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Bioequivalence and Pharmacokinetics of 70 mg Alendronate Sodium Tablets by Measuring Alendronate in Plasma

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The bioequivalence and pharmacokinetics of alendronate sodium tablets were examined by determining the plasma concentration of alendronate. Two groups, consisting of 24 healthy volunteers, each received a 70 mg reference alendronate sodium tablet and a test tablet in a 2×2 crossover study. There was a 6-day washout period between doses. The plasma alendronate concentration was monitored for 7 h after the dose, using HPLC-Fluorescence Detector (FD). The area under the plasma concentration-time curve from time 0 to the last sampling time at 7 h (AUC_{0-7h}) was calculated using the linear-log trapezoidal rule. The maximum plasma drug concentration (C_{max}) and the time to reach C_{max} (T_{max}) were derived from the plasma concentration–time data. Analysis of variance was performed using logarithmically transformed AUC_{0-7h} and C_{max}, and untransformed T_{max}. For the test medication versus the reference medication, the AUC_{0-7h} were 87.63 ± 29.27 vs. 102.44 ± 69.96 ng·h·mL^{-1} and the C_{max} values were 34.29 ± 13.77 vs. 38.47 ± 24.39 ng·mL^{-1}, respectively. The 90% confidence intervals of the mean differences of the logarithmic transformed AUC_{0-7h} and C_{max} values were log 0.8234–log 1.1597 and log 0.8222–log 1.1409, respectively, satisfying the bioequivalence criteria guidelines of both the US Food and Drug Administration and the Korea Food and Drug Administration. The other pharmacokinetic parameters for the test drug versus reference drug, respectively, were: $t_{1/2}$, 1.87 ± 0.62 vs. 1.77 ± 0.54 h; V/F, 2061.30 ± 986.49 vs. 2576.45 ± 1826.05 L; CL/F, 835.32 ± 357.35 vs. 889.48 ± 485.87 L·h^{-1}; K_{el}, 0.42 ± 0.14 vs. 0.40 ± 0.18 h^{-1}; K_a, 4.46 ± 3.63 vs. 3.80 ± 3.64 h^{-1}; and T_{lag}, 0.19 ± 0.09 vs. 0.18 ± 0.06 h. These results indicated that two alendronate formulations(70-mg alendronate sodium) were biologically equivalent and can be prescribed interchangeably.

Key words: Alendronate, Human plasma, Bioequivalence, HPLC-FD, Pharmacokinetics

INTRODUCTION

Alendronate [(4-amino-1-hydroxybutylidene)bisphosphonate] is currently the most effective inhibitor of bone resorption, and was first used clinically to treat Paget's disease (Rodan *et al.*, 2000); it is the drug of choice in treating hypercalcemia of malignancy (Rodan *et al.*, 2000) and postmenopausal osteoporosis (Liberman *et al.*, 1995). Alendronate is also being evaluated for the treatment of inflammation-related bone loss and fibrous dysplasia (Lane *et al.*, 2001), as well as other disorders of the musculoskeletal system such as osteogenesis imperfecta (Astrom *et al.*, 1998). As the key pharmacological action of alendronate is the inhibition of osteoclastic bone resorption, some studies have investigated the effects of alendronate on osteoclasts, and this mechanism of action has been well described (Schmidt *et al.*, 1996; Sato *et al.*, 1991). Alendronate binds avidly to exposed bone mineral and is released in the acidic clear zone created by osteoclasts (Sato *et al.*, 1991).

The bioequivalence and pharmacokinetic data for alendronate are limited because of the difficulty in measuring plasma concentrations. The plasma concentration of alendronate cannot be measured by LC-MS/MS due to its molecular specificity (Lin, 1996). We recently developed an analytical method for the simultaneous determination of alendronate in human plasma (Yun *et al.*, 2005). The purpose of this study was to determine the pharmacokinetic parameters of two brands of alendronate sodium 70 mg tablets using our method (Yun *et al.*, 2005) and then to compare these parameters statistically in order to evaluate the bioequivalence between the two medications. Typical

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bioavailability parameters for alendronate sodium, including the area under the plasma concentration–time curve from time 0 to the last sampling time at 7 h (AUC_{0-7h}) and the maximum plasma concentration (C_{max}) were compared for the bioequivalence evaluation.

