Original Articles

Bioequivalence of Glucosamine 1,500 mg Sachet in Healthy Thai Male Volunteers

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Objvetive: *To compare the rate and extent of absorption of generic glucosamine powder formulation manufactured by Defence Pharmaceutical Factory with that of a reference product when given as equal labeled doses.* ***Materials and Methods:*** *A randomized, open label, single dose, two-treatment, two-period, two-sequence crossover design with 14 days washout period between phase I and phase II dosing was performed in 24 subjects randomly selected from healthy Thai male volunteers. Seventeen blood samples were drawn from 0 to 48 h. Plasma glucosamine concentrations were assayed using a validated LC-MS/MS method.* ***Results:*** *All of enrolled volunteers completed the study. Safety assessment was monitored throughout the study period. Treatments were generally well tolerated. No serious adverse effects were observed. The mean C_{max} for the reference and the test formulation were 782.88 ng/mL and 838.98 ng/mL, respectively. The mean AUC_{0-t} was 2,723.21 ng/mL for the reference and 2,719.95 ng/mL for the test formulation while the mean ($AUC_{0-\alpha}$) were 3,123.49 ng/mL and 3,051.63 ng/mL, respectively. The secondary pharmacokinetic parameters; T_{max}, (AUC_{0-t} /$AUC_{0-\alpha}$) %, $t_{1/2}$ and K_e were reported and ANOVA was used for statistical analyses.* ***Conclusion:*** *The 90% confidence intervals for the log-transformed ratios (Test/Reference) for the C_{max}, AUC_{0-t} and $AUC_{0-\alpha}$ were within the 80-125%. Therefore, the bioequivalence in term of the rate and the extent of drug absorption of the two products can be concluded.*

Key Words: ● *Bioequivalence* ● *Glucosamine* ● *LC-MS/MS* ● *Defence Pharmaceutical Factory*

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Introduction

Glucosamine is an amino monosaccharide, biosynthesized endogenously both in animals and humans by amination of glucose. It is structurally incorporated into mucopolysaccharides, glycoproteins and proteoglycans, notably those of the articular cartilage and synovial fluid, as it serves as an intermediate substrate in the synthesis of these molecules. In vitro studies have shown that glucosamine stimulate the production of proteoglycans and increase sulfate uptake by articular cartilage[1-3]. Glucosamine supplements are widely used for the treatment of osteoarthritis (OA). As a therapeutic agent for human use, glucosamine is supplied as crystalline glucosamine sulfate. Several clinical studies have indicated that glucosamine sulphate is effective in controlling

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osteoarthritis symptoms and disease progression. Most clinical trials of glucosamine supplementation in treating OA have used glucosamine sulfate because it is well absorbed from the gastrointestinal tract in its crystalline form, with linear pharmacokinetics reported at doses between 750 mg and 1,500 mg/day[4-5]. However, the bioavailability of glucosamine products in human is rarely determined. So, there is a need to accurately determine the bioavailability, especially because numerous products are marketed for the treatment of OA[6].

This study is designed to evaluate the quality of generic sachet formulation of glucosamine compared with the reference formulation. The generic can be prescribed interchangeably to the reference if this study shows the bioequivalence of the two formulations. The bioequivalent factors are the rate and extent of absorption. Considerably, this *in vivo* bioequivalence study is necessary for market application of the generic formulation. Furthermore, the benefit of availability of generic products in the market is the increasing of choices for drug prescription to the patients.

Meterials and Methods

Product information

Test Product: Glucosamine GPO® Sachet, Batch No. 953105, Expiration date 24/08/2012, manufactured by Defence Pharmaceutical Factory (DPF), Thailand.

Reference Product: Viartril-S® Sachet, Batch No. G10051A, Expiration date 28/02/2013, manufactured by Rottapharm Ltd., Ireland.

