Bioequivalence Study of the Two 1.5 g Cefoperazone and Sulbactam IM Injections in Thai Healthy Male Volunteers

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Objective: *To perform a bioequivalence study of the two 1.5 g cefoperazone (1.0 g) and sulbactam (0.5 g) between Cefper® and Sulperazon® injections.*
Material and Method: *The present study was performed in 24 Thai healthy male volunteers who were intramuscularly injected a single dose of 1.5 g cefoperazone and sulbactam. A single dose, two periods, two sequences, double blind randomized crossover with a one-week washout period was used. Blood samples were collected before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after intramuscular injection and determined for cefoperazone and sulbactam plasma concentration by validated HPLC- UV methods. The pharmacokinetic parameters were analyzed by noncompartmental analysis and the ANOVA was carried out.*
Results: *T_{max} of both cefoperazone and sulbactam for volunteers who were injected with either Cefper® or Sulperazon® injection were not significantly different ($p > 0.05$). The 90% confidence intervals of the log of ratio of either C_{max} or AUC_{last} or AUC_{inf} of both cefoperazone and sulbactam between 1.5 g Cefper® and Sulperazon® injections were within the bioequivalence range of 0.80-1.25.*
Conclusion: *The 1.5 g cefoperazone and sulbactam injection of Cefper® and Sulperazon® used in the present study are bioequivalent.*

Keyword: *Bioequivalence, Cefoperazone, Sulbactam, Cefper®, Sulperazon®*

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Sulperazon® is a fixed combination of the sodium salts of cefoperazone and sulbactam (a beta lactamase inhibitor); sulbactam synergistically expands cefoperazone's spectrum of activity against many strains of beta lactamase-producing bacteria[1-3].

Combination of cefoperazone sodium and sulbactam sodium is used parenterally for the treatment of skin and skin structure, intra-abdominal (including peritonitis), urinary tract, respiratory tract, gynecologic (including pelvic inflammatory disease and endometritis) infections, and septicemia caused by susceptible bacteria[3,4].

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Combination of cefoperazone sodium and sulbactam sodium is commercially available for parenteral administration as a sterile powder containing a 1:1 ratio of cefoperazone to sulbactam and 1:0.5 ratio of cefoperazone to sulbactam.

As cefoperazone sodium and sulbactam sodium are very useful in the treatment of skin and skin structure, intra-abdominal, and gynecologic infections, there are many preparations of cefoperazone and sulbactam injections available in the market including Sulperazon® injection, an innovative product, and products made in Thailand. Bioequivalence study between the products made in Thailand and Sulperazon® injection will give information to physicians, pharmacists, and drug consumers for appropriate selection of drug. Confidence in therapeutic efficacy will be

enhanced. If the test products are bioequivalent, the patients can have an alternative drug for use, giving rise to cheaper treatment.

The objective of the present study was to perform a bioequivalence study between the product made in Thailand, Cefper® injection from Biolab Co., Ltd., Thailand and the innovative product, Sulperazon® injection from Pfizer.

Material and Method

Subjects

Twenty-four healthy Thai male volunteers aged between 19-26 years old and their body mass indexes within 19-24 kg/m^2 participated in the present study. Volunteers were in good health based on medical history, physical examination, routine blood test including complete blood count with differential count and blood chemistry profiles as well as having a negative screening test for hepatitis B surface antigen and anti-HIV. Volunteers with known contraindication or hypersensitivity to either cefoperazone or sulbactam were excluded as well as those with a known history of alcohol consumption or cigarette smoking. No drug was allowed 1 week before the study period to avoid the effects of inducing or inhibiting hepatic metabolizing enzyme and the risk of drug interactions. The present study was approved by the Ethical Review Committee, Faculty of Pharmacy, Chiang Mai University, Thailand. All volunteers signed the informed consent forms prior to participating in the present study.

Study drug

Test product: Cefper® injection, Lot No. CPI-5 MFD: 01/03/2008, Exp. Date 01/03/2010, Biolab Co, Ltd. Thailand, in the dosage of 1.5 g (cefoperazone 1 g + sulbactam 0.5 g)

Reference product: Sulperazon® injection, Lot No. 739431, MFD. 04-2007, Exp. Date 04-2009, Pfizer Italia, Italy, in the dosage of 1.5 g (cefoperazone 1 g + sulbactam 0.5 g)

Method of drug administration

The present study was performed in 24 Thai healthy male volunteers who were intramuscularly injected a single dose of 1.5 g cefoperazone and sulbactam (1.0 g of cefoperazone and 0.5 g of sulbactam). A single dose, two treatments, two periods, two sequences, double blind randomized crossover with one-week washout period was used. After the over night fast, each volunteer received a single intramauscular injection of either Cefper® or Sulperazon®. Blood samples were collected at pre-dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after drug administration and, then, the plasma was separated immediately by centrifugation. The plasma samples were stored at -40°C and analyzed for cefoperazone and sulbactam content within 5 days after blood samples collection.