MATERIALS AND METHODS

Test and reference medications

The test medication, Alenmax 70 mg® (70 mg alendronate sodium, batch no. PAlen 70-1; Hanmi Pharm. Co., Ltd., Seoul, Korea), and the reference medication, Fosamax 70 mg® (70 mg alendronate sodium, batch no. F1037; Merck & Co., Inc., Whitehouse Station, NJ, U.S.A.), were supplied in 70 mg tablet form.

Experimental design

The bioequivalence study of alendronate involved 24 healthy volunteers ranging in age from 19 to 31 years (23.00 ± 3.41 years), in weight from 48 to 90 kg (67.30 ± 10.51 kg), and in height from 151 to 187 cm (173.91 ± 7.58 cm). All volunteers were selected after passing a clinical screening, including a physical examination and laboratory tests (blood analysis: hemoglobin, hematocrit, WBC, platelets, WBC differential, blood urea nitrogen, total bilirubin, cholesterol, total protein, albumin, alkaline phosphatase, fasting glucose, ALT, and AST; and urine analysis: specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC, and casts). Any volunteer who was potentially sensitive to this type of medication, had a history of any illness of the hepatic, renal, or cardiovascular system, or had used alcohol or other drugs for a long period of time was excluded. This was done to ensure that the existing degree of variation was not influenced by illness or other medications. All volunteers avoided using other medications for at least one week prior to the study and until after its completion. They also refrained from alcoholic beverages and xanthine-containing foods and beverages for 48 h before each dose, until collection of the last blood sample. Each volunteer received an oral dose of 70 mg (1 tablet) of alendronate sodium in a standard 2×2 crossover model, in randomized order. There was a 6-day washout period between doses. The study protocol was approved by the local ethics committee. All participants signed a written informed consent after being informed of the nature and details of the study, in accordance with the Korea Guidelines for Bioequivalence Tests (KGBT 1998).

Clinical experiment

The subjects were hospitalized (Sun Hospital, Daejeon, Korea) at 1900 h the day before each drug administration. At 07:00 h, the median cubital vein was cannulated, and 1 mL of heparinized injectable normal saline solution was flushed into the cannula to prevent blood clotting. The doses were taken with 240 mL of tap water at 0800 h on each dosing day. Four hours after the oral administration, all subjects were given standardized meals. The subjects were not allowed to remain in a supine position or to sleep until 4 h after oral administration. Approximately 7 mL blood samples were collected *via* the cannula before the dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, and 7 h after administration. The cannula was flushed with 1 mL of heparinized injectable normal saline solution after each blood sampling. The blood sample was centrifuged immediately, and the plasma was frozen at -70°C until the HPLC-FD analysis.

HPLC-FD assay of alendronate in plasma

The alendronate concentration in plasma was analyzed using the reported HPLC-FD method (Yun *et al.*, 2005). In brief, the sample preparation involved coprecipitation with calcium phosphate, separation on a diethylamine (DEA) solid-phase extraction (SPE) cartridge, and derivatization with 9-fluorenylmethyl chloroformate in a sodium carbonate buffer (pH 11.9). Liquid chromatography was performed on a Capcell Pak C_{18} stationary phase column (150×4.6 mm i.d., 5 μm particles; Shiseido Co., Ltd., Tokyo, Japan). The mobile phase was a series of steps in a gradient consisting of a mixed organic solution (solvent A: acetonitrile: methanol, 1:1) and buffer (solvent B: 25 mM citric acid and 25 mM sodium pyrophosphate tetrabasic without pH adjustment). The total run time was 25 min. The fluorimetric detector was operated at an excitation wavelength of 260 nm and emission wavelength of 310 nm. Pamidronate was used as the internal standard. The limit of quantitation was 1 $ng \cdot mL^{-1}$ using 3 mL of plasma.

