Bioequivalence Study of 500 mg Glucosamine Sulfate in Thai Healthy Volunteers

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Background and Objective: *Glucosamine sulfate is widely used to relieve symptoms from osteoarthritis. The present study was conducted in order to determine pharmacokinetic and assess the in-vivo bioequivalence of two different hard capsule formulations of glucosamine sulfate when administered as equal dose of 500 mg. The two formulations contained different salt form where reference product is NaCl and test product is KCl.*
Material and Method: *A randomized, single dose, two-treatment, two-period, two-sequence crossover study was conducted. Twenty-four healthy volunteers were recruited at Siriraj Clinical Research Unit. Each subject received a dose of 500 mg glucosamine sulfate of both formulations with at least one-week washout period. Blood samples were collected over 24 h after the oral administration. The plasma fractions were analyzed for glucosamine using a liquid chromatography-mass spectrometry (LC-MS/MS).*
Results: *Twenty-four volunteers were enrolled in the present study. Pharmacokinetic parameters were determined using the non-compartment model. The 90% confidence intervals of the mean ratios (test/ reference) of* C_{max} *(93.69%; ranged from 86.68%-113.32%) and* AUC_{0-t} *(97.73; ranged from 87.38%- 112.62%) fell within the acceptable range (80-125%) for bioequivalent eligibility. Both preparations were well tolerated and had a few non-serious adverse events.*
Conclusion: *The glucosamine sulfate containing KCl (test product) is bioequivalent to glucosamine sulfate containing NaCl (reference product) in terms of rate and extent of absorption.*

Keywords: *Bioequivalence, Liquid chromatography-mass spectrometry, Glucosamine, Pharmacokinetics*

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Glucosamine is widely used to relieve symptoms from osteoarthritis[1-4]. The actual mechanism of action of glucosamine is not known. Glucosamine, 2-amino-2-deoxy-D-glucose, is an amino monosaccharide that is an essential component of mucopolysaccharides, and chitin. Glycosaminoglycans (mucopolysaccharides) are large complexes of negatively-charged carbohydrate chains that are incorporated into mucous secretions, connective tissue, skin, tendons, ligaments, and cartilage. Glucosamine and its acetylated derivative, N-acetylglucosamine, are readily synthesized in the body from glucose. Because of its high concentration in joint tissues, the hypothesis that glucosamine supplements would provide symptomatic relief for osteoarthritis was developed more than 30 years ago[5]. Many clinical trials have tested this hypothesis[6] and glucosamine supplements are widely used to relieve arthritic complaints[7]. Its safety and effects on glucose metabolism are critically evaluated in several reviews[2-4].

Very few studies on the pharmacokinetic of glucosamine have been published. After oral administration, 90% of glucosamine sulfate is absorbed. The AUC after oral administration is only 26% of that after intravenous or intramuscular administration[8]. A significant fraction of orally administered glucosamine undergoes first-pass metabolism in the liver, which metabolizes a notable proportion of glucosamine into

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smaller molecules and ultimately to CO_2, water and urea[9]. Blood levels achieved after oral glucosamine are only 20% of those achieved with intravenous glucosamine. Serum glucosamine concentrations were approximately 0.04 mmol/L when they are not consuming supplemental glucosamine. Intravenous infusion of approximately 9.7 g of glucosamine produced steady state serum glucosamine concentrations of approximately 0.65 mmol/L. Infusion of 30.45 g of glucosamine produced steady state serum glucosamine concentrations of approximately 1.42 mmol/L. Intake of usual oral doses of glucosamine in humans would achieve serum levels of approximately 0.06 mmol/L.

The main objective of the present study was to compare bioavailability of two pharmaceutical alternative formulations of glucosamine sulfate. The two formulations are different in salt form and strength, test formulation contains KCl in strength of 500 mg while reference formulation contains NaCl in strength of 250 mg. The dosage of glucosamine sulfate 500 mg for both formulations were administered as a single dose to 24 healthy volunteers under a two-treatment, two-period and two-sequence crossover study design with a minimum of one-week washout period.

Material and Method

Glucosamine preparations

Test preparation: Flexsa® (Mega Lifesciences Company Ltd. Thailand) containing 500 mg glucosamine sulfate KCl /capsule (Lot no. 510126, Mfg. date 12 October 2005, Exp. date 12 September 2007).

