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Bioequivalence Study of 1,500 mg Glucosamine Sulfate in Thai Healthy Volunteers

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Abstract

Glucosamine sulfate is widely used to relieve symptoms from osteoarthritis. This study was conducted in order to determine pharmacokinetic and assessed the in-vivo bioequivalence of two different hard capsule formulations of glucosamine sulfate when administered as equal dose of 1,500 mg. The two formulations contain different salt form where reference product is NaCl and test product is KCl. A randomized, single dose, two-treatment, two-period, two-sequence crossover study was conducted. Twenty-six healthy volunteers were recruited at Siriraj Clinical Research Unit. Each subject received a dose of 1,500 mg glucosamine sulfate of both formulations with at least a week washout period. Blood samples were collected over 24 hrs after the oral administration. The plasma fractions were analyzed for glucosamine using LC-MS/MS. Twenty-six volunteers 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} (111.19%; ranged from 93.01%-132.92%) and AUC_{0-t} (107.24; ranged from 87.16%-131.93%) was not contained within the equivalence criteria of 80.00-125.00% (USFDA, 2003). However, this study showed the high intra-individual CV calculated from ANOVA for C_{max} and AUC_{0-24} (≥ 30%). Thus, based on equivalence limits of USFDA (2003), the test product is not bioequivalent to the reference product in terms of rate and extent of absorption. However, concerning the wider equivalence criteria for highly variable drug (EMEA, 2008), the test product is bioequivalent to the reference formulation in terms of rate and extent of absorption.

Keywords: Glucosamine; LC MS/MS; Bioequivalence; Pharmacokinetic

Introduction

Several clinical studies have indicated that glucosamine sulphate is effective in controlling osteoarthritis (OA) symptoms and disease progression [1-4]. In particular, two randomised, placebo-controlled, double-blind trials of 3-year duration in knee osteoarthritis (OA) patients, showed that this symptom-modifying effect is sustained over long-term treatment courses [5,6]. Moreover, many studies suggested that the drug also has a structure-modifying effect, as assessed by measurement of joint space narrowing using validated techniques on standardised plain radiographs [4,5,7-9]. Another recently completed trial (the GUIDE study) [10], confirmed the symptomatic results described above and indicated that, at the dose of 1500 mg once-a-day, crystalline glucosamine sulphate provided a symptomatic effect that was significantly superior to that observed after the administration of placebo [11]. On the other, some studies did not detect any benefit of glucosamine [12,13].

The formulation used in several studies [4,7,10] is the original crystalline glucosamine sulphate 1,500 mg once-a-day soluble powder preparation which is a prescription drug in most European and extra-European countries [11]. However, in United States, glucosamine is marketed as a dietary supplement to enhance the repair and synthesis of cartilage and connective tissue. It is reported that the U.S. retail market for nutritional supplements containing glucosamine or chondroitins is more than $1 billion per year; the demand for bulk glucosamine has been growing in excess of 20% annually, and global consumption exceeds 5000 metric tons [14].

Our previous studies [15] show that glucosamine sulfate containing KCl (500 mg capsule) is bioequivalent to glucosamine sulfate containing NaCl (2×250 mg capsules) in terms of rate and extent of absorption. This study is designed to evaluate the quality of the generic sachet formulation of glucosamine sulfate 1,500 mg in compare with the original formulation, Viartril®-S. The dosage of glucosamine sulfate 1,500 mg for both formulations were administered as a single dose to 26 healthy volunteers under a two-treatment, two-period, and two-sequence crossover study design with a minimum of one week washout period.

Materials and Methods

Glucosamine preparations

Test preparation: Flexsa® (Mega Lifesciences Company Ltd. Thailand) containing 1,500 mg glucosamine sulfate KCl powder for oral solution in sachet (Lot no. 8185, Mfg. date February 2007, Exp. date February 2009).

Reference preparation: Viartril®-S (Rottapharm Company Ltd., Ireland) containing 1,500 mg glucosamine sulfate NaCl powder for oral solution in sachet (Lot no. G07043A, Mfg. date 12 February 2007, Exp. date 28 February 2010).

