Songklanakarin J. Sci. Technol.
35 (3), 325-331, May - Jun. 2013

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Pharmacokinetics of alendronate in a combined Alendronate/Vitamin D_3 tablet in healthy volunteers using plasma and urine data

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Received 1 September 2012; Accepted 2 April 2013

Abstract

Alendronate is indicated for treatment of a variety of bone metabolism disorders. In spite of quite low plasma concentrations following oral administration, pharmacokinetic study of alendronate based on plasma concentrations had normally been reported for up to 8 h. Due to much longer detectable periods in urine, a number of pharmacokinetic studies have been based upon urinary excretion data. The objective of this study was to determine pharmacokinetics of alendronate following oral administration of combined Alendronate/Vitamin D_3 tablet. Blood and urine samples were collected for 10 h after oral administration of a combined Alendronate/Vitamin D_3 tablet to healthy volunteers. Alendronate levels in plasma and urine samples were determined by high-performance liquid chromatography (HPLC) with fluorescence detection. The analytical method involved co-precipitation of alendronate in plasma or urine samples with calcium and followed by solid-phase extraction (SPE) process. Pharmacokinetic parameters were analyzed by the non-compartmental method using WinNonlin. Pharmacokinetics parameters, i.e. C_{max}, T_{max}, AUC_{0-10}, $T_{1/2}$, were 56.6±11.8 ng/mL, 1.29±0.39 h, 132.4±46.9 ng·h/mL, and 3.43±2.24 h, respectively. Amount excreted in 10 h urine and maximum excretion rate (R_{max}) were 578.59±308.9 µg and 143.3±72.8 µg/h, respectively. Although these parameters were different from previously reported values, R_{max} obtained at 1.29±0.76 h agreed well with T_{max} observed from plasma data. Based upon urinary excretion data, enhanced absorption of alendronate after administration of a combined Alendronate/Vitamin D_3 tablet is suggested.

Keywords: alendronate, alendronate and vitamin D_3, HPLC determination, pharmacokinetics, plasma and urine

1. Introduction

Alendronate (4-amino-1-hydroxybutylidine-1,1-bisphosphonate) is indicated for use in a variety of bone diseases, including postmenopausal osteoporosis, hypercalcemia of malignancy, and Paget's disease (Hosking *et al.*, 1998; Fleisch, 1999). Alendronate has a strong affinity for the solid-phase calcium phosphate of the bone, where it has its primary action. It is an effective inhibitor of osteoclast and bone resorption. About 40% of an oral dose was excreted within 8-12 h, while the remaining was gradually released from the bone, depending on the rate of bone turnover, and was eventually eliminated in the urine (Lin, 1996; Porras *et al.*, 1999). The plasma half-life of alendronate was 1.7-1.8 h in healthy individuals (Lin, 1996; Porras *et al.*, 1999). Its terminal half-life of more than 10 years reflects skeletal retention as reported in postmenopausal women (Khan *et al.*, 1997).

Alendronate is currently available in a tablet combined with cholecalciferol (vitamin D_3) either 2800 IU or 5600 IU in a once-weekly dose (Fosamax Plus® D, package insert). Since low vitamin D level is a common feature among post-

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menopausal women and osteoporotic patients (Moniz *et al.*, 2005; Gallacher *et al.*, 2005), the extra vitamin D in addition to dietary sources, can be beneficial to osteoporotic patients. Although pharmacokinetics of alendronate after oral administration of alendronate plus vitamin D_3 tablet based on urinary excretion were recently reported (Denker *et al.*, 2010), the data based upon plasma levels have not been addressed. Several plasma or urine pharmacokinetic data have been revealed employing reliable HPLC-fluorescence methods (Ptacek *et al.*, 2002; Roldan *et al.*, 2005; Kang *et al.*, 2006; Yun *et al.*, 2006; Tarcomnicu *et al.*, 2007; Rhim *et al.*, 2009; Thudi *et al.*, 2009).

This study aims to examine pharmacokinetics of alendronate in both human plasma and urine after a single oral dose of a tablet of combined Alendronate/Vitamin D_3 using a validated HPLC method with fluorescence detection.