Determination of the plasma cefoperazone and sulbactam concentrations

Plasma cefoperazone and sulbactam contents were analyzed using a validated High Performance Liquid Chromatography[5-8]. The HPLC system consisted of a C18 column (Hypersil®, 250 x 4 mm, 5 μm (Agilent Technologies, USA) with column temperature of 25°C. Column eluate was monitored for cefoperazone at 230 nm wavelength and for sulbactam at 220 nm wavelength. The isocratic mobile phase were acetonitrile: methanol: 5 mM tetrabutylammonium hydroxide (13:9:78), pH = 6.4 for cefoperazone and acetonitrile: methanol: 5 mM tetrabutylammonium hydroxide (18:5:77), pH = 6.0 for sulbactam. The plasma sample was prepared by liquid-liquid extraction. Validation of the analysis method e.g. specificity, accuracy and precision, lower limit of quantification (LLOQ), linearity, stability, extraction recovery, was performed before using for drug analysis. Standard curves were performed every day of analysis.

Pharmacokinetic parameters and statistical analysis

Plasma concentration - time curves were plotted. Pharmacokinetic parameters were determined. Maximum plasma concentration (C_{max}) which represents the maximum extent of the drug approached blood circulation and time to reach the peak concentration (T_{max}) which represents the rate of the drug approached blood circulation were taken from the raw data. Area under the concentration time curve (AUC), which represents the extent of the drug approached blood circulation was determined using trapezoidal rule. The 90% confidence interval[9] was calculated as follows.

$$90\%\ CI = \Delta \pm t_{0.10}, \sqrt{EMS\ (2/n)}$$

Where Δ is a difference in means of log transformed pharmacokinetic parameters (C_{max} or AUC) between the test product and the reference, t0.10, ν is the tabulated two-tail t value for a 90% CI, ν is a degree of freedom of the error mean square obtained from the ANOVA table, EMS is the error mean square from the ANOVA table, and n is the number of subjects. Anti-logarithm of the calculated confidence interval will yield an exact confidence interval for the ratio. Bioequivalence between the test and reference products would be

stated if 90% CI of the ratio of the log transform of the pharmacokinetic parameters, i.e. C_{max} and AUC, were in the range of 0.80 –1.25 (USP 28, Thai FDA)[1,10].

Results

Bioequivalence study of cefoperazone between Cefper® and Sulperazon® injections

No side effect was noticed in all the volunteers who received any of Cefper® or Sulperazon® injection. For the analysis of cefoperazone by HPLC, retention time of cefoperazone and rosigitazone maleate (internal standard) were about 8.7 and 10.8 minutes respectively. Intraday variation (1-150 μg/mL) and interday variation (3-150 μg/mL) of the analysis method were validated. The lower limit of quantification (LLOQ) was less than 1.0 μg/mL.

Average cefoperazone concentration - time curves of Cefper® and Sulperazon® injections are shown in Fig. 1. Pharmacokinetic parameters, i.e. AUC_{last}, AUC_{inf}, C_{max}, T_{max} are collated in Table 1.

T_{max} of cefoperazone for volunteers who were injected with either Cefper® (1.40 ± 0.84 hr) or Sulperazon® (1.16 ± 0.64 hr) injections were not significantly different ($p > 0.05$, Wilcoxon Signed Rank test). AUC_{last} were 227.25 ± 58.74 and 228.77 ± 68.93 μg.hr/mL and AUC_{inf} were 250.94 ± 65.57 and 249.28 ± 77.13 μg.hr/mL for Cefper® and Sulperazon® injections, respectively. Average C_{max} were 57.65 ± 19.10 μg/mL and 57.69 ± 17.12 μg/mL for Cefper® and Sulperazon® injections, respectively. The 90% CI of the ratio of AUC_{last}, AUC_{inf} and C_{max} between Cefper® and Sulperazon® were 0.955-1.065, 0.945-1.110, and 0.935-1.055, respectively

Bioequivalence of sulbactam between Cefper® and Sulperazon® injections

No side effect was noticed in all the volunteers who received any of Cefper® or Sulperazon® injection. For the analysis of sulbactam by HPLC, retention times of sulbactam and enalapril maleate (internal standard) were about 5.7 and 7.0 minutes respectively. Intraday variation (0.5-90 μg/mL) and interday variation (1.5-90 μg/mL) of the analysis method were validated. The lower limit of quantification was less than 0.5 μg/mL.