Clinical Study Design

A randomized, open label, single dose, two-treatment, two-period, two-sequence crossover design with 14 days washout period was performed. The study protocol and the related materials were approved by the Ethics Committee at Faculty of Medicine, Chulalongkorn University before the initiation of the study. Twenty four healthy adult Thai male volunteers were enrolled into the study. The enrolled subjects were required for 3 visits at the Railway Hospital (Burachatchaiyakorn) to complete the study; first visit was for screening, second and third visit were for period 1 and period 2 studies, respectively. Subjects were carefully monitored of vital signs and all adverse events throughout the study and all data were recorded in source document. If any adverse events occurred, the appropriate treatment and further investigation were performed.

In each period, each subject was physically examined, vital signs measured and re-assessed for eligibility to enroll into the study and randomly received a single dose of assigned formulation with 250 mL of water after an overnight fasting of at least 10 hours. In period 2 study, subjects were crossed over to the alternative product and the same study procedures as period 1 were performed. Five mL of blood samples were withdrawn for determination of glucosamine plasma concentration in each period. Blood samples were collected prior to dosing and then 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 9, 12, 24, 36 and 48 h after dosing. The whole blood was centrifuged and plasma samples were stored at -70°C until analysis of glucosamine level.

Pharmacokinetic Parameters analysis

The plasma concentrations of glucosamine were analyzed to evaluate the bioequivalence of two formulations. Analysis of glucosamine was performed using a validated ultra performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. Individual plasma drug concentration-time curve was plot and the pharmacokinetic parameters were calculated by noncompartmental methods. The maximal concentration (C_{max}) was determined directly from the obtained data of plasma concentration throughout the observed period of 48 h. The highest plasma concentration observed in each

period was therefore designated as C_{max}. The corresponding time that gave rise to the C_{max} was designated as T_{max}. The total area under plasma concentration-time curve ($AUC_{0-\alpha}$) was obtained from the summation of the area under plasma concentration-time starting from 0 to 48 h (AUC_{0-t}) calculated using the trapezoidal's rule and the extension of the area from 48 h to infinity ($AUC_{0-\alpha}$). Computed by C*/Ke; where, C* is the last measurable plasma drug concentration and Ke is the first order terminal elimination rate constant.

Statistical Analyses

Analysis of variance (ANOVA) for 2 x 2 crossover design was performed for log-transformed data; (AUC_{0-t}, $AUC_{0-\alpha}$ and C_{max}) and used to assess the effect of formulations, periods, sequences and subjects (sequence) on these parameters. The difference between two corresponding parameters was considered statistically significant for *p*-value equal to or less than 0.05. Ninty percents confidence interval (CI) for the ratios of geometric mean Test/Reference (T/R) for AUC_{0-t}, $AUC_{0-\alpha}$ and C_{max} was calculated based on least squares means from the ANOVA of log-transformed data.

The 90% confidence interval for the ratios of AUC_{0-t}, $AUC_{0-\alpha}$ and C_{max} values of the test preparation over those of the reference product were estimated. A non-parametric statistical analysis, Wilcoxon's signed rank test (for a paired experiment) was performed on T_{max} and considered to be significant between test and reference formulation when $p < 0.05$.

Equivalence Criteria

The 90% geometric confidence intervals of the ratios (T/R) of least squares means of log-transformed AUC_{0-t}, $AUC_{0-\alpha}$ and C_{max} should be within 80.00% to 125%.

Assay Methodology and Validation

A sensitive and specific liquid chromatographic-electrospray ionization mass spectrometric (LC-MS/MS) method was developed and validated for the determination of unchanged glucosamine sulfate in human plasma using ^{13}C glucosamine as the internal standard. The method was validated for specificity/ selectivity, linearity, precision, accuracy, recovery of extraction and stability according to the ASEAN guidelines for the conduct of bioavailability and bioequivalence studies 2009[7].

Study Phase Validation

In consideration of the analysis run, standard curve and sufficient QC samples should be used to ensure control of the assay. The number of QC samples to ensure proper control of the assay should be determined based on the run size. The placement of QC samples should be judiciously considered in the run. The accuracy and precision with which known concentrations of analyte in biological matrix should be demonstrated. This can be accomplished by analysis of replicate sets of analyte samples of known concentrations (QC samples) from an equivalent biological matrix. At a minimum, three concentrations representing the entire range of the standard curve should be studied: one within 3x the lower limit of quantification (LLOQ) (low QC sample), one near the center (middle QC), and one near the upper boundary of the standard curve (high QC). The acceptable limit determination at 4 from 6 QC sample should be within $\pm$ 15% of the nominal value and 2 from 6 QC sample should be exceed $\pm$ 15% of the nominal value but should not be same concentrations.