Reference preparation: Viartril®-S (Rottapharm Company Ltd, Ireland) containing 250 mg glucosamine sulfate NaCl/capsule (Lot no. 40131, Mfg. date 18 June 2004, Exp. date 18 May 2009).

Volunteers

Twenty-four healthy Thai volunteers aged between 18-45 years with a body mass index between 18-24 kg/m^2 were recruited at Siriraj Clinical Research Center, Siriraj Hospital. After explaining the details and the purposes of the present study, all healthy volunteers provided written informed consents. They were non-smoking, non-alcoholic, and free from significant cardiac, hepatic, renal, gastrointestinal, and hematological diseases, as assessed by physical examination and the following laboratory investigations: complete blood count, BUN, creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase, fasting blood sugar, serum electrolyte, and hepatitis B surface antigen. Urine pregnancy tests were negative in all female volunteers. Volunteers did not have a history of allergy to glucosamine and/or its constituents and did not receive other medicines within 14 days before the first study drug administration.

Study design

Randomized, single dose, fasting, two-period, two-sequence, crossover study with at least a one-week washout period was conducted. Volunteers were allocated into two equal groups. Each volunteer was assigned to a particular study group using a pre-printed randomization table generated by Microsoft Excel. During each period, the volunteers were admitted to the Siriraj Clinical Research Center, Siriraj Hospital. After overnight fasting for at least 8 hours, they received a single dose of test formulation (500 mg capsule) or reference formulation (2 x 250 mg capsules) along with 240 ml of drinking water. Volunteers continued fasting for 2 and 4 h (water and food, respectively) after drug administration.

The subjects were closely observed to assess the adverse events. As test product containing KCl 2.18 mmol/500 mg, serum potassium was monitored at pre-dose, 12, and 24 h after test and reference products administration.

The present study was approved by the independent Ethics Committee of the Faculty of Medicine, Siriraj Hospital, Mahidol University prior to commencing and was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guideline. All subjects were individually gave written informed consent prior to starting the study procedures.

Sample collection and glucosamine analysis

Ten ml of each blood sample was collected by catheterized venupuncture at forearms from each subject. Sodium heparinized vacutainer tubes were used for sample collection. Thirteen samples were collected: 0 (before the dosing), 5, 10, 15, 30, 80, 90 min and 2, 4, 6, 8, 12 and 24 h after administration. The blood samples were centrifuged. Then, the plasma fractions were collected and kept at -70°C until analysis.

Propranolol was used as an internal standard. Glucosamine was extracted by liquid-liquid extraction technique, using acetronitrile and triethylamine. All of organic phase was evaporated to dryness under nitrogen gas. The residual was re-dissolved and injected to LC-MS/MS. The mobile phase consisted of acetroni-trile and formic acid. The analytical equipment used included a HPLC device coupled with a mass

selective detector. The multiple reaction monitoring (MRM) mode used the range from m/z transition 179.90 to 143.70 for glucosamine and from m/z transition 260.00 to 116.00 for internal standard. Validation of this method was performed as recommended by the USFDA. Calibration curve was linearity in the range of 0.1 to 10 μg/mL with $r^2 = 0.998979$ and the lower limit of quantification for the validated assay was 0.1 μg/ml. Mean recovery of extraction was 89.83-96.99% and 106.45% for glucosamine and internal standard, respectively. The intra- and inter-assay precision were 2.81%-13.80% and 5.08-9.26%, respectively. The percentage average of intra- and inter-assay recovery was between 88.20%-119.60% and 96.40%-108.00%, respectively. Stability of glucosamine in plasma during sample processing and 30 days storage in -70°C were within the acceptable range. Glucosamine level was calculated using MassLynx version 4.0.