Pharmacokinetic analysis

The pharmacokinetic analysis was performed using non-compartmental and compartmental methods. The non-compartmental analysis was performed for each subject, using standard methods. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined by inspecting the individual plasma concentration–time profiles of the drugs. The AUC_t was calculated using the linear trapezoidal rule and was extrapolated to infinity using the relationship

$$AUC_{inf} = (AUC_t + C_t)/k_{el},$$

where AUC_{inf} is the area under the plasma concentration–time curve from zero time to infinity, C_t is the last plasma concentration measured, and k_{el} is the elimination rate constant at the terminal phase. The half-life ($t_{1/2}$) of alendronate was calculated as half-life = ln $2/k_{el}$. The clearance (CL/F) was calculated as CL/F = dose/AUC_{inf}. The apparent volume of distribution (V/F) was estimated

from the equation, $V/F = (CL/F)/k_{el}$.

In the compartmental analysis, we used a one-compartment model with first-order absorption and lag time. Models were fitted to the data using the WinNonlin 2.1 Pro software (Pharsight Co., Inc., Mountain View, CA, U.S.A.). Fitting with individual data was performed using weighted least squares estimation.

Statistical analysis of the data

ANOVA was performed using logarithmically transformed AUC_t and C_{max}. Schuirmann's two one-sided *t*-test for logarithmically transformed AUC_t and C_{max} was used to test the bioequivalence of the pharmacokinetic characteristics between the medications (Kang *et al.*, 2005). The range of bioequivalence for the parametric analysis was set to the commonly accepted 80-125% of the pharmacokinetic parameters obtained from the reference medication, and the range of equivalence for the nonparametric analysis was set to 20% of the reference mean. All statistical comparisons were made using the K-BEtest program (Korea Food and Drug Administration, Korea).

RESULTS AND DISCUSSION

Measurement of plasma alendronate

With the HPLC-FD method, no interfering substances were observed in human plasma. The respective retention times for alendronate and the internal standard (pamidronate) were approximately 7.5 and 6.4 min, respectively. The quantification limit for alendronate in human plasma was 1 $ng \cdot mL^{-1}$, based on a signal-to-noise ratio of 5.0. The intraday and inter-day coefficients of variation for human plasma were less than 8.99 and 10.19%, respectively, for alendronate plasma concentration ranges from 1 to 200 $ng \cdot mL^{-1}$. A typical chromatogram of blank plasma and the chromatogram of a plasma sample are shown in Fig. 1.

Clinical observations

No significant adverse effects resulting in withdrawal from the study occurred in any of the volunteers in the alendronate groups. Minor adverse events, most often general muscle pains (n = 10), fatigue (n = 8), and gastro-

