MATERIALS AND METHODS

1. SUBJECTS

A total of 24 healthy volunteers, twelve in each group participated in the pharmacokinetic study of cefoxitin and ceftazidime. Their median ages were 26 years old (Table 1 and 2). All had no history of severe adverse reactions to cephalosporins and/or penicillins. None had evidence of any illness or underlying disease based on physical examination and blood chemistry (liver function test, blood urea nitrogen, creatinine and complete blood count) (Table 1 and 2). They were instructed to be abstinent from any drugs that might interfere with the pharmacokinetics of cefoxitin and ceftazidime for at least one week before and during the study days. Alcohol and xanthine-containing foods or drinks were not allowed for 24 hours before and during the study days. All twelve female volunteers were not pregnant, confirmed by a urine pregnancy test and using an adequate method of contraception during the study period. Subjects were enrolled in the studies after given written informed consent approved by The Human Experimentation Committee of the Faculty of Medicine, Chiang Mai University.

2. STUDY PLAN AND METHOD OF DRUG ADMINISTRATION

The two studies were designed as randomized double-blind two period crossover studies, with 1 week washout period between each visit. Twelve subjects participated in each study. Cefoxitin (Cefoxin® or Cefxitin®) and ceftazidime (Fortum® or Cef-4®) were given intramuscularly to the subjects in each group through a randomized computer-generated list. Only the investigator, who did not participate in the volunteers care and/or measurement of drug levels, kept the code and prepared the administered drug during visit 1 and visit 2. Other investigators

as well as all volunteers were blind to the allocated treatment until the end of the studies.

Drug preparations for intramuscular administration ;

- 1,000 mg of cefoxitin (Cefoxin® or Cefxitin®) was constituted with 2 ml of 1 % lidocaine hydrochloride solution
- 1,000 mg of ceftazidime (Fortum® or Cef-4®) was constituted with 3 ml of 1% lidocaine hydrochloride solution.

Cefoxitin administration and serial blood sampling

Visit 1. After admitted to the Clinical Pharmacology Unit, subjects were required to fast at least 2 hours before and after drug administration. Each volunteer was randomized to receive an intramuscular 1,000 mg dose of either Cefoxin® or Cefxitin®. The drug was deeply injected into the gluteus maximus muscle. Seven milliliters of blood samples were collected before drug administration and at 10, 20, 30, 45, 60, 90, 120, 180, 240 and 360 min after dosing. Blood samples were collected through an indwelling catheter cannulated onto forearm vein and kept patent with 50 unit/ml of heparin. The blood samples were allowed to clot at room temperature and then centrifuged for 10 min at 1,500 rpm. The serum samples were frozen at -20°C until analysis. After one week wash out period, subjects were admitted for a second visit.

Visit 2. The other preparation of cefoxitin was administered intramuscularly to each subject and the same procedure as visit 1 was performed.

Table 1 Demographic characteristics of subjects receiving 1000 mg dosage of cefoxitin.

Characteristics	Subject number												mean ± SD
	1	2	3	4	5	6	7	8	9	10	11	12	
Age (yr.)	22	22	44	26	27	27	21	25	26	20	34	28	26[a]
Sex	M	M	F	M	F	F	M	F	F	M	M	F	-
Height (cm)	179.5	173	150	166	158	153	167	156	154	176	172	162	163.9 ± 9.8
Weight (kg)	63.5	61	48	62	50	44	60	60	47	60	75	53	57.0 ± 8.8
Hct (M=40-50%,F=36-45%)	37	39	40	43	39	35	40	38	38	43	39	38	39.1 ± 2.3
Hb (10-15 gm/dl)	-	-	13.8	14.9	12.7	12	12.3	12.1	12.2	13.4	13.5	13	13.0 ± 0.9
WBC (5,000-10,000 /cm3)	-	-	6,300	7,400	9,300	7,500	5,800	7,300	8,530	7,600	6,800	8,500	7,503 ± 1064
Platelets	-	-	Adequate										-
AST (0-40 u/L)	20	14	18	20	23	15	27	23	24	20	29	17	20.8 ± 4.6
ALT (0-40 u/L)	18	18	14	29	19	11	38	13	21	20	36	14	20.9 ± 8.8
Total bilirubin (0.2-1 mg/dl)	0.78	0.63	0.77	0.69	0.7	0.56	0.6	0.8	0.8	1.67	0.8	0.51	0.8 ± 0.3
Direct bilirubin (0-0.2 mg/dl)	0.23	0.22	0.24	0.23	0.24	0.2	0.2	0.1	0.1	0.44	0.33	0.22	0.2 ± 0.1
Creatinine (0.6-1.7 mg/dl)	1.2	1.0	0.9	0.8	1	0.8	1.3	0.6	0.9	1.3	1	0.9	1.0 ± 0.2
BUN (7-27 mg/dl)	12	13	11	18	13	13	14	17	16	20	18	14	14.9 ± 2.8