2. Material and Methods

2.1 Study designs

Healthy male volunteers aged 18-35 years and body mass index (BMI) 18-25 kg/m^2 were recruited to the study. They were screened based on medical history, physical examination, and standard laboratory test results (complete blood count, fasting blood glucose, blood urea nitrogen, biochemical profile, and urinalysis). They were excluded if they smoked, had a history of alcohol and/or substance abuse, had an allergic history to alendronate, had used bisphosphonates (or other structurally similar substance) 4 weeks prior to the study period, had participated in any clinical studies or were using any medications within at least 4 weeks prior to this study. In addition, the volunteers were asked to refrain from drinking alcohol for at least 1 week prior to and throughout the study. All participants were informed about the risks and benefits, and details of the study. Written informed consent was obtained from all volunteers before any study procedures were performed. The study was conducted in accordance with the principles of good clinical practice and was approved by Ethics Committee, Phramongkutklao College of Medicine and Phramongkutklao Hospital, Thailand.

2.2 Blood sampling

After an overnight (or at least 10 h) fast, each volunteer was administered a combined tablet containing 70 mg of alendronate sodium and 2,800 IU of vitamin D_3 (Fosamax Plus®, Merck Sharp & Dome de Mexico; Mexico) orally with 250 mL water. The volunteers continued to fast for 4 h, after which a standard lunch was served. Approximately 10 mL of blood sample was collected through a heparin-locked indwelling catheter placed in a forearm vein before the dose and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 10 h after administration. The catheter was flushed with 1 mL of heparin in normal saline solution after each blood sampling. Blood samples were centrifuged immediately, and plasma samples were frozen at -20°C until the analysis. Urine samples were collected before drug administration, and at 0-2, 2-4, 4-8, and 8-10 h after administration. After collection, urine samples were monitored for volume, pH and appearance prior to storage at -20°C until assayed.

2.3 Preparation of plasma and urine samples

The analytical method for the determination of alendronate in plasma and urine used in this study was modified from that reported by Yun *et al.*, 2006. Method modifications included sample preparation, derivatization conditions, and chromatographic conditions. The frozen samples were allowed to thaw at room temperature before processing. Three mL of the plasma sample was pipetted into a 15-mL polypropylene centrifuge tube, briefly shaked with added 25 mL I.S. (24 mg/mL pamidronate disodium). Three mL of trichloroacetic acid (6%) was added to precipitate plasma proteins during vortex-mixing for 45 sec. The protein precipitate was sedimented at 4,000 rpm (1,914 g) for 10 min at 10°C. The supernatant was carefully transferred to a new tube. Then 200 µL of 0.1 M KH_2PO_4 and the same amount of 0.1 M $CaCl_2$ were added and the tube was briefly shaked for 10 sec. After subsequent addition of 4.0 M NaOH and shaking for 30 sec, the solution became cloudy under vortex mixing. The calcium co-precipitate was collected by centrifugation at 4,000 rpm (1,914 g) for 10 min at 10°C. After removal of supernatant, the precipitate was completely dissolved in 0.5 mL of 0.2 M acetic acid. A repeat precipitation was obtained by gradual addition of 4.0 M NaOH. The resulting precipitate was finally dissolved in 1 mL of 0.2 M acetate buffer (pH 6.0) and 40 µL of 0.2 M acetic acid. Prior to solid phase extraction, the solution was diluted with 2.0 mL distilled water and 250 µL of 0.2 M EDTA. Three mL of the sample solution was loaded onto a DEA SPE cartridge (Bond Elute-DEA, Varian, USA) pre-washed with water. The cartridge was washed with 1.0 mL distilled water and alendronate was eluted with 1.0 mL of 0.2 M sodium citrate. An aliquot of the eluate was taken for the derivatization.

The derivatization procedure involved addition of 200 µL of 1 M Na_2CO_3 (pH 11.9) to 540 µL of the sample. Subsequently, 200 µL of FMOC solution was added and the reaction was allowed to take place for 5 min, at room temperature, with constant stirring. The reaction was stopped by the addition of 20 µL glycine solution (360 mmole/mL) and 200 µL citric acid. One hundred µL of the derivatized solution was injected into the chromatographic system.

