro et al.[12] suggests that multicompartment models are only applied when the natural log of plasma drug concentration versus time curve is not straight line. Furthermore, in this study, natural log of plasma drug concentration versus time was a straight line. However, multicompartmental models have been preferred for the evaluation of drugs in subjects with impaired renal function[15,16]. In addition, most elderly patients suffering from osteoporosis have renal dysfunction[17]. Thus, further modeling of alendronate should be performed using different compartmental model in elderly individuals. Because the model used a hypothetical value for K_a reflecting an apparent absorption rate, this value appears to be very large compared with that used in a previous study[13]. Moreover, the compartmental parameters such as the apparent volume of distribution and absorption rate constant were similar to those obtained in a non-compartmental analysis. In this study, the $K_{non\text{-}ur}$ was about 100-fold the rate of urinary elimination, indicating that the drug may be cleared quickly by rapid uptake into the avid bone (non-renal clearance) and retained in the skeleton[18].

Limitations

This study had some limitations because it included few healthy adult Korean volunteers, used single oral-dose administration and applied an older version of ADAPT. A large-scale study including different sexes, ethnicities, routes of administration and pharmacokinetic model analysis (NONMEM (ICON Development Solutions, Ellicott city, MD) and ADAPT V) needs to be carried out in the future. Pharmacokinetic of plasma and urine of alendronate was estimated using one-compartment analysis. However, this concentration of plasma and the amounts of urine of alendronate does not directly indicate the response or behavior of drug in plasma. So, further study was needed concerned the pharmacokinetics/pharmacodynamics modeling to predict the therapeutic or response of drug.

Conclusion

The pharmacokinetics of alendronate have been characterized based exclusively on plasma concentrations and urinary excretion data. We successfully measured alendronate in the plasma and urine of healthy men and developed a one-compartment open model that included the urinary excretion compartment. This simple model could be reference for predicting the plasma concentration of orally administered alendronate based on urinary excretion data in patients having osteoporosis in future.

Declaration of interest

The authors report no declarations of interest.

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