[a] median

Table 2 Demographic characteristics of subjects receiving 1000 mg dosage of ceftazidime.

Characteristics	Subject number												mean ± SD
	1	2	3	4	5	6	7	8	9	10	11	12	
Age (yr.)	26	26	27	26	27	24	22	21	25	34	27	20	26[a]
Sex	M	F	F	F	F	F	M	M	F	M	M	M	-
Height (cm)	166	157	158	154	153	163	173	167	156	172	167	176	163.5 ± 7.8
Weight (kg)	62	60	50	47	44	50	61	60	60	75	63	60	57.7 ± 8.5
Hct (M=40-50%,F=36-45%)	43	40	39	38	35	34	40	40	38	39	43	43	39.3 ± 2.9
Hb (10-15 gm/dl)	14.9	14.0	12.7	12.2	12.0	11.4	13.4	12.3	12.1	13.5	14.3	13.4	13.0 ± 1.1
WBC (5000-10000 /cm^3)	7400	12500	9300	8530	7500	6860	10400	5800	7300	6800	6800	7600	8065.8 ±1863.7
Platelets	adequate												-
AST (0-40 u/L)	20	13	23	24	15	24	14	27	23	29	20	20	21 ± 5.0
ALT (0-40 u/L)	29	9	19	21	11	16	10	38	13	36	9	20	19.2 ± 10.2
Total bilirubin (0.2-1 mg/dl)	0.69	0.58	0.70	0.8	0.56	0.8	0.62	0.6	0.8	0.8	0.7	1.67	0.8 ± 0.3
Direct bilirubin (0-0.2 mg/dl)	0.23	0.19	0.24	0.1	0.20	0.1	0.23	0.2	0.1	0.33	0.25	0.44	0.2 ± 0.1
Creatinine (0.6-1.7 mg/dl)	0.8	0.8	1.0	0.9	0.8	0.8	1.1	1.3	0.6	1.0	1.3	1.3	1.0 ± 0.2
BUN (7-27 mg/dl)	18	10	13	16	13	9	17	14	17	18	16	20	15.1 ± 3.3

[a] median

<u>Ceftazidime administration and serial blood sampling</u>

Visit 1. The same procedure was applied to ceftazidime study except that 5 ml of blood samples were collected before drug administration and at 5, 15, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after dosing.

Visit 2. The other preparation of ceftazidime was administered intramuscularly to each subject and the same procedure as visit 1 was performed.

3. ANALYTICAL METHODS

Analytical method of cefoxitin

Drug concentrations were determined by HPLC technique modified from the method previously described (Yost and Derendorf, 1986).

Apparatus and chromatographic system

The HPLC system consisted of a Series 410 LC Pump, an ISS-200 Advanced LC Sample Processor and an LC-235 diode Array Detector set at 255 nm (Perkin-Elmer Corp., Norwalk, USA). The chromatographic separation was performed on a spherisorb 10 um C_{18}, 250 × 4.6 mm i.d. analytical column (Phenomenex®) and a 30 × 4.6 mm i.d. precolumn used to prolong column life. The mobile phase consisted of 91.5 % 0.01 M phosphate buffer, pH 3 and 8.5% acetonitrile. It was degassed for 20 min with helium gas and pumped through the column at 1 ml/min. The separation was carried out at 40° C. Peak areas were calculated through PC integrator (Perkin Elmer, Model 2100).

Drug and chemicals

Standard powder of cefoxitin was supported from Siam Pharmaceutical (Bangkok, Thailand). The 1000 mg vials preparations of Cefxitin® were supported from Siam Pharmaceutical. The 1000 mg vials preparations of Cefoxin® were purchased from Maharaj Nakhon Chiang Mai Hospital.