Volunteers

Twenty-six healthy Thai volunteers aged between 18-45 years with a body mass index between 18-25 kg/m^2 were recruited at Siriraj Clinical Research Center, Siriraj Hospital. After explaining the details

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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 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 (1,500 mg sachet) or reference formulation (1,500 mg sachet) along with 240 ml of drinking water. Volunteers continued fasting for 2 and 4 hrs (water and food, respectively) after drug administration.

The subjects were closely observed to assess the adverse events. As test product containing KCl 6.58 mmol /1,500 mg, serum potassium was monitored at pre-dose, 12 and 24 hrs after test and reference products administration.

The study was approved by the independent Ethics Committee of 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 given written informed consent prior to starting study procedures.

Sample collection and glucosamine analysis

Nine 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), 10, 20, 30 min and 1, 1.5, 2, 3, 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.

Bioanalytical of plasma glucosamine was performed by using a validated high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) method in accordance with the USFDA guidelines [16]. Sample preparation was extracted by liquid-liquid extraction technique. Propanolol Hydrochloride was used as an internal standard (10 µg/ml). Briefly, 10 µl of internal standard was added in 500 µl of standard spiked sample (Calibrators and QC sample) and plasma unknown sample and then was mixed for 10 seconds. After well mixed, all samples were added and mixed with 1,000 µl of extract solvent (a mixture of acetronitrile and triethylamine, 3:1 (v/v)). Then, the samples were centrifuged at 10,000 rpm for 10 min. The organic layer was transferred into a new vial. All of organic phase was evaporated to dryness under nitrogen gas. The residual was re-dissolved with a dilution solvent (triethylamine : acetonitrile, 10 : 90 (v/v)) and injected into the LC-MS/MS system. The chromatographic separation was carried out on LC-MS/MS with C18, 2.5 µm (50×3.00 mm i.d.). A mobile phase consisting of acetonitrile and 0.025% formic acid (Gradient condition) was delivered with a flow rate of 0.2 ml/min. Mass spectra were obtained using a Quattro Micro™ mass spectrometer (Micromass Technologies, UK) equipped with electrospray ionisation (ESI) source in positive mode. The mass transition ion-pair for glucosamine $[M+H]^+$ ions was selected as m/z 179.90>161.71 and 179.90>143.70. The mass transition ion-pair for propanolol $[M+H]^+$ ions was selected as m/z 260.00>116.00. The data acquisition was ascertained by Masslynx 4.0 software. All validated results of our new developed LC-MS/MS method were found in the acceptable limit criteria of US FDA guidance with exhibit good accuracy and reproducibility. Calibration curve was linearity in the range of 0.05-10 µg/mL. The best linear fit was achieved with a 1/x weighting factor, showing a mean correlation coefficient (r^2) ≥ 0.999800. The lower limit of quantification (LLOQ) for the validated assay was 0.05 µg/ml. The limit of detection (LOD) was 0.0075 µg/ml. Mean recovery of extraction were 89.83-96.99% and 106.45% for glucosamine and internal standard, respectively. The intra- and inter-assay precision was 0.84%-9.79% and 1.20-3.49%, respectively. The percentage average of intra- and inter-assay accuracy was between 93.65%-102.67% and 97.25%-101.41%, respectively. The stability of glucosamine in plasma during sample processing at room temperature after 6 hours for short term stability and 30 days storage in −70°C for long term stability was within the acceptable limit of standard criteria. The % of variation of glucosamine for post-preparative stability was also showing no significant loss in the quantified values, indicating that samples should be processed within this period of time (10 hours.).

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, our 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, we 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 transformed 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 [17].

Results and Discussion

Twenty-six volunteers (13 males, 13 females) completed the study. Demographic characteristics of subjects among 2 groups seemed similar and shown in Table 1. The average plasma concentrations of at each time point from 26 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 t1/2 is 15.650 and that for reference is 23.231 which show less different. Because the distribution of t1/2 might not be a normal distribution, it may be better to use to geometric mean