Average sulbactam concentration - time curves of Cefper® and Sulperazon® injections are shown in Fig. 2. Pharmacokinetic parameters, i.e. AUC_{last}, AUC_{inf}, C_{max}, T_{max} are collated in Table 2.

T_{max} of sulbactam for volunteers who were injected with either Cefper® (0.66 ± 0.34 hr) or Sulperazon® (0.63 ± 0.30 hr) injections were not significantly different ($p > 0.05$, Wilcoxon Signed Rank test). AUC_{last} were 22.04 ± 4.23 and 21.98 ± 4.29 μg.hr/mL and AUC_{inf} were 23.78 ± 4.51 and 23.71 ± 4.22 μg.hr/mL for Cefper® and Sulperazon® injections, respectively. Average C_{max} were 13.97 ± 4.11 μg/mL and 14.37 ± 4.43 μg/mL for Cefper® and Sulperazon® injections, respectively. The 90% CI of the ratio of AUC_{last}, AUC_{inf} and C_{max} between Cefper® and Sulperazon® were 0.940-1.070, 0.941-1.068, and 0.895-1.066, respectively.

Discussion

Bioequivalence study of cefoperazone and sulbactam in Cefper® and Sulperazon®, 1.5 g IM injections, in 24 Thai healthy male volunteers were determined. The present study was conducted with

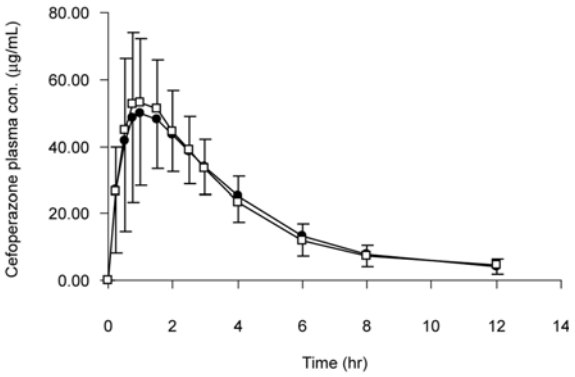

Fig. 1 Average cefoperazone plasma concentration at various sampling times of all volunteers after 1.5 g IM injection. (●) Cefper® injection, (□) Sulperazon® injection (n = 24)

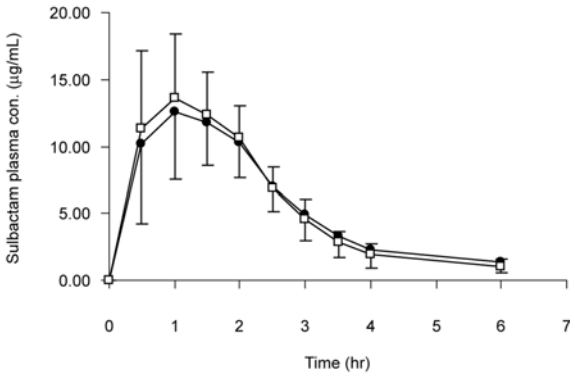

Fig. 2 Average sulbactam plasma concentration at various sampling times of all volunteers after 1.5 g IM injections. (●) Cefper® injection, (□) Sulperazon® injection (n = 24)

Table 1. Pharmacokinetic parameters for cefoperazone from Cefper® and Sulperazon® injections and 90% confidence interval (n = 24)

Pharmacokinetic parameters	Cefper®	Sulperazon®	90% confidence interval
AUC_{last}* (μg.hr/mL)	227.25 ± 58.74	228.77 ± 68.93	0.955-1.065
AUC_{inf}* (μg.hr/mL)	250.94 ± 65.57	249.28 ± 77.13	0.945-1.110
C_{max}* (μg/mL)	57.65 ± 19.10	57.69 ± 17.12	0.935-1.055
T_{max} (hr)	1.40 ± 0.84	1.16 ± 0.64	

* Log (base 10) data transformation
Data were presented with mean ± standard deviation

Table 2. Pharmacokinetic parameters of sulbactam from Cefper® and Sulperazon® injections and 90 % confidence interval (n = 24)

Pharmacokinetic parameters	Cefper®	Sulperazon®	90% confidence interval
AUC_{last}* (μg.hr/mL)	22.04 ± 4.23	21.98 ± 4.29	0.940-1.070
AUC_{inf}* (μg.hr/mL)	23.78 ± 4.51	23.71 ± 4.22	0.941-1.068
C_{max}* (μg/mL)	13.97 ± 4.11	14.37 ± 4.43	0.895-1.066
T_{max} (hr)	0.66 ± 0.34	0.63 ± 0.30	