Reagent-grade acetonitrile and methanol were purchased from J.T. Baker Company (USA). Dipotassium hydrogen phosphate and potassium dihydrogen phosphate were perchased from Merck Company (Germany).

Sample preparation procedure

The solid phase extraction technique was used to prepare serum samples before injected into the system. This step was performed to remove the large quantities of interfering materials and prevent contamination of the analytical column. It was carried out using C_{18} disposable cartridge from Waters Associates (Sep-Pak®). The extraction procedure was as follows.

(1) Wash the cartridge with 5 ml of methanol to solvate the sorbent.

(2) Condition the cartridge with 5 ml of water.

(3) Load 1 ml of serum sample.

(4) Wash the cartridge with 1 ml of water to remove early eluting impurities.

(5) Elute with 1 ml of acetonitrile.

(6) Evaporate the eluent to dryness under room air and reconstitute with 1 ml of water.

(7) Inject 40 μl of the extract into the system.

Under the chromatographic condition already described, the chromatogram of cefoxitin was free from any interference peak, with well resolution, sharp peak and optimal retention time (Fig.3). The peak area of the chromatograms were

quantitated with PC integrator. Calculations were performed by using calibration curve of peak areas versus known concentrations of aqueous standard, then corrected by percentage of mean recoveries.

Limitation of detection and linearity

The lowest limit of quantitation under this condition was 0.25 ug/ml, adequate for assay drug levels for the pharmacokinetic study of the drug. Standard calibration curves for the assay were linear (r = 0.999) over a concentration range of 1-50 μg/ml.

Precision

The precision of the method was determined by repeated analysis of 6 aliquots of control samples at 2 different drug concentrations. The intraday and interday coefficient of variation were less than 10 % (Table 3).

Recovery

The assay recovery of cefoxitin was determined by comparing the peak area of cefoxitin in serum after the extraction procedure with the peak area of cefoxitin in water from 2 sets of 6 samples. The mean recoveries as determined from this assay were 84.3 % and 86.2 % at the drug concentrations of 12.5 ug/ml and 50 ug/ml , respectively (Table 4).

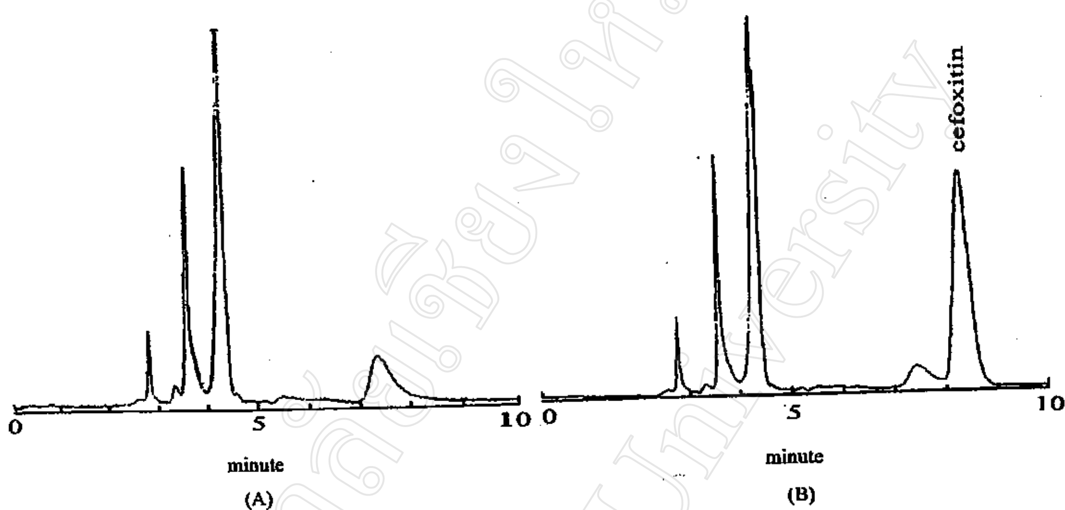

Figure 3 Typical chromatogram of cefoxitin in the serum after an extraction procedure; (A) before and (B) 1.5 hr after intramuscular administration of cefoxitin.

Table 3 Precision of the HPLC analytical procedure of cefoxitin.

