เภสัชจลนศาสตร์ของยาอะเลนโดรเนตในผู้ป่วยไตเรื้อรัง: การศึกษานำร่อง

Pharmacokinetics of alendronate in patients with chronic kidney disease: A preliminary study

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บทคัดย่อ: อะเลนโดรเนตเป็นยาในกลุ่มบิสฟอสโฟเนตที่ใช้รักษาความผิดปกติของเมตาบอลิสมของกระดูกที่พบได้ในโรคหลายชนิด การศึกษาเภสัชจลนศาสตร์ของยาอะเลนโดรเนตที่มีรายงานส่วนใหญ่เป็นการศึกษาในอาสาสมัครสุขภาพดี ยังไม่มีการศึกษาในผู้ป่วยโรคไตเรื้อรัง การศึกษานี้ จึงมีวัตถุประสงค์เพื่อศึกษาเภสัชจลนศาสตร์ของยาอะเลนโดรเนต จากระดับยาในเลือดและปัสสาวะในผู้ป่วยโรคไตเรื้อรัง (Chronic kidney disease, CKD) ผู้ป่วยที่เข้าร่วมการศึกษา คือ ผู้ป่วยโรคไตเรื้อรังระยะที่ 3-5 ที่มีค่าการทำงานของไตน้อยกว่า 60 ml/min/1.73 m^2 และได้รับยาอะเลนโดรเนตในรูปแบบรับประทานมาไม่ต่ำกว่า 6 เดือน มีการเก็บตัวอย่างเลือดและปัสสาวะที่เวลาต่างๆ เป็นเวลา 10 ชั่วโมงหลังรับประทานยา ตัวอย่างที่ได้จะผ่านกระบวนการสกัด และกระบวนการที่ทำให้ตัวอย่างสะอาดขึ้นก่อนวิเคราะห์ปริมาณโดยเทคนิค high-performance liquid chromatography (HPLC) ที่ตรวจวัดโดย fluorescence มีการตรวจสอบความถูกต้องของวิธีวิเคราะห์ที่พัฒนาขึ้นในด้านความเป็นเส้นตรง (linearity) ความจำเพาะ (specificity) ความถูกต้องและความแม่นยำ (accuracy and precision) ความไว (sensitivity) และความคงตัว (stability) ผลการศึกษาพบว่า $AUC_{0\text{-inf}}$ และ C_{max} ในผู้ป่วย CKD มีความแปรปรวนสูง เมื่อเปรียบเทียบค่าพารามิเตอร์ทางเภสัชจลนศาสตร์กับข้อมูลในอาสาสมัครสุขภาพดีที่เคยมีรายงานมาก่อนหน้า พบว่า $AUC_{0\text{-inf}}$ และ C_{max} ในกลุ่ม CKD มีค่าสูงกว่าค่าในอาสาสมัครสุขภาพดีหลายเท่า ในขณะที่ค่า T_{max} ของทั้ง 2 กลุ่มมีค่าใกล้เคียงกัน ค่าครึ่งชีวิตในการขจัดยา (elimination half-life, $t_{1/2}$) ในกลุ่ม CKD มีค่ามากกว่าในอาสาสมัครสุขภาพดี ในขณะที่อัตราการขับออกสูงสุดทางปัสสาวะ(Maximum urinary excretion rate) รวมทั้งปริมาณอะเลนโดรเนตที่ขับออกมาทางปัสสาวะ (cumulative amount excreted in urine) มีค่าใกล้เคียงกัน เนื่องจากอะเลนโดรเนตถูกขจัดจากร่างกายโดยการขับทางปัสสาวะเป็นหลัก ดังนั้น การขจัดยาอะเลนโดรเนตทางไตจึงไม่เปลี่ยนแปลงมากนักใน CKD ปัจจัยอื่นๆ ที่อาจส่งผลต่อ $AUC_{0\text{-inf}}$ และ C_{max} ในกลุ่ม CKD ได้แก่ องค์ประกอบของยาที่บริหาร

รวมถึงการเปลี่ยนแปลงทางพยาธิสรีรวิทยาในกลุ่มผู้ป่วย CKD ที่มีผลต่อการดูดซึม หรือ การกระจายตัวของยา จึงควรศึกษาเพื่ออธิบายปัจจัยที่มีผลต่อเภสัชจลนศาสตร์ของยาอะเลนโดรเนตในผู้ป่วย CKD ต่อไป