Pharmacokinetic and statistical analysis

A non-compartmental pharmacokinetic model was used to determine the pharmacokinetic parameters of glucosamine. The pharmacokinetic parameters, *i.e.*, $AUC_{0\to t}$, $AUC_{0\to\infty}$, C_{max}, t_{max}, $t_{1/2}$ were determined using WinNonlin edition version 3.1. Statistical comparisons between pharmacokinetic parameters of the two products were analyzed using two-way ANOVA with $p < 0.05$ for statistical significance to assess the effect of formulation, periods, sequence, subjects within sequence. The variation in estimation of terminal slope as can be seen in lamda_z or $t_{1/2}$ calculation (0.693/ lamda_z), the $AUC_{0\to\infty}$ might not be a good parameter to be compared. Moreover, the authors' first previous bioanalytical method was not sensitive enough to detect the concentration of glucosamine. There are many BQL data even also at baseline level. Thus, it may not be possible to obtain reliable $AUC_{0\to\infty}$ parameters. Thus, the authors did the statistical analysis for $AUC_{0\to t}$ instead. The 90 percent confidence intervals of the test/reference ratio of C_{max}, and $AUC_{0\to t}$ using log trans-formed data were determined. The bioequivalence between the two formulations would be accepted if the 90 percent confidence intervals (CI) of the log transformed C_{max}, and $AUC_{0\to t}$ of test fell within 80-125% of the original product[10,11].

Results and Discussion

Twenty-four volunteers (16 males, 8 females) completed the present study. Demographic characteristics of subjects between the two groups seemed similar and shown in Table 1. The average plasma concentrations of at each time point from 24 Healthy Volunteers after administration of the reference and test product are tabulated in Table 2. No significant difference was observed in any of the analyzed pharmacokinetic parameters (Table 3). The geometric mean for test $t_{1/2}$ is 15.650 and that for reference is 23.231, which show less difference. Because the distribution of $t_{1/2}$ might not be a normal distribution, it may be better to use the geometric mean for more log-normal distribution. The generic formulation had C_{max} at 0.99 μg/ml, t_{max} at 1.42 h while the original formulation had C_{max} at 1.12 μg/ml, t_{max} at 2.00 h (Table 1). Ninety percent CI of the mean ratios (generic/original) of the log transformed of the C_{max} and $AUC_{0\to t}$ were 93.69% (ranged from 86.68%-113.32%) and 97.73% (ranged from 87.38%-112.62%), respectively. Since the 90% CI for C_{max} and $AUC_{0\to t}$ fell within the predefined bioequivalence acceptance limits (80-125% of the innovator); the generic and original formulations were considered bioequivalent in terms of the rate and extent of absorption.

The plots of average plasma concentration of glucosamine (ng/ml; mean ± SD) vs. time over the 24 h sampling period after oral administration of 500 mg of the test and reference capsules are presented in Fig. 1. It was found that the plasma profiles of the glucosamine concentration of both formulations exhibited close similar patterns, which were nearly super imposable. The amounts of glucosamine in plasma at pre-dose were detected by the fact that glucosamine is a normal constituent of the extracellular matrix of mammalian

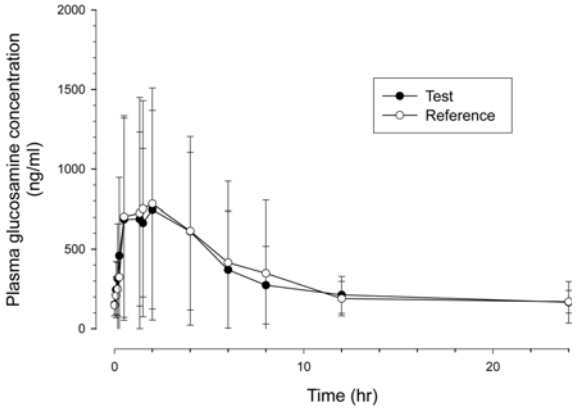

Fig. 1 Average plasma concentration (ng/mL; mean ± SD) vs. time curves of glucosamine after oral administration of 500 mg of the test (□) and reference capsules (%)