Known cefoxitin concentrations (ug/ml)	Calculated cefoxitin concentrations determined by HPLC (ug/ml)			
	Intraday value		Interday value	
	mean ± SD	% CV	mean ± SD	% CV
12.5 (n=6)	12.36 ± 0.31	2.51	12.64 ± 0.40	3.17
50 (n=6)	50.55 ± 0.67	1.32	50.30 ± 2.64	5.25

Table 4 Percent recovery of cefoxitin from serum following the extraction procedure.

Cefoxitin concentration (ug/ml)	Apparent cefoxitin concentration (ug/ml)	% Recovery
12.5 (n = 6)	10.5 ± 0.3[a]	84.3 ± 2.1
50 (n = 6)	43.1 ± 0.6	86.2 ± 1.1

[a] mean ± SD

***In vitro* quantitative study of cefoxitin**

Cefoxin® and Cefxitin® (1000mg/vial) were dissolved with 100 ml water in volumetric flask, thereafter diluted 400 folds with water. Cefoxitin concentrations were analyzed using HPLC method described previously. The amount of the drug in each vial was calculated by a formulation below.

Amount of drug (mg) = measured concentration (ug/ml) × 40

Analytical method of ceftazidime

Ceftazidime concentrations were determined by HPLC technique modified from the method previously described (Yost and Derendorf, 1986).

Apparatus and chromatographic system

The system consisted of a DGU-3A degasser and LC-10 AS pump and SPD-10A UV-VIS detector set at 254 nm. Separation was performed at 40°C on a spherisorb 10 µm C_{18}, 250 × 4.6 mm i.d. analytical column (Phenomenex®) and a 30 × 4.6 mm i.d. precolumn. The mobile phase consisted of 0.182 g% HDTA in 0.01 M phosphate buffer pH 3 (70 %) and acetonitrile (30%) with a flow rate of 1 ml/min. Peak areas were calculated through C-R 6A chromatopac.

Drug and chemicals

Standard powder of ceftazidime was purchased from The United States Pharmacopeial Convention, Inc. (USA). The Cef-4® preparations in 1000 mg vials were supported from Siam Pharmaceutical. The Fortum® preparations in 1000 mg vials were purchased from Maharaj Nakhon Chiang Mai Hospital.

Reagent-grade acetonitrile and methanol were purchased from J.T. Baker Company (USA). Dipotassium hydrogen phosphate and potassium dihydrogen phosphate were perchased from Merck Company (Germany). Hexadecyl-trimethylammonium bromide, an ion-pairing agent was purchased from Sigma Chemical Company (USA).

Sample preparation procedure

The precipitation technique was used to prepare serum samples before injected into the system. This step was performed to remove the large quantities of interfering materials and to prevent contamination of the analytical column. Five hundred µl of serum sample was pipetted into centrifuge tube, then spike with 1,000 µl acetonitrile. The mixture was vortexed for 20 sec, stood at room temperture for 10 min, vortexed again and centrifuged for 6 min at 13,000 rpm. Ten ul of supernatant was injected into the system. Under the chromatographic condition already described, the chromatogram of ceftazidime was free from any interference peak, with well resolution, sharp peak and optimal retention time (Fig.4). The peak areas of the chromatograms were quantitated using C-R 6A Chromatopac. Calculations were performed using calibration curve of peak areas versus known concentrations of aqueous standard, thereafter the drug levels were corrected by percentage of mean recovery.

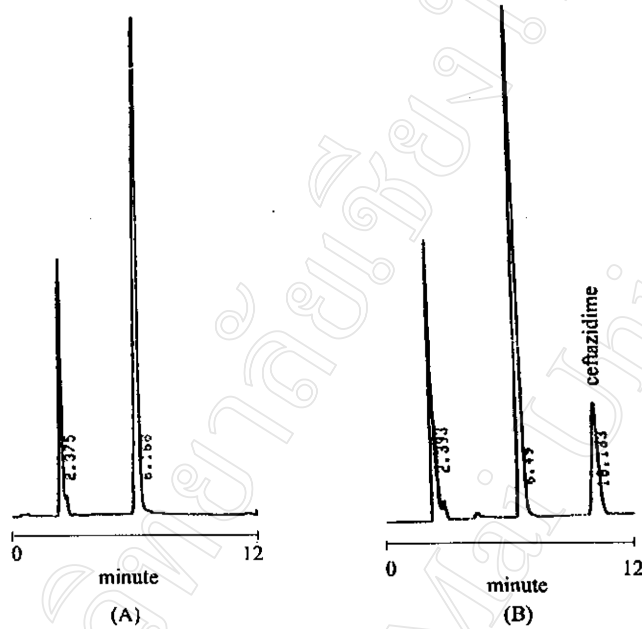

Figure 4 Typical chromatogram of ceftazidime in the serum after an extraction procedure; (A) before and (B) 15 min after intramuscular administration of ceftazidime.