Characteristics		Group 1 (TR Group) (*n*=13)	Group 2 (RT Group) (*n*=13)
Gender	Male Female	5 8	8 5
Age (years ± SD))		25 ± 4.8	25.4 ± 5.4
Weight (kg. ± SD)		60.3 ± 8.3	59.3 ± 8.8
Height (cm. ± SD)		170.4 ± 8.3	168.8 ± 6.6
Body Mass Index (kg/m^2 ± SD)		20.7 ± 1.8	20.7 ± 2.1
Vital signs	Temperature (ºC ± SD) Pulse (beat/minutes ± SD) Respiratory Rate (times/minute ± SD) Systolic Blood Pressure (mmHg ± SD) Diastolic Blood Pressure (mmHg ± SD)	36.7 ± 0.2 69.2 ± 12.6 19.9 ± 0.3 110.1 ± 10.2 69.7 ± 9.2	36.8 ± 0.2 70.1 ± 9.2 19.2 ± 1.5 105.4 ± 10.8 69.3 ± 12.2
Clinical laboratory	Hemoglobin (g/dl) Hematocrit (%) BUN (mg/dl) Creatinine (mg/dl) AST(units/L) ALT(units/L) ALP(units/L) LDH(units/L) Total bilirubin (mg/dL)	13.9 ± 1.2 41.9 ± 3.6 9.8 ± 2.1 0.8 ± 0.1 21.2 ± 4.9 16.5 ± 5.0 63.7 ± 21.2 279.4 ± 41.7 0.7 ± 0.3	13.9 ± 1.6 41.7 ± 4.8 12.0 ± 2.4 0.8 ± 0.2 20.2 ± 3.9 15.9 ± 4.4 60.4 ± 20.5 294.4 ± 36.3 0.7 ± 0.4
Serum electrolyte	Blood sugar (mg/dL) Na+(mmol/L) K+(mmol/L) Cl-(mmol/L) HCO3- (mmol/L)	79.1 ± 7.3 139.85 ± 0.95 4.05 ± 0.19 103.38 ± 1.82 27.31 ± 1.59	82.2 ± 5.4 140.08 ± 1.51 4.09 ± 0.29 101.92 ± 2.02 28.92 ± 2.81

Table 1: Demographic Data and Mean clinical laboratory of 26 Volunteers.

Time (Hr)	Mean ± S.D. (µg/ml)	
	Test Product	Reference Product
0 min	0.3926 ± 0.3010	0.3977 ± 0.3902
10 min	0.7294 ± 0.4687	0.7498 ± 0.5054
20 min	1.3282 ± 0.7588	1.3221 ± 0.7000
30 min	2.3212 ± 1.0313	2.1334 ± 1.0920
1 hr	3.5808 ± 1.8990	3.1109 ± 1.8677
1.5 hr	3.9149 ± 1.8257	4.1001 ± 2.5635
2 hr	4.3860 ± 2.5859	4.4126 ± 2.5472
3 hr	4.3759 ± 3.6053	3.8045 ± 2.5497
4 hr	3.5139 ± 3.0156	3.1042 ± 2.5472
6 hr	1.4454 ± 1.0619	1.2028 ± 0.8092
8 hr	0.7346 ± 0.4929	0.8093 ± 0.4353
12 hr	0.4910 ± 0.4152	0.4998 ± 0.2874
24 hr	0.5733 ± 0.4504	0.3938 ± 0.1627

Table 2: Average Plasma Concentration of Glucosamine from 26 Healthy Volunteers after Administration of Test and Reference Product.

for more log-normal distribution. The generic formulation had C_{max} at 0.99 µg/ml, t_{max} at 1.42 hrs while the original formulation had C_{max} at 1.12 µg/ml, t_{max} at 2.00 hrs (Table 1). Ninety percent CI of the mean ratios (generic/original) of the log transformed of the C_{max} and $AUC_{0\rightarrow t}$ 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 $AUC_{0\rightarrow t}$ 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 24 hrs sampling period after oral administration of 500 mg of the test and reference capsules are presented in Figure 1. It was found that the plasma profiles of the glucosamine concentration of both formulations exhibited closely 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 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 test product, most of subjects (87.50%) have normal level of serum potassium (3.5-5.0 mmol/L). Two adverse events of hypokalemia (once after taking

Pharmacokinetic Parameters	Product (Mean)		90% confidence interval (CI) of the mean ratios (generic/original) of log transformed values
	Reference (Viartril®-S)	Test (Flexsa®)	
t_{max} (h)	2.00	1.50	-
$t_{1/2}$ (h)	6.46	3.64	-
C_{max}(µg/ml)	4.51	4.95	111.19% (93.01% - 132.92%)
$AUC_{0\rightarrow\infty}$(obs) (µg.h/ml)	23.1	22.8	98.64% (78.77% - 123.52%)
$AUC_{0\rightarrow 24}$(µg.h/ml)	19.7	21.0	107.24% (87.16% - 131.93%)

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; $AUC_{0-\infty(obs)}$ = Area under the concentration time curve form time zero to infinity; AUC_{0-24} = Area under the concentration-time curve from time zero to 24 hrs, where plasma concentration can be measured

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.