Results and Discussion

Clinical Study Results

All volunteers gave written, informed consent before any study-related screening procedures were performed. The screening and clinical period was on 6-22 November 2010. Twenty four subjects aged between 20 to 34 years (mean 24.50 $\pm$ 4.04 years), weight 54 to 79 kg (mean 65.42 $\pm$ 6.63 kg), height between 1.65 to 1.85 (mean 1.74 $\pm$ 0.06) and the body mass index between 18.91 to

23.94 (mean 21.53 ± 1.55) were enrolled and randomly divided into 2 groups, TR and RT groups. Each group consisted of 12 subjects. None of them were smoking or drinking and currently taking anyone of medicine.

Safety assessment included the incidence of adverse events were monitored and recorded in case report forms based on volunteer interview and physical examination. No abnormality was observed in terms of blood pressure, heart rate and body temperature.

Validation and Analytical Results

This assay was used to assess a bioequivalence study of two sachet formulations of glucosamine sulfate given as a single oral dose of 1,500 mg to healthy Thai volunteers. The summary of validation and analytical results were shown in Table 1.

Pharmacokinetic Analysis

The linear plot of the mean drug concentrations versus time in 24 study subjects were illustrated in Figure 1. The individual and average pharmacokinetic parameters of glucosamine for test and reference product included the maximum observed plasma concentration (C_{max}), the area under the plasma concentration-time curves from 0 to 48 h (AUC_{0-t}), the area under the plasma concentration-time curves from 0 to infinity ($AUC_{0-\alpha}$), the time taken to peak (T_{max}), plasma elimination half life ($t_{1/2}$) and the terminal rate constant (l_z) are reported (Table 2). The mean C_{max} for the reference and the test formulation were 782.88 ng/mL and 838.98 ng/mL, respectively. The mean AUC_{0-t} was 2,723.21 ng/mL for the reference and 2,719.95 ng/mL for the test formulation while the mean ($AUC_{0-\alpha}$) were 3,123.49 ng/mL and 3,051.63 ng/mL, respectively. The mean $t_{1/2}$ was 1.75 h and 1.42 h for the reference and the test formulation with the K_{el} of 0.47 and 0.57, respectively. The mean T_{max} for the reference was 2.25 h and 2.58 h for the test formulation. The mean ratios between test and reference formulation (T/R) was 1.0771 for (C_{max}) whereas these ratios were 1.0299 and 1.0110 for (AUC_{0-t})

Table 1 Summary of validation and analytical results

Information Requested	Data
Analyte	Glucosamine
Internal standard (IS)	^{13}C-Glucosamine
Limit of quantitation	80.22 ng/mL
Average recovery of drug (%)	77.79%
Average recovery of IS (%)	76.83%
Standard curve concentrations (units/mL)	80-2000 ng/mL
Regression analysis	Linear regression with weighting $1/x^2$
QC concentrations (units/mL)	240, 800, 1800 ng/mL
QC Intraday precision range (%)	1.59 % - 4.53 %
QC Intraday accuracy range (%)	90.10 %-114.57 %
QC Interday precision range (%)	1.21 % - 7.29 %
Freeze-thaw stability (cycles)	3 cycles
Long-term stability (days)	12 weeks @ 70°C
Short-term stability (hrs)	6 hours @ room temperature
Post-preparative stability (hrs)	12 hours @ room temperature
Stock-solution stability (days)	6 hours and 12 weeks for glucosamine and internal standard, respectively @ 4°C
Selectivity	No interfering peaks noted in blank plasma samples