Urine sample was prepared according to the procedures described above for the plasma sample except for protein precipitation step. Each 1 mL of urine was diluted to 5 mL with distilled water prior to sample preparation.

2.4 Tolerability

Adverse events (nausea, vomiting, diarrhea, abdominal bloating, gastric pain, headache, musculoskeletal and

joint pain, and fatigue) were monitored via observation and interview. Vital signs were monitored over 10 h after drug administration. All other adverse events were recorded on the clinical record form for up to 1 week after the study.

2.5 Chromatographic conditions

The HPLC system (CTO-10AS VP, Shimadzu, Tokyo, Japan) consisted of two LC-20AD VP pumps, a RF-10AXL fluorescence detector, an SIL-10ADVP autosampler, an SCL-10AVP controller, and a DGE-14A degassing unit. Separation was performed on a C18 column (Vertisep® GES 150 mm x 4.6 mm i.d., 5 mm). The detector operated with an excitation wavelength of 260 nm and an emission wavelength of 310 nm.

The mobile phase consisted of a mixture of an organic part (acetonitrile: methanol = 50: 50 v/v, solvent A), and a buffer solution (25 mM citric acid: 25 mM sodium pyrophosphate buffer pH 4.3, solvent B). Gradient elution was performed by increasing the percentage of the organic part from 32% to 60% (solvent A), as follows: 32: 68 at 0-17 min; 60: 40 at 17-22 min; and 32: 68 at 22-40 min. The condition was operated at 35°C with a constant flow rate of 1.5 mL/min. Validation of this HPLC method was performed according to the Guidance for Industry for Bioanalytical Method Validation (US FDA, 2001). The calibration curves were obtained by plotting the ratio of the alendronate peak area to the IS peak area against the alendronate concentration in ng/mL. The calibration curve was constructed over the range of 5-100 ng/mL for plasma and 5-200 ng/mL for urine. The lower limit of quantification (LLOQ) was determined for both plasma and urine.

To assess the accuracy and precision, three different levels of plasma quality control (QC) samples of (15, 40, 80 ng/mL) and urine QC samples (5, 15, 40 ng/mL) were prepared. The intraday accuracy and precision were determined by repeated analyses of the QC samples on the same day ($N \geq 5$). The interday accuracy and precision were determined by repeated analyses of the QC samples on 5 different days. Recovery was determined by comparing the peak area of the alendronate (80 and 40 ng/mL in the plasma and urine, respectively) and pamidronate (200 ng/mL) after extraction and derivatization to that of each analyte obtained in the un-extracted standard solution.

The stability of alendronate in plasma samples was evaluated at 2 predetermined concentrations. Human plasma samples spiked with alendronate at 50 and 500 ng/mL were stored at -20°C. At least 3 aliquots of each concentration were analyzed, and the obtained concentrations were compared to the mean of the back-calculated values for the standards from the first day of long-term stability testing.

2.6 Pharmacokinetic analysis

Pharmacokinetic parameters were analyzed by the non-compartmental method using WinNonlin (Pharsight Corporation, California). The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule and extrapolated to infinity. The elimination half-life ($t_{1/2}$) was calculated using the relationship $t_{1/2} = \ln 2/k$, where k was the elimination rate constant obtained from WinNonlin. Urinary excretion was evaluated as the total (cumulative) amount excreted in the urine ($U_{0\text{-}10}$) and maximum excretion rate (R_{max}). Data were reported as mean and standard deviation.