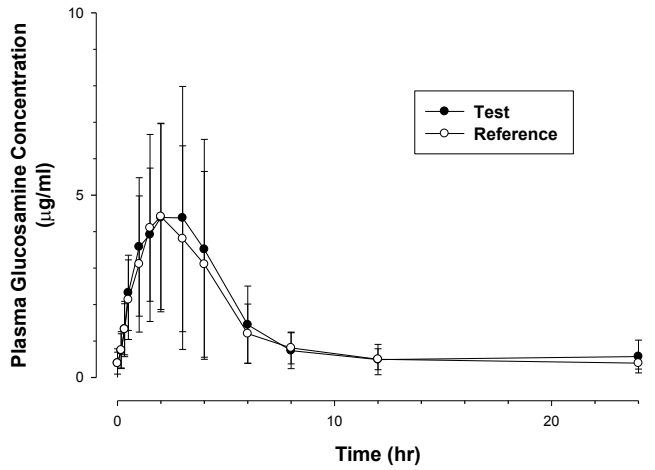

Figure 1: Average plasma concentration (µg/mL; mean ± SD) vs time curves of glucosamine after oral administration of 1,500 mg of the test (•) and reference capsules (○).

reference product and once after taking test product) and one event of dizziness (after taking test formulation) were reported in 3 volunteers. There were no clinically significant found and these three subjects received appropriate treatment and finally recovered. All of the adverse events were judged to be mild in intensity and were possibly related to the study drug. These events were also reported to Ethics Committee of Faculty of Medicine Siriraj Hospital, Mahidol University.

Conclusions

The bioequivalence study of two formulations of glucosamine sachet between the test product (Flexsa®-1500) and the reference product (Viartril®-S Sachet) in 25 healthy male and female volunteers was completed. This study was unbalanced and the ANOVA type III was calculated which demonstrated no significant sequence and treatment effects for all parameters. There was no significant period effect for $AUC_{0-\infty\ (obs)}$ but there were significant period effect for C_{max} and AUC_{0-24}. Moreover, subject nested in sequence effect was significant for all parameters that may be due to the fact that the bioequivalence was performed in small sample size. Non-parametric Friedman's test for T_{max} was demonstrated no significantly different between both formulations ($p>0.05$). The 90% confidence interval of the geometric mean ratio (test/reference) of C_{max}, AUC_{0-24} and $AUC_{0-\infty\ (obs)}$ was not contained within the equivalence criteria of 80.00-125.00% [18]. However, this study showed the high intra-individual CV calculated from ANOVA for C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ that may be due to the fact that glucosamine is endogenous substance which those concentration varied considerably between individuals (high inter-subject variability), especially between women [19,20]. Therefore, it can be indicated that glucosamine sulfate is a "highly variable drug" because a within subject variability of ≥ 30% in terms of the ANOVA-CV [21]. Based on the bioequivalence limit of highly variable drugs [22-24], it is acceptable for widening of confidence interval from 80.00%-125.00% to 75.00%-133.00% for C_{max} parameter. Therefore, it is reasonable to be accepting that C_{max} was entirely within equivalence limits of 75.00%-133.00%. Moreover, a wider range of AUC may be acceptable [25] since glucosamine occurs naturally in human tissues and results of previous studies show the very well tolerability profile [4-5]. Using this guideline, the 90% CI for AUC_{0-24} and $AUC_{0-\infty\ (obs)}$ ratios were also within the acceptance bioequivalence range of 75.00%-133.00%. Thus, Based on equivalence limits of USFDA [18], it can be concluded that the test product is not bioequivalent to the reference product in terms of rate and extent of absorption. However, concerning the wider equivalence criteria for highly variable drug [22-24], it may be accepted that the test product was bioequivalent to the reference formulation in terms of rate and extent of absorption.

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