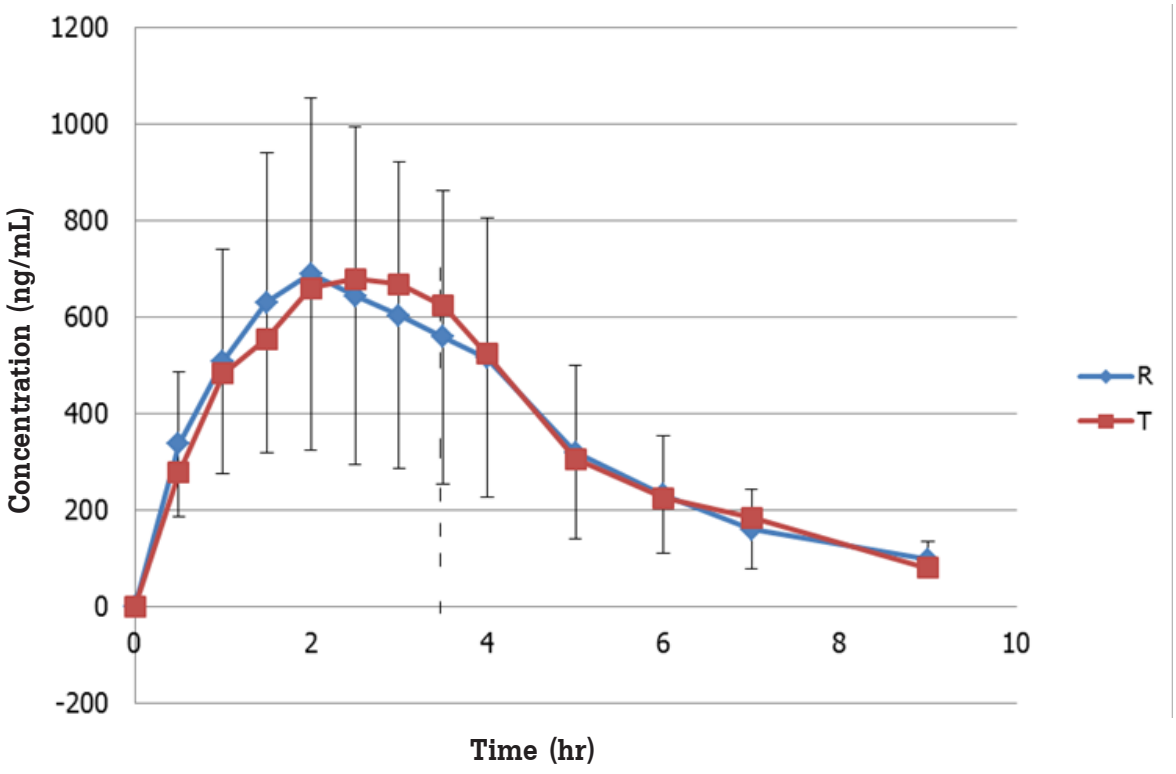

Figure 1 Geometric Mean of Plasma Concentration-time Profile of Glucosamine; Normal Plot (N = 24)

Table 2 Descriptive Statistics

Product/Statistics	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-t} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)	%ext. AUC	$t_{1/2}$ (h)	K_{el} (1/h)
Formulation T							
Mean	2.58	838.98	2,719.95	3,051.63	12.86	1.42	0.57
CV%	31.61	64.67	60.19	54.73	71.92	39.59	45.92
N	24	24	24	24	24	24	24
Formulation R							
Mean	2.25	782.88	2,723.21	3,123.49	14.60	1.75	0.47
CV%	34.68	45.62	49.01	41.95	89.09	59.42	31.01
N	24	24	24	24	24	24	24
Ratio of least Square mean							
T/R (%)	-	100.01	99.44	99.08	-	-	-
90% Confidence Intervals (T/R)							
Lower Limit:	-	88.17	84.66	80.95	-	-	-
Upper Limit:	-	113.04	107.43	106.04	-	-	-
Power(%)	-	0.80	0.95	0.95	-	-	-
Intra-subjects CV(%)	-	25.46	24.50	27.85	-	-	-

*Log-transformed parameters, the anti-logarithm of the geometric mean is reported

and ($AUC_{0-\alpha}$), respectively.