3. Results

Seven healthy males were recruited in the study. Demographic data (mean ± SD) of the participants are shown in Table 1: age 22.3±2.5 years, height 171.6±4.7 (range 168-181) cm, weight 63.1±9 (range 52-74) kg, and BMI 21.4±3.0 kg/m^2. All participants had normal GFR. Linearity can be described by $y = 0.0050\ (\pm 0.0003)x - 0.023\ (\pm 0.0078)$ ($r^2 = 0.9986$) at 5-100 ng/mL plasma; and $y = 0.0215\ (\pm 0.00625)x - 0.0944\ (\pm 0.0821)$ ($r^2 = 0.9980$) at 5-200 ng/mL urine. The lower limits of quantification (LLOQ) were 18.34 ng/mL (88±9.50 % accuracy) is in plasma, and 18.33 ng/mL (109.9±16.0 % accuracy) in urine. The LLOQ in plasma was higher than the previously reported values 1 and 2 ng/mL plasma (Yun *et al.*, 2006 and Rhim *et al.*, 2009), but LLOQ in urine was slightly lower than previously reported values by Kang *et al.*, 2006 (25 ng/mL).

The precision in the plasma ranged from 6.38-18.6% (intraday) and 9.93-13.2% (interday). The precision in the

Table 1. Demographic data of study participants

Participants	Gender	Age (year)	Weight (kg)	Height (cm)	BMI	GFR (mL/min/1.73 m^2)
1	M	27	74	168	26.22	106.25
2	M	21	53	168	18.78	101.33
3	M	22	72	174	23.78	111.66
4	M	23	64	181	19.54	93.99
5	M	23	69	171	23.60	101.21
6	M	19	52	168	18.42	101.74
7	M	21	58	171	19.84	98.70

M = male, BMI = body mass index, GFR = glomerular rate filtration

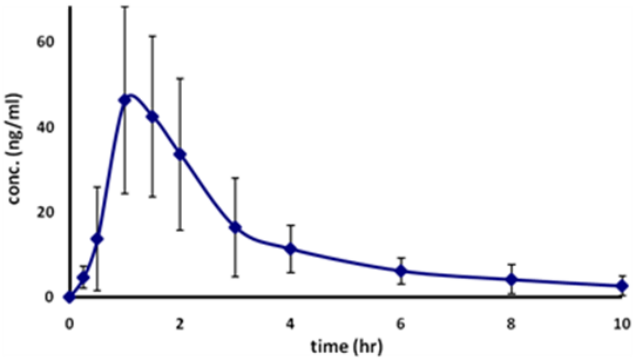

Figure 1. Plasma concentration-time profiles from healthy volunteers after oral administration of single combined tablet of Alendronate sodium 70 mg/Vitamin D_3 (N = 7)

urine ranged from 1.54-16.2% (intraday) and 2.58-24.3% (interday). The recovery of alendronate from the plasma and urine samples were 80.75±4.80% and 109.1 ± 9.04%, respectively. Alendronate remaining in plasma after 45 days storage at -20°C were 97.0±22.0% and 97.7±18.3% of the original amount at 50 and 150 ng/mL, respectively. These validation data indicated that the analytical method had good linearity, sensitivity, accuracy, and precision, and therefore was appropriate for pharmacokinetic study.

Plasma alendronate concentration-time profiles from seven patients are shown in Figure 1. Alendronate was readily absorbed into the plasma with a measurable concentration at the first sampling time (0.25 h) in all volunteers. A maximum plasma concentration (56.6±11.8 ng/mL) was attained at 1.29 ±0.39 h with terminal half life ($t_{1/2}$) of 3.43±2.24 h (Table 2). The areas under the plasma-time curve AUC_{0-10} and $AUC_{1-\infty}$ were 132.40±46.9 and 150.9±52.7 (ng×h/mL), respectively, which extrapolated to an AUC of approximately 11.52±12.1% of the total AUC.

Urinary excretion data, summarized in Table 3, revealed maximum excretion rate (R_{max}) during the first interval (0-2 h) were found in most of the participants. Total amount excreted in 10 h (U_{0-10}) of 578.6±309 µg, and maximum excretion rate (R_{max}) of 143.32±72.8 µg/h, were several fold higher than previously reported values (Kang *et al.*, 2006). Plot of excretion rate of alendronate versus the mid-point of the interval after a dose of Fosamax Plus® tablet (70 mg) was shown in Figure 2. When natural log of excretion rate was plotted against time of mid point, the linear relationship was obtained (Figure 3). The terminal slope of this line (representing this elimination rate constant, *k*) was 0.382±0.056 h^{-1}, and the corresponding half life calculated from *k* was 1.844±0.272 h.