Limitation of detection and linearity

The lowest limit of quantitation under this condition was 1 μg/ml, adequate for assay drug levels for the pharmacokinetic study of the drug. Standard calibration curves for the assay were linear (r =0.999) over a concentration of 1-50 μg/ml.

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Precision

The precision of the method was determined by repeated analysis of 6 aliquots of control samples at 3 different drug concentrations. The intraday and interday coefficient of variation were less than 10 % (Table 5).

Recovery

The assay recovery of ceftazidime was determined by comparing the peak area of ceftazidime in serum after the extraction procedure with the peak area of cefoxitin in water from 3 sets of 6 samples. The mean recoveries as determined from this assay were 96.60 % , 88.51% and 88.71 % at the drug concentrations of 2.5 ug/ml, 12.5 ug/ml and 50 ug/ml , respectively (Table 6).

Table 5 Precision of the HPLC analytical procedure of ceftazidime.

Known ceftazidime concentrations (μg/ml)	Calculated ceftazidime concentrations determined by HPLC (μg/ml)			
	Intraday value (n = 8)		Interday value (n = 6)	
	mean ± SD	% CV	mean ± SD	% CV
2.5	2.69 ± 0.12	4.40	2.72 ± 0.20	7.45
12.5	11.79 ± 0.40	3.35	11.16 ± 0.64	5.77
50	49.27 ± 0.57	1.15	44.61 ± 2.74	6.15

Table 6 Percent recovery of ceftazidime from serum following the extraction procedure.

Ceftazidime concentration (μg/ml)	Apparent ceftazidime concentration (μg/ml)	% Recovery
2.5 (n = 6)	2.42 ± 0.10^{a}	96.60 ± 4.07
12.5 (n = 6)	11.06 ± 0.22	88.51 ± 1.79
50 (n = 6)	44.35 ± 1.80	88.71 ± 3.61

[a]mean ± SD

***In vitro* quantitative study of ceftazidime**

Fortum® or Cef-4® (1000mg/vial) were dissolved with 100 ml water in volumetric flask, thereafter diluted 400 folds with water. Ceftazidime concentrations were analyzed using HPLC method previously described. The amount of the drug in each vial was calculated by a formulation below.

Amount of drug (mg) = measured concentration (ug/ml) × 40

4. PHARMACOKINETIC ANALYSIS

In order to extract the maximum information from raw data of concentration-time profile, mathematical techniques are used to describe the biological behavior of the body system in terms of pharmacokinetic model. An important concept in modeling the time profiles of drug and metabolite concentrations as well as their pharmacological effects is a compartmental concept. Compartments are location in the body and can be represented with certain simplifications during modeling, i.e. input compartment (sites of absorption), central compartment (blood and plasma), disposition compartments (sites which are involved in processes of distribution, biotransformation and excretion). In the present study, the great majority of individual concentration-time profiles of both

drugs were best fitted with one compartment with regard to statistical tests (F-ratio test, Akaike information criterion, etc..), visual inspection of the analysis of residuals (the difference between measured values and calculated values), inspection of conventional linear and semilogarithmic plots of the fitted data. Therefore the present study used one compartment model to calculate pharmacokinetic parameters of both drugs with the aid of the TOPFIT, a pharmacokinetic data analysis program for PC. The common measures used to assess differences in absorption are the area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the time to reach the maximal plasma concentration (T_{max}). The pharmacokinetic parameters include $AUC_{0-\infty}$, T_{max}, maximal plasma concentration (C_{max}), plasma elimination half-life ($t_{1/2}$), plasma clearance (Cl), volume of distribution (V_d), absorption rate constant (K_a), and elimination rate constant (K_e) were calculated with the use of TOPFIT.

5. STATISTICAL ANALYSIS

Data were expressed as mean ± SD, and were compared using two tails t-test. P value of < 0.05 was considered statistically significant.