Abstract: Alendronate is a bisphosphonates indicated for treatment of a variety of bone metabolism disorders. Most of pharmacokinetic data were previously examined in healthy subjects receiving alendronate, no study in chronic kidney disease (CKD) patient have been reported. The purpose of this study is to investigate pharmacokinetic of alendronate in patients with chronic kidney disease (CKD), using plasma and urine data. Patients diagnosed with CKD stage 3-5 (GFR < 60 ml/min/1.73 m^2) who have administered alendronate sodium 70 mg once weekly for at least 6 months were enrolled in the study. Blood and urine samples were collected for 10 hours after administration. High-performance liquid chromatographic (HPLC) technique with fluorescence detection was used for the determination of alendronate in plasma and urine. The method was validated for linearity, specificity, accuracy, precision, sensitivity, and stability. Pharmacokinetic parameters obtained from CKD were highly variable except T_{max}. The results showed elevated $AUC_{0\text{-}inf}$ and C_{max} in CKD patients compared to those from normal group. Elimination $t_{1/2}$ in CKD patients were longer whereas urinary excretion profile (including maximum urinary excretion rate and cumulative amount excreted in urine) were similar to those from healthy volunteers. Renal excretion tend to be unaltered in CKD patients. Changes in absorption or distribution, due to different product administration and pathophysiology associated with CKD, might affect pharmacokinetics of alendronate in CKD. Further investigation is required to delineate pharmacokinetic of alendronate in CKD patients.

Introduction: Alendronate is an aminobisphosphonate indicated for a variety of bone disease, including osteoporosis, hypercalcemia of malignancy, and Paget's disease.[1,2] Alendronate usually presented in plasma at the level of low ng/mL, which can be due to limited oral absorption (< 1% of the dose) as well as extensive accumulation in bone tissue.[3] Difficulty in measuring plasma levels has limited essential pharmacokinetic study. Although a number of pharmacokinetic study have been reported recently.[4-8] Those were performed in healthy subjects. Alendronate is not metabolized to any extent but eliminated exclusively via renal excretion. Detailed pharmacokinetic of alendronate in impaired renal function have not been investigated. Some researchers have suggested dose reduction to half of normal dose in hemodialysis patients.[9] Thus, in this study we aimed to determine pharmacokinetic of alendronate in patients with chronic kidney disease (CKD), using plasma and urine data.

Methodology: Chronic kidney disease (CKD) was defined as GFR < 60 ml/min/1.73 m^2, estimated by Modification of Diet in Renal Disease (MDRD) equation. Patients diagnosed with CKD stage 3-5 were enrolled in the study. Based on medical history, eligible subjects were those who have administered alendronate sodium 70 mg once weekly for at least 6 months. Inclusion criteria were also based on physical examination and laboratory testing. Hemodialysis patients were not included in the study. Subjects were informed about the purpose and risks of the study, which informed consent was obtained from all patients before screening. Clinical data of the patients were shown in *Table 1*

Table 1 Clinical data of the patients

Patient	Sex	Age (year)	Weight (kg)	Height (cm)	GFR (ml/min/1.73 m^2)	Primary renal disease
1	F	43	44	150	39.02	Uncontrolled hypertension
2	F	74	54	150	59.09	Hypertension
3	M	74	49	162	46.69	Hypertension and DM
4	M	80	91	168	49.50	Hypertension and DM
5	F	78	47	147	48.63	Hypertension and DM
6	F	75	69	165	59.84	Hypertension and DM

The study protocol was approved by Ethic committee, Office of Research Development, Phramongkutklao College of Medicine & Phramongkutklao Hospital, Bangkok Thailand. Subjects were instructed to fast at least 8 hours overnight. At about 8:00 am on the experiment day, subjects administered a 70 mg alendronate sodium tablet (Fosamax Plus®, MSD) orally with 250 mL water. Subjects were asked to remain in the upright position for at least 60 minutes and continue fasting for at least 2 hours after drug administration. Water was allowed as needed during 2 hours post-dose. Food and beverage intake were allowed 4 hours after drug administration. Subjects remained under continuous medical supervision at the study site to monitor adverse events. Approximately 10 mL of blood samples were 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 hours after administration. The catheter was flushed with 1 mL of heparinized normal saline solution after each blood sampling. The blood sample was centrifuged immediately, and the plasma was frozen at -20°C until the analysis. Urine samples were collected before drug administration, during 0-2, 2-4, 4-8, and 8-10 hours after administration. After collection, urine samples were monitored for volume, pH and appearance prior to storage at -20°C until assayed.