3.1 Tolerability

No adverse event was observed during the study period or one week after study. Thus, the combined tablet was well tolerated in these healthy male volunteers.

4. Discussion

Total amount excreted in urine obtained in our study (0.578±0.31 mg) was consistent with 24-h excreted in urine (U_{0-24}) of 0.59±0.38 mg reported by Roldan (Roldan *et al.*, 2005). However, the inconsistency between as urinary data and these of other studies was noted. Among the previous related studies, only the study by Denker had employed the same product as our study (Denker *et al.*, 2010). Their study, however, has shown lower excretion data (median U_{0-36} 209.4 µg; range 3617.7, 11.3 µg) than our finding (U_{0-10} 578.6±309

Table 2. Pharmacokinetic parameters in healthy volunteers after oral administration of a single combined tablet of Alendronate sodium 70 mg/Vitamin D_3 (N = 7)

Parameters	Mean ± SD (N = 7)
C_{max} (ng/mL)	56.59 ± 11.8
T_{max} (h)	1.29 ± 0.39
AUC_{0-10} (ng.h/mL)	132.40 ± 46.9
$AUC_{1-\infty}$ (ng.h/mL)	150.98 ± 52.7
k (h^{-1})	0.28 ± 0.14
$t_{1/2}$ (h)	3.43 ± 2.24
Cl/F (L/h)	510.4 ± 162.0
V_d/F (L)	2,496 ± 1,814

Table 3. Urinary excretion data of alendronate in healthy volunteers after oral administration of 70 mg alendronate tablet (Fosamax®) and combined Alendronate/Vitamin D_3 tablet (Fosamax Plus®)

Urinary parameters	Current study results			Kang *et al.* (*N* = 7)
	Fosamax Plus® (*N* = 7)[a]	Fosamax® (*N*=5)[b]	*p*-value	
Urine volume (mL)	979.71 ± 269.5	984.0 ± 550.2	0.493	-
Maximum excretion rate [R_{max}] (µg/h)	143.32 ± 72.8	52.842 ± 14.56	0.011	65.67 ± 20.8
Total amount excrete [U_{0-t}] (µg)	578.59 ± 308.9	178.69 ± 51.16	0.002	198.4 ± 81.2
T_{max} (h)	1.286 ± 0.756	1.400 ± 0.894	0.408	0.93 ± 0.59

a: 10 h urine collection
b: 12 h urine collection

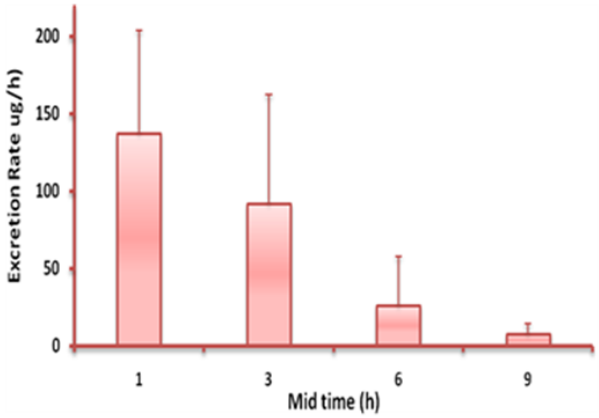

Figure 2. Urinary excretion profile of Alendronate from healthy volunteers after oral administration of single combined tablet of Alendronate sodium 70 mg/Vitamin D_3 (N = 7)