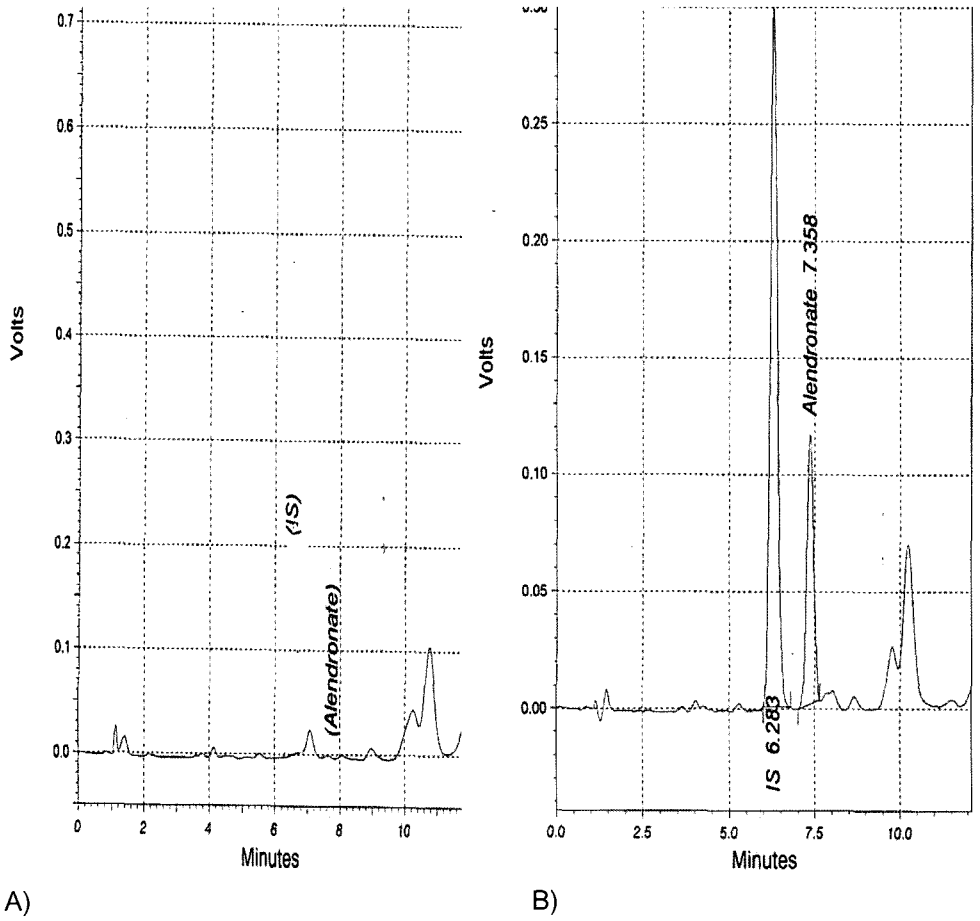

Fig. 1. A: Typical chromatogram of drug-free human plasma. B: Chromatogram of the plasma sample collected from volunteer No. 7 0.5 h after administering 70 mg of alendronate.

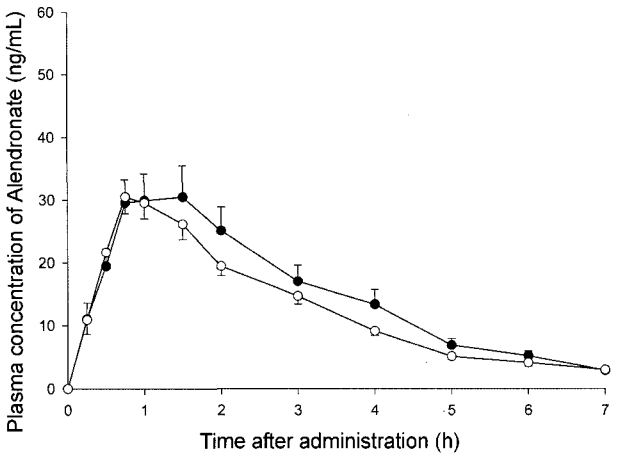

Fig. 2. The mean plasma alendronate concentration–time profiles of healthy subjects following oral administration of Fosamax 70 mg® (reference tablet: ●) or Alenmax 70 mg® (test tablet: ○). The vertical bars represent the standard error of the mean (n = 24).

intestinal symptoms (n = 6), were common in each group.

Plasma concentrations and Pharmacokinetic characteristics

The plasma alendronate (reference and test medication) mean concentration-time profiles are shown in Fig. 2. There were no significant differences in plasma concentrations of each observed time points between reference and test medication. Table I shows the parameters for the non-compartmental pharmacokinetic analysis (AUC_{0-7h}, C_{max}, T_{max}, V/F, and CL/F) and for the one-compartment pharmacokinetic analysis with lag time (K_{el}, K_a, $t_{1/2}$, and T_{lag}), which were calculated using WinNonlin 2.1 Pro (Pharsight).

For the reference drug versus the test drug, the means of the AUC_{0-7h} were 102.44 ± 69.96 vs. 87.63 ± 29.27 ng·h mL^{-1} and the means of the C_{max} were 38.47 ± 24.39 vs. 34.29 ± 13.77 ng mL^{-1}, respectively. It is noticed that the AUC and C_{max} were observed to be higher in the reference tablet without statistical significance ($p>0.05$). The mean terminal half-life values for the reference and test medications were 1.87 ± 0.62 and 1.77 ± 0.54 h, respectively (total mean terminal half-life: 1.82 ± 0.58 h). All of the parameter values were similar to previously reported values (Yun *et al.*, 2005).