Sample preparation and HPLC analysis: Based on the high-performance liquid chromatographic (HPLC) technique with fluorescence detection, 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.[5] Method modifications included sample preparation, derivatization, and chromatographic conditions. Three milliliter of plasma sample was pipetted into a 15-mL polypropylene centrifuge tube, 25 μL I.S. (24 μg/mL pamidronate disodium, which served as an internal standard) was added. After a brief vortex mixing, 3.0 mL trichloroacetic acid (6%) was added. Plasma protein was then precipitated and isolated by centrifugation. Supernatant was added with each 200 μL of 0.1 M KH_2PO_4 and 0.1 M $CaCl_2$. With subsequent added 400 μL of 4.0 M NaOH, the solution became cloudy under vortex mixing. The calcium co-precipitate was collected by centrifugation at 4,000 rpm, 10°C for 10 minutes. After supernatant discarding, the precipitate was dissolved in 0.5 mL of 0.2 M acetic acid. Repeat precipitation was obtained by addition of 4.0 M NaOH. The precipitate was finally dissolved in 1.0 mL of 0.2 M acetate buffer (pH 6.0) and 40 μL of 0.2 M acetic acid. Prior to solid phase extraction (SPE), the solution was added with 2.0 mL distilled water and 250 μL of 0.2 M EDTA. Three milliliter of sample solution was loaded on the SPE cartridge (diethylamine 100 mg/3 mL, Varian, USA) after prewashed with water. The cartridge was washed with 1.0 mL distilled water and alendronate was subsequently eluted with 1.0 mL of 0.2 M sodium citrate. The analyte was subjected to derivatization prior to HPLC analysis. 9-fluorenylmethyl chloroformate (FMOC) solution (0.25 mg/mL acetonitrile) 0.2 mL was added to 0.54 mL sample solution at pH 11.9 (1.0 M Na_2CO_3). The reaction was allowed to occur at room temperature for 5 minutes with constant stirring. The reaction was stopped by

the addition of 20 μL glycine solution (360 μmole/mL) and 0.2 mL citric acid. One hundred microliter of the derivatized solution was injected into the chromatographic system. Urine was diluted to 25% and prepared by the procedures used for plasma sample except trichloroacetic acid addition.

Chromatographic conditions: The HPLC system (CTO-10AS VP, Shimadzu, Tokyo, Japan) consists of a high-pressure pump, an autosampler, a column thermostat, and a fluorescence detector operated at excitation wavelength of 260 nm and emission wavelength of 310 nm. The separation was performed on a C18 column (Vertisep® GES 150 mm x 4.6 mm i.d., 5 μm) at 35°C. Mobile phase consists of a mixture of acetonitrile and methanol (50:50 v/v, solvent A), and buffer solution (25 mM citric acid : 25mM sodium pyrophosphate buffer pH 4.3, solvent B). Gradient elution was performed by increasing the organic solvent percentage from 32% to 60% v/v at a constant flow rate of 1.5 ml/min: 0-17 minute 32:68; 17-22 minute 60:40; 22-40 minute 32:68. The method was validated in our analytical laboratory according to the Guidance for Industry, Bioanalytical Method Validation. The accuracy in plasma were 99.11 ± 13.0%, 105.6 ± 11.4% and 104.9 ± 10.4% at 15, 40 and 80 ng/ml, respectively. The accuracy in urine were 103.4 ± 25.2 % and 106.9 ± 2.76 % at 15 ng/ml and 40 ng/ml, respectively. Limit of detection were 3.87 and 3.19 ng/mL for plasma and urine, respectively. The lower limit of quantifications (LLOQ) were 18.34 ng/mL (%accuracy= 87.99%) in plasma and 17.49 ng/mL (%accuracy= 104.92%) in urine. The precisions in plasma ranged from 6.38-18.6% (within-run) and 9.93-13.2% (between-run). The precisions in urine ranged from 1.54-16.2% (within-run) and 2.58-24.3% (between-run). The calibration curve was linear over the concentration range of 0.0-100 ng/mL and 0.0-200 ng/mL with $r^2 > 0.99$ for plasma and urine, respectively. Alendronate remained in plasma 96.97 ± 22.0% and 97.75 ± 18.3% of original amount at 50 and 150 ng/mL, respectively, when stored at -20°C for 45 days. According to this analytical method validation data, this modified method for the determination of alendronate in plasma and urine was reliable and suitable for pharmacokinetic study.