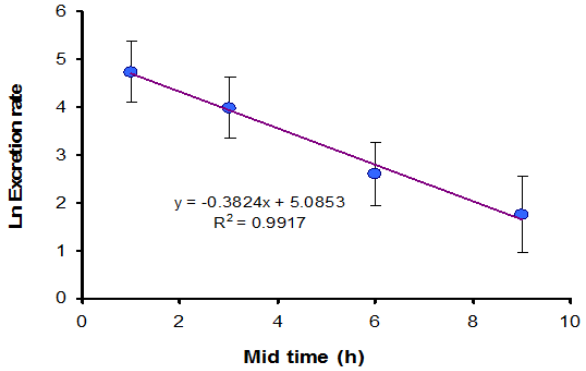

Figure 3. Urinary excretion rate (Ln) of Alendronate versus Mid time from healthy volunteers after oral administration of single combined tablet of Alendronate sodium 70 mg/ Vitamin D_3 (N = 7)

µg). Two differences between our study and that of Denker *et al.* were noticed. Firstly, the fasting period post dosing (4 h) in our study was longer than that in the study by Denker (2 h). Owning to the low oral bioavailability and its ability to form complexes with multivalent cations, timing of meals can affect absorption of alendronate (Porras *et al.*, 1999). Drug administration either immediately or 2 h after breakfast lowered bioavailability by about 85-90% (Gertz *et al.*, 1995). We expected minimal food effects in our study by using a 4-h fasting period, while a shorter fasting period could contribute to the result discrepancy. Secondly, both males and females were included in the study by Denker while only males were enrolled in our study. A number of issues related to gender differences are discussed herein. Since GI absorption of alendronate occur mainly via the paracellular pathway, the soluble alendronate should be available at the absorption site long enough to maximize the absorption (Lin, 1996). Therefore, an empty GI tract after drug administration is critical for alendronate absorption and clinically recommended. Slower gastric emptying in females than in males was reported (91.7 h female vs. 44.8 h males) (Stephen *et al.*, 1986; Gandhi *et al.*, 2004; Soldin and Mattison, 2009). This was partly due to GI motility inhibition by estrogen (Gandhi *et al.*, 2004). Paracellular absorption of alendronate was postulated to occur at the jejunum and duodenum (Lin, 1996), therefore, the slow gastric emptying would diminish absorption. Oral bioavailability of alendronate was 0.76% in postmenopausal women (Porras *et al.*, 1999). Under fasting condition, oral bioavailability was 0.64 % in women after taking 5-70 mg alendronate, and 0.59 % in men after taking 10 mg alendronate (Physician Desk Reference). Thus, gender could lead to biological variability that affect bioavailability of the molecule absorbed via the intricate paracellular pathway and thus explain the discordance of the results of the two studies.

In addition to the above addressed factors, body position post dosing might affect alendronate absorption. Transit time from mouth to stomach (mouth to stomach transit time, MST) was examined in participants in standing and lying position after alendronate administration (Acotto *et al.*, 2012). Results revealed longer MST in participants in lying position compared to those in standing position post administration (73.1s *vs.* 30.9 s, respectively; p <0.09) (Acotto *et al.*, 2012). Although no statistical significance obtained in this small sample size, the results implied longer time a tablet traversing from esophagus to stomach in persons in the lying position. Thus, a recommendation for patients remaining upright after drug administration would be prudent for therapeutic success (Acotto *et al.*, 2012). In our study, we have strictly followed this specific instruction for drug administration. However, additional information which can affect the study results, i.e. participant's activity post administration, urine collection procedures, had not been described in the study by Denker. Thus, the disparity of the data from two studies could not be simply ascribed.

In our study, plasma derived data, T_{max} (1.29±0.39 h), agreed well with urinary excretion data, R_{max} (1.29±0.76 h) Considering plasma parameters, larger C_{max}, AUC, and $t_{1/2}$ than previously reported values by Rhim were obtained. For example, elimination rate constants (k) of 0.28 ± 0.14 h^{-1} *vs.* 0.37±1.58 h^{-1}, corresponding to $t_{1/2}$ of 3.43±2.24 h *vs.* 1.85± 0.44 (Rhim *et al.*, 2009). Significant differences could not be examined due to different drug product used. However, $t_{1/2}$ calculated from urinary excretion data (1.84 ± 0.27 h) in this study was similar to those obtained from plasma data in other previous studies. The discordance between plasma derived k and urine derived k might be due to the multicompartment nature of alendronate. This is evident from plasma time profile of alendronate showing a biphasic decline after peak time (Figure 1). Estimation of k from those 3-4 points after T_{max} might not reflect true elimination constant. Alendronate is eliminated solely by renal excretion so that renal clearance represents systemic clearance. Excretion data can provide accurate elimination parameters during the first 12 h after administration (Porras *et al.*, 2009).