Table 1. Demographic data and mean clinical laboratory of 24 volunteers

Characteristics		Group 1 (TR* group) (n = 12)	Group 2 (RT* group) (n = 12)
Gender	Male	7	9
	Female	5	3
Age (years ± SD)		22.20 ± 5.87	21.30 ± 1.78
Weight (kg ± SD)		57.46 ± 9.15	57.09 ± 8.47
Height (cm ± SD)		165.67 ± 9.29	166.67 ± 8.46
Body mass index (kg/m^2 ± SD)		20.82 ± 1.88	20.57 ± 1.57
Vital signs	Temperature (°C ± SD)	36.60 ± 0.21	36.60 ± 0.30
	Pulse (beat/minutes ± SD)	67.00 ± 11.76	68.00 ± 11.94
	Respiratory rate (times/minute ± SD)	20.00 ± 0.85	20.00 ± 0.58
	Systolic blood pressure (mmHg ± SD)	106.90 ± 9.61	114.00 ± 6.00
	Diastolic blood pressure (mmHg ± SD)	68.50 ± 10.44	70.30 ± 7.69
Clinical laboratory	Hemoglobin (g/dl)	13.91 ± 1.44	14.57 ± 1.31
	Hematocrit (%)	41.26 ± 3.86	43.26 ± 4.55
	BUN (mg/dl)	10.81 ± 2.50	12.75 ± 4.28
	Creatinine (mg/dl)	0.64 ± 0.23	0.74 ± 0.22
	AST (units/L)	17.17 ± 8.20	17.33 ± 6.43
	ALT (units/L)	17.67 ± 7.28	18.83 ± 10.19
	ALP (units/L)	64.92 ± 17.74	73.92 ± 16.81
	Total bilirubin (mg/dL)	0.63 ± 0.19	0.69 ± 0.24
	Blood sugar (mg/dL)	85.33 ± 7.48	82.33 ± 6.64
Serum electrolyte	Na^+ (mmol/L)	138.50 ± 1.73	140.08 ± 1.51
	K^+ (mmol/L)	4.22 ± 0.28	4.09 ± 0.29
	Cl^- (mmol/L)	102.00 ± 1.60	101.92 ± 2.02
	HCO_3^- (mmol/L)	27.92 ± 2.64	28.92 ± 2.81

* The sequence of product taken, TR = test-reference group and RT = reference-test group

Table 2. Average plasma concentration of glucosamine from 24 healthy volunteers after administration of test and reference product

Time (hr)	Mean ± SD (ng/ml)	
	Test product	Reference product
0 min	147.5730 ± 17.01	154.2593 ± 69.12
5 min	262.6906 ± 186.27	222.1618 ± 131.55
10 min	351.7290 ± 354.99	252.6047 ± 167.91
15 min	514.4770 ± 537.65	355.7727 ± 357.97
30 min	699.7758 ± 653.66	709.3775 ± 656.03
80 min	754.2704 ± 594.34	746.0196 ± 747.98
90 min	701.3929 ± 496.09	776.4454 ± 700.41
2 hr	797.7508 ± 643.06	871.9308 ± 840.48
4 hr	656.0250 ± 552.99	613.2088 ± 591.09
6 hr	389.6422 ± 371.42	424.4091 ± 509.57
8 hr	275.5496 ± 238.87	353.2981 ± 457.17
12 hr	222.0422 ± 118.10	190.4390 ± 104.29
24 hr	180.2893 ± 126.71	168.6388 ± 66.62

articular cartilage and synovial fluid, and, therefore, endogenous concentrations of glucosamine may be present in blood as a result of this and other connective tissue turnover.

Glucosamine was well tolerated. The clinical tolerability was good with both formulations. No serious adverse events were registered in the course of the trial. For effects of potassium contained in the test product, most of the subjects (87.50%) had normal level of serum potassium (3.5-5.0 mmol/L). Three subjects (12.50%) were reported to be abnormal serum potassium. Two subjects reported hypokalemia (one after taking the test and one after taking reference product) and another subject reported hyperkalemia (after taking the reference product). However, EKG was normal. Another adverse event found was palpitation (after taking the test product) but with no clinical significance. These events were determined not related to the study drug either test or reference products. These events were also reported to the

Table 3. Pharmacokinetic parameters of Reference (Viartril®-S) and Test (Flexsa®) with 90% confidence interval (CI) of the mean ratios (generic/original) of log transformed values

Pharmacokinetic parameters	Product (mean)		90% confidence interval (CI) ofthe mean ratios (generic/original)of log transformed values
	Reference (Viartril®-S)	Test (Flexsa®)	
t_{max} (h)	2.00	1.42	-
$t_{1/2}$ (h)	56.311	25.38	-
C_{max}(µg/ml)	1.12	0.99	93.69% (86.68%-113.32%)
$AUC_{0\rightarrow t}$(µg.h/ml)	6.56	6.24	97.73% (87.38%-112.62%)

t_{max} = Time to reach the peak plasma concentration (presented as median (range)); $t_{1/2}$ = Elimination half-life; C_{max} = Maximal plasma observed concentration; AUC_{0-t} = Area under the concentration-time curve from time zero to the last time where plasma concentration can be measured; $AUC_{0-\infty}$ = Area under the concentration time curve form time zero to infinity

Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University.