Statistical Analyses

The pharmacokinetic parameters were subjected to a comparative statistical evaluation by determining the position of the 90% confidence intervals for the individual ratios "test/reference" by least square means of ANOVA of logarithmically transformed data for C_{max} and AUC to obtain the residual error. The ANOVA model included sequence, subject nested within sequence and period as factors.

The ANOVA results (Table 3) demonstrated no significant sequence and formulatiom effects for the log-transformed data of C_{max}, AUC_{0-t} and $AUC_{0-\alpha}$. However, subject nested within sequence effect was significant for all parameter which may result form high intersubject variability. Significant of period effect may be due to the specimen transport to the laboratory is not good enough.

Two drug products are considered to be bioequivalent if they exhibit a comparable rate and extent of absorption when administered in the same molar dose and under similar experimental conditions. Bioequivalent formulations are usually considered to be therapeutically equivalent[8]. AUC is accepted as a good indicator of the extent of absorption. US FDA 2003generally accepts that the AUC and C_{max} of a test formulation should lie within the 20% deviation of the reference formulation, so that the ratio of AUC and C_{max} should be between 0.80 and 1.25 for logarithm-transformed data[9].

Bioequivalence statistics 90% confidence interval of geometric mean ratio of bioavailability parameters between the test and reference formulation was presented

Table 3 ANOVA table

Sources	**Cmax (Ln transformed date)**					
	D.F.	**SS**	**MS**	**Fc**	**Ft**	**Sig**
Subject (Sequence)	22	11.4006	0.5182	8.2488	2.05	S
Sequence	1	0.0142	0.0142	0.0274	4.30	NS
Period	1	0.3722	0.3722	5.9246	4.30	S
Treatment (Formulation)	1	0.000033	0.000033	0.000521	4.30	NS
Error	22	1.3821	0.0628			
Total	47	13.1691				
Sources	**AUC0-t (Ln transformed data)**					
Subject (Sequence)	22	12.9771	0.5899	10.1162	2.05	S
Sequence	1	0.0189	0.0189	0.0320	4.30	NS
Period	1	0.4549	0.4549	7.8015	4.30	S
Treatment (Formulation)	1	0.0270	0.0270	0.4630	4.30	NS
Error	22	1.2828	0.0583			
Total	47	14.7609				
Sources	**AUC0-∞(Ln transformed data)**					
Subject (Sequence)	22	9.4356	0.4289	5.7429	2.05	S
Sequence	1	0.0001	0.0001	0.0002	4.30	NS
Period	1	0.3573	0.3573	4.7843	4.30	S
Treatment (Formulation)	1	0.0700	0.0700	0.9373	4.30	NS
Error	22	1.6430	0.0747			
Total	47	11.5060				

Table 4 Ratio of Log-tranformed Least Square Mean and 90% confidence interval table

Parameter	Ratio of Least Square Mean	90% C.I.
Ln Cmax	101.01	88.17 - 113.04
Ln AUC0-t	99.44	84.66 - 107.43
Ln AUC0-inf	99.08	80.95 - 106.04

in Table 4. The statistical analysis obtained from this study showed that the point estimate (90% confidence interval) of the geometric mean ratio (test/reference) of C_{max}, AUC_{0-t} and $AUC_{0-\alpha}$ was entirely within the equivalence criteria (80.00 - 125.00%) which was 101.01% (88.17 - 113.04%) for C_{max} ratios, 99.44% (84.66 - 107.43%) for AUC_{0-t} ratios and 99.08% (80.95 - 106.04%) for $AUC_{0-\alpha}$ ratios. Accordingly, this study confirms that the sample size was adequate with the power of all parameters were above 80%. It can be concluded that both glucosamine sachet formulations established bioequivalence in terms of rate and extent of absorption.