Table I. Pharmacokinetic parameters between reference and test medications for alendronate after 70 mg (one tablet) oral administration in healthy humans (n = 24)

Parameter (unit)	Value	
	Reference medication	Test medication
Non-compartmental analysis		
AUC_{0-7h} (ng·h mL^{-1})	102.44 ± 69.96	87.63 ± 29.27
AUC_{inf} (ng·h mL^{-1})	110.24 ± 72.40	96.68 ± 34.30
C_{max} (ng mL^{-1})	38.47 ± 24.39	34.29 ± 13.77
T_{max} (h)	0.99 ± 0.51	0.93 ± 0.28
CL/F (L h^{-1})	889.48 ± 485.87	835.32 ± 357.35
V/F (L)	2576.45 ± 1826.05	2061.30 ± 986.49
Compartmental analysis		
K_{el} (h^{-1})	0.40 ± 0.18	0.42 ± 0.14
K_a (h^{-1})	4.46 ± 3.63	3.80 ± 3.64
$t_{1/2}$ (h)	1.87 ± 0.62	1.77 ± 0.54
T_{lag} (h)	0.19 ± 0.09	0.18 ± 0.06

AUC: area under the concentration–time curve; C_{max}: peak concentration; T_{max}: time to reach peak concentration; CL/F: oral clearance; V/F: apparent volume of distribution; k_{el}: elimination constant; K_a: first order absorption rate constant; $t_{1/2}$: elimination half-life; T_{lag}: lag time

Table II. Analysis of variance test (á = 0.05) for AUC_{0-7h} (ln-transformed) and C_{max} (ln-transformed) for the alendronate sodium 70-mg tablets

ANOVA	ln -transformed AUC_{0-7h} (F-value)	ln -transformed C_{max} (F-value)
Group or Sequence	0.562 (4.301)	0.391 (4.301)
Subjects/Group	3.428 (2.048)	4.255 (2.048)
Period	0.097 (4.301)	0.327 (4.301)
Drug	0.053 (4.301)	0.112 (4.301)

Standard bioequivalence analysis

No significant sequence effect was found for any of the bioavailability parameters (Table II), indicating that the crossover design was properly performed. Significant F-values were found between subjects and the subjects' nested sequence (SEQ) for AUC_{0-7h} and C_{max}, indicating substantial inter-subject variation in the pharmacokinetics of the alendronate formulation (Table II). No significant period effect in AUC_{0-7h} or C_{max} was detected in this study.

Table III. The 90% confidence intervals and results of Schuirmann's test on the target pharmacokinetic parameters of alendronate 70 mg

	Mean			90% C.I.	Result of Schuirmann's test			
	Test (T)	Reference (R)	T/R		Side I		Side II	
					t	P	t	P
C_{max}	34.29	38.47	0.86	0.82–1.16	2.91	0.008	–3.10	0.005
AUC_{0-7h}	87.63	102.44	0.89	0.82–1.14	3.02	0.006	–3.15	0.005

The means of the parameters are given separately for the test and reference formulations of alendronate for each period and as combined estimates. The parametric point estimates for the ratio of the test medication mean to the reference medication mean for the AUC_{0-7h} and C_{max} were 0.86 and 0.89, respectively, and the parametric 90% confidence intervals for AUC_{0-7h} and C_{max} were 0.8234-1.1597 and 0.8222-1.1409, respectively (Table III), which were within the commonly accepted bioequivalence range of 0.80-1.25. Schuirmann's two one-sided *t*-test also showed significant *P*-values at the lower and upper limits for rejecting nonequivalence.

In conclusion, the results indicate that the test drug is bioequivalent to the reference drug and that the two formulations can be prescribed interchangeably.

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