* Log (base 10) data transformation
Data were presented with mean ± standard deviation

a single dose, two treatments, two periods, two sequences, double blind randomized crossover with one-week washout period. The results demonstrated that T_{max} of both cefoperazone and sulbactam for volunteers who were injected with either Cefper® or Sulperazon® injection were not significantly different ($p > 0.05$), and 90% confidence interval of the ratio of AUC_{last}, AUC_{inf} and C_{max} for both cefoperazone and sulbactam between Cefper® and Sulperazon® injections were in the range of 0.80 to 1.25 as required by Thai FDA and USP 28. Therefore, bioequivalence of cefoperazone and sulbactam are indicated between Cefper® and Sulperazon® IM injections.

Conclusion

The pharmacokinetic parameters of cefoperazone and sulbactam from Cefper® and Sulperazon®, 1.5 g IM injection, in 24 Thai healthy male volunteers were determined. The results demonstrated that T_{max} of both cefoperazone and sulbactam for the volunteers who were injected with either Cefper® or Sulperazon® injection were not significantly different. The 90% confidence interval of the ratio of AUC_{last}, AUC_{inf} and C_{max} for both cefoperazone and sulbactam between Cefper® and Sulperazon® were in the range of 0.80 to 1.25 as required by Thai FDA and USP 28. Therefore, bioequivalence is indicated between Cefper® and Sulperazon® (1.5 g, 1.0 g cefoperazone and 0.5 g sulbactam) in terms of the rate and extent of drug approached the systemic circulation. It should be noted that this finding was limited only to the lot used in the present study. In addition, the present study was designed as a single dose administration in healthy volunteers, therefore the therapeutic effect of long-term use in patients should be considered.

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การศึกษาชีวสมมูลของยาฉีดเข้ากล้ามสูตรผสม cefoperazone และ sulbactam 1.5 กรัม ในอาสาสมัครชายไทยสุขภาพดี

สยาม แก้ววิชิต, ทรงวุฒิ ยศวิมลวัฒน์, วรรณดี แต้โสตถิกุล, วิรัตน์ นิวัฒนนันท์, ชฎารัตน์ ดวงรัตน์, โชคชัย วงศ์สินทรัพย์, ศตวรรษ ทองสวัสดิ์, ปาริชาติ สาลี

วัตถุประสงค์: *เพื่อศึกษาชีวสมมูลของยาสูตรผสม cefoperazone (1 กรัม) และ sulbactam (0.5 กรัม) 1.5 กรัม ระหว่างยา Cefper® กับ Sulperazon®*

วัสดุและวิธีการ: *ทำการศึกษาในอาสาสมัครชายไทยสุขภาพดี 24 คน โดยการฉีดยาสูตรผสม cefoperazone และ sulbactam ในขนาด 1.5 กรัม เข้ากล้ามให้อาสาสมัคร การศึกษาเป็นแบบการให้ยาเพียงครั้งเดียว สองช่วง โดยเว้นระยะห่างกัน 1 สัปดาห์ สองลำดับ สุ่มสลับ ปกปิดสองด้าน เก็บตัวอย่างเลือด ของอาสาสมัคร ก่อนให้ยา และที่เวลา 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8 และ 12 ชั่วโมงหลังการให้ยา วิเคราะห์หาความเข้มข้นของยาในพลาสมาโดยวิธี ไฮเพอฟอร์มานลิควิดโครมาโตกราฟี และวิเคราะห์ค่าตัวแปร ทางเภสัชจลนศาสตร์แบบ noncompartment และ ANOVA*

ผลการศึกษา: *ระดับยาในเลือดสูงสุด (T_{max}) ของยา cefoperazone และ sulbactam จากการให้ยา Cefper® และ Sulperazon® ไม่แตกต่างกัน ($p > 0.05$) และมีช่วงค่าความเชื่อมั่นที่ 90% ของอัตราส่วนของความเข้มข้นสูงสุดของยาในพลาสมา (C_{max}) หรือ พื้นที่ใต้เส้นโค้งของกราฟ ความสัมพันธ์ระหว่างความเข้มข้นของยาในพลาสมากับเวลาถึงเวลาที่เก็บตัวอยางครั้งสุดท้าย (AUC_{last}) หรือ ถึงเวลาอนันต์ (AUC_{inf}) ในรูปลอการิทึมระหว่างยา Cefper® และ Sulperazon® อยู่ในช่วงที่กำหนดว่าเท่าเทียมกัน (0.80-1.25)*

สรุป: *ยา Cefper® และ Sulperazon® ในขนาด 1.5 กรัม ที่ใช้ในการศึกษาครั้งนี้มีชีวสมมูลกัน*