Results, Discussion and Conclusion: Plasma alendronate concentration-time profiles from six patients are shown in *Figure 1*. *Table 2* shows the parameters for the non-compartmental pharmacokinetic analysis of plasma data and urinary data. Pharmacokinetic parameters in CKD were highly varied except T_{max}, which mean values were approximately the same as those obtained from healthy subjects. Mean $AUC_{0\text{-}10}$, $AUC_{0\text{-}inf}$ and C_{max} in CKD patients were several folds higher than those from normal group. As shown in *Figure 2*, maximum urinary excretion rate was comparable to those in normal groups. Total alendronate recovered in 10 h urine was also similar between CKD patients and normal group.

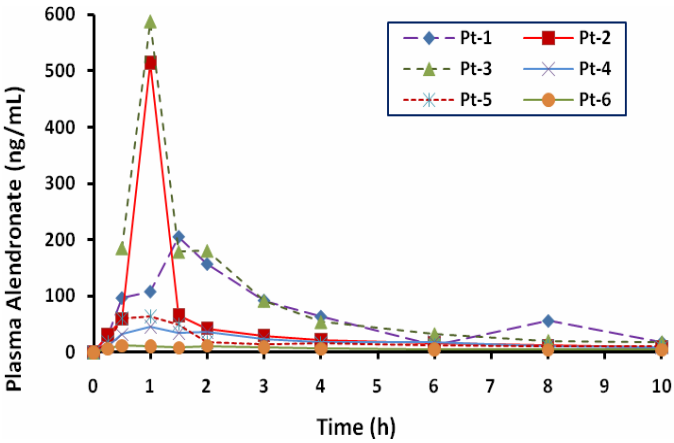

Figure 1 Alendronate plasma concentration–time profiles in CKD patients following oral administration of Fosamax Plus 70 mg (n =6)

Table 2 Plasma and urinary excretion of alendronate in CKD patients (n=6) after oral administration of a 70 mg alendronate sodium tablet

PK parameters	CKD	Healthy volunteers
$AUC_{(0\text{-}10)}$ (ng·h/ml)	420.32 ± 323.59	102.44 ± 69.96 [a]
$AUC_{(0\text{-}inf)}$ (ng·h/ml)	532.01 ± 300.31	110.24 ±72.40 [a]
C_{max} (ng/ml)	237.9 ± 251.8	38.47 ± 24.39 [a]
T_{max} (h)	1.00 ± 0.32	0.99 ± 0.5 [a]
CL/F (L/h)	170.17 ± 87.41	889.48 ± 485.87[a]
λ_z (h^{-1})	0.135 ± 0.081	0.40 ± 0.18[a]
$T_{1/2}$ λ_z (h)	9.303 ± 9.83	1.87 ± 0.62 [a]
Maximum urinary excretion rate (µg/h)	49.2 ± 26.8	52.8 ± 14.6[b]
Cumulative amount excreted (µg)	242.3 ± 107.3	178.7± 51.2 [b]

[a] Data from Yun et al[5] in this study, Fosamax® 70 mg were administered single dose, and plasma were collected for 7.0 h

[b] Data from preliminary study in healthy volunteers (n=5)

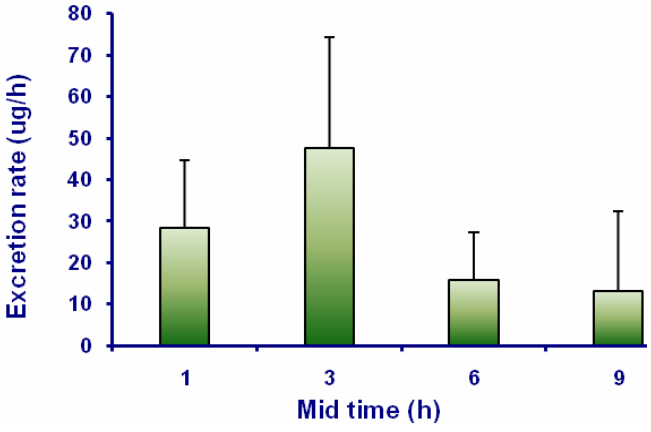

Figure 2 Urinary excretion profile of alendronate in CKD patients following oral administration of Fosamax Plus 70 mg (n = 6)

From plasma data, longer elimination $t_{1/2}$′s in CKD patients compared to healthy volunteers was observed. However, urinary excretion profile between two groups were comparable. This might suggest that elimination of alendronate was minimally affected in CKD. Whether volume of distribution (V/F) is affected remained inconclusive. Elevated extent of absorption and C_{max} indicated that absorption of alendronate can be affected in CKD patients. This can be related to either drug product effect (Fosamax Plus® used in CKD *vs*. Fosamax® used in healthy group) or pathophysiological changes associated with CKD. Further investigation is required to delineate pharmacokinetic of alendronate in CKD patients.

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