Conclusion

The bioequivalence study of 500 mg glucosamine sulfate capsule formulations in 24 healthy Thai male and female volunteers showed that glucosamine from test product (Flexsa) was equivalent to the reference product (Viartril®-S) in terms of rate and extent of absorption. The 90% confidence interval of ratio of logarithmically transformed C_{maxt} and $AUC_{0\rightarrow t}$ of glucosamine from both the test and reference products were in the accepted range of 80.00%-125.00%. The nonparametric statistical analysis for T_{max} was used to evaluate the difference between the median T_{max} as untransformed data of the two formulations. The criteria in the Friedman's test is 3.375 and the Chi-Square (0.95-1) is 3.841. There was no statistically significant difference of t_{max} between test and reference formulations ($p > 0.05$). There were no evidences of abnormal plasma serum potassium related to the study drug in both test and reference products although the test product (Flexsa®) contained KCl 2.18 mmol/500mg. Thus, test and reference glucosamine formulations can be considered bioequivalent from the obtained plasma glucosamine concentrations and their corresponding pharmacokinetic parameters.

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Conflict of interest

Dr. Pravit Akarasereenont was a principal investigator of the present study under Siriraj Clinical Research Unit, which received an independent research grant from Mega Lifesciences Company Ltd. Thailand.

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การศึกษาชีวสมมูลของยาแคปซูลกลูโคซามีนซัลเฟตขนาด 500 มิลลิกรัม ในอาสาสมัครไทยที่มีสุขภาพแข็งแรง

ประวิทย์ อัครเสรีนนท์, สมฤดี ฉัตรสิริเจริญกุล, ปิยาภัทร พงศ์นรินทร์, กอบธัม สถิรกุล, สุพรชัย กองพัฒนากูล

วัตถุประสงค์: *เพื่อศึกษาชีวสมมูลของยาแคปซูลกลูโคซามีนซัลเฟตขนาด 500 มิลลิกรัม ระหว่างผลิตภัณฑ์ยาสามัญกับผลิตภัณฑ์ยาต้นแบบ*

วัสดุและวิธีการ: *อาสาสมัครสุขภาพดีจำนวน 24 คน ได้รับการคัดเลือกให้มาเข้าร่วมการศึกษาที่ศูนย์วิจัยคลินิกศิริราช รูปแบบการศึกษาที่ใช้คือ randomized, single dose, two treatments, two periods, two sequences cross-over study อาสาสมัครแต่ละคนได้รับประทานยาแคปซูลกลูโคซามีนซัลเฟตขนาด 500 มิลลิกรัมทั้งสองตำรับ โดยมีระยะเวลา washout period นานอย่างน้อย 1 สัปดาห์ มีการเก็บตัวอย่างเลือดในช่วงเวลา 24 ชั่วโมง ตัวอย่างเลือดจะได้รับการวิเคราะห์โดยวิธี liquid chromatography-mass spectrometry ที่ได้รับการตรวจสอบความถูกต้องแล้ว*

ผลการศึกษา: *อาสาสมัครจำนวน 24 คนได้เข้ามาร่วมในการศึกษา ผลการศึกษาค่าทางเภสัชจลนศาสตร์โดยใช้การวิเคราะห์แบบ non-compartmental analysis โดยมีค่า 90% ความเชื่อมั่นของค่า log ของค่าเฉลี่ยของยาสามัญต่อยาต้นแบบ ดังนี้ ค่า C_{max} เท่ากับ 93.69% (86.68%-113.32%) และค่า AUC $_{0\rightarrow t}$ เท่ากับ 97.73% (87.38%-112.62%) ซึ่งทุกค่าอยู่ในเกณฑ์ของการยอมรับความเท่าเทียมกันในการศึกษาชีวสมมูล (80-125%) ยาทั้ง 2 รูปแบบมีความปลอดภัยและพบเหตุการณ์ไม่พึงประสงค์ที่ไม่รุนแรงเพียงเล็กน้อย*

สรุป: *ยาแคปซูลกลูโคซามีนซัลเฟตทั้ง 2 ตำรับมีชีวสมมูลซึ่งกันและกันในแง่ของเภสัชจลนศาสตร์ เมื่อศึกษาในอาสาสมัครไทยสุขภาพดี*