Taking account of urinary excretion data, increased alendronate absorption after administration of combined Alendronate/Vitamin D_3 tablet was suggested. In this study,

we have conducted a preliminary study in healthy male volunteers administering alendronate 70 mg without vitamin D_3. The result revealed data consistent with than previously reported by Kang (Kang *et al.*, 2006) (Table 3). In this preliminary study, the amount of alendronate presented in 12 h urine and R_{max} were reduced compared to those values from alendronate plus vitamin D_3 administration (Table 3). Significant increase calcium absorption has been demonstrated in a randomized controlled trial including 56 postmenopausal women administered combined Alendronate/Vitamin D_3 tablet (Shapes *et al.*, 2011). An *in vitro* study has shown the stimulation of paracellular calcium influx by vitamin D_3 and its analogs in Caco-2 cell culture (Chirayath *et al.*, 1998). This was associated with a reduction of transepithelial electric resistance (TEER) by about 50% in the presence of 1a,25-dihydroxyvitamin D_3 at 10^{-5} to 10^{-3} mM (Chirayath *et al.*,1998). Since alendronate binds strongly to Ca^{2+}, greater permeability of Ca^{2+} during reduced TEER might facilitate alendronate traversing tight junctions of the intestinal epithelium. This could partly contribute to the enhanced alendronate absorption in these healthy volunteers.

Since approximately 45% of the alendronate dose was excreted in 36 h, and 54% was excreted in the first week (Khan *et al.*, 1997). Approximately 46% of the drug administered was retained in the skeleton, which could be slowly released from bone during elimination phase (Lin *et al.*, 1996). Because of high affinity to bone, alendronate plasma concentration was quite low and undetectable after 8-10 h of oral administration. Therefore, pharmacokinetics data derived from plasma data were normally reported up to 7 h (Yun *et al.*, 2006a; Yun *et al.*, 2006b; Rhim *et al.*, 2009). Because plasma concentrations after 10 h are undetectable, the data up to 10 h was reported in our study. One drawback of the analytical method presented here was high LLOQ in plasma analysis, compared to the reported analytical method (1 ng/ml by Yun). However, the method of determining LOD and LLOQ was not clearly addressed in the study by Yun. Instrument is related problems, such as detector (light source), analytical column, could be the resource of such errors. In our study, initial LLOQ was estimated from LOD + 10*SD, the predicted value was then verified with spiked samples. While the accuracy and precision were within the acceptable range of ±20 %, the LLOQ was large due to the large SD.

For urine samples, LLOQ of 18 ng/mL was lower than that reported by Kang (25 ng/mL) (Kang *et al.*, 2006). Due to the fact that a large amount of alendronate was excreted in urine in 10 h, the sensitivity of the method was sufficient in determining alendronate concentration in urine. Since only 1 ml of urine sample was required for each analysis, the sensitivity for the analytical method can be improved by increasing urine volume.

We attempted to use plasma and urine data in examining pharmacokinetics of alendronate from combined Alendronate/Vitamin D_3. No interference from vitamin D_3 was found in the analysis of either plasma or urine samples, confirming specificity of the analytical method. The sensitivity of the analytical method was adequate in determining amount of alendronate in urine sample, while plasma levels determination was still challenging. Urinary excretion data, which is the pharmacologically relevant parameter, was demonstrated in this study and suggested enhanced absorption of alendronate in these volunteers. Finally, since the parameters from this study were obtained from healthy young volunteers, other considerations should be taken into account before drawing inferences from the data to an actual clinical situation in a particular separate population, such as postmenopausal women.

Acknowledgements

We would like to thank the Graduate School, Faculty of Pharmaceutical Sciences, and Prince of Songkla University, for financial support.

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