Statistical Analysis for T_{max}

The nonparametric statistical method as Wilcoxon's signed rank test was used to evaluate the difference between the mean of untransformed data of two formulations. The null hypothesis of test (H_0) is signified that there is no difference between means. The result showed that there was no statistical difference of the mean T_{max} between the test and reference formulation ($p > 0.05$)

Conclusion

The bioequivalence study of a single dose of the test product glucosamine sulfate 1,500 mg sachet (Glucosamine GPO®), compared to the reference product (Viatril-S®) in 24 healthy male volunteers was completed. The ANOVA table results demonstrated no significant sequence and treatment effects for the log-transformed data of C_{max}, AUC_{0-t} and $AUC_{0-\alpha}$. However, period effects and subject nested within sequence effect were significant for all parameter which may result in the high intersubject variability in subject being assigned between 2 sequences. The 90% confidence interval of the logarithmic transformed of C_{max}, AUC_{0-t} and $AUC_{0-\alpha}$ were entirely contained in 80.00-125.00% with the power more than 80%. Non-parametric Wilcoxon's signed rank test (for a paired experiment) for T_{max} was also demonstrated no significantly different between both formulations ($p > 0.05$). In conclusion, these findings indicate that the test formulation is bioequivalent to reference formulation according to both the rate and extent of absorption.

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การศึกษาชีวสมมูลของยาผงกลูโคซามีนซัลเฟต ขนาด 1,500 มิลลิกรัม ในอาสาสมัครชายไทยที่มีสุขภาพแข็งแรง

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วัตถุประสงค์การศึกษา: *เพื่อเปรียบเทียบอัตราและปริมาณการดูดซึมยาเข้าสู่ร่างกายของยาผงกลูโคซามีนซัลเฟต ขนาด 1,500 มิลลิกรัม ที่ผลิตโดยโรงงานเภสัชกรรมทหารเปรียบเทียบกันยาต้นแบบในขนาดเดียวกัน* ***วัสดุและวิธีการ:*** *ทำการศึกษาแบบข้ามสลับแบบสองทาง (Two-Way Crossover Design with two-period, two-sequence and two-treatment) ในอาสาสมัครชายไทยสุขภาพดี 24 คน โดยอาสาสมัครแต่ละคนจะได้รับการสุ่มเพื่อให้ยาแต่ละตำรับ ระยะห่างของการศึกษาแต่ละครั้ง (wash out period) อยู่ที่ 14 วัน ทำการเก็บตัวอย่างเลือดในแต่ละครั้งของการศึกษาทั้งหมด 17 ครั้งตั้งแต่เวลา 0 ถึง 48 ชั่วโมง การวิเคราะห์หาปริมาณกลูโคซามีนในพลาสมาใช้ LC-MS/MS ตามวิธีการวิเคราะห์ที่ผ่านการตรวจสอบความถูกต้อง* ***ผลการศึกษา:*** *อาสาสมัครได้รับการเฝ้าระวังความปลอดภัยจากแพทย์ในระหว่างการศึกษา ไม่พบรายงานการเกิดอาการข้างเคียงที่รุนแรง ค่าเฉลี่ย C_{max} ของยาต้นแบบและยาที่ศึกษามีค่า 782.88 ng/mL และ 838.98 ng/mL ตามลำดับ ค่าเฉลี่ย AUC_{0-t} ของยาต้นแบบและยาที่ศึกษามีค่า 2,723.21 ng/mL และและ 2,719.95 ng/mL และค่าเฉลี่ย ($AUC_{0-\alpha}$) ของยาต้นแบบและยาที่ศึกษามีค่า 3,123.49 ng/mL และ 3,051.63 ng/mL ตามลำดับ สำหรับค่าพารามิเตอร์ทางเภสัชจลศาสตร์ของ T_{max} ($AUC_{0-t}/AUC_{0-\alpha}$)% $t_{1/2}$ และ K_e มีการรายงานผลและ ใช้ ANOVA ในการคำนวณค่าทางสถิติ* ***สรุปผลการศึกษา:*** *90% confidence intervals สำหรับ log-transformed ratios (Test/Reference) ของค่า C_{max} AUC_{0-t} และ $AUC_{0-\alpha}$ อยู่ในช่วง 80-125% จึงสรุปได้ว่าผลิตภัณฑ์ที่ทำการศึกษามีชีวสมมูลกันทั้งในแง่ของอัตราและปริมาณการดูดซึมยาเข้าสู่ร่างกาย*

Key Words: ● *Bioequivalence* ● *Glucosamine* ● *LC-MS/MS* ● *Defence Pharmaceutical Factory*

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