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Bioequivalence Evaluation of Two Brands of Cefixime 100 mg Capsule (Suprax and Alpha-Cefixime) in Korean Healthy Volunteers

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Abstract – Cefixime is an orally absorbed cephalosporin with a broad spectrum of activity against Gram-negative bacteria and is highly resistant to beta-lactamase degradation. The purpose of the present study was to evaluate the bioequivalence of two cefixime capsules, Suprax capsule (Dong-A Pharmaceutical Co., reference drug) and Alpha-Cefixime capsule (Alpha Pharmaceutical Co., test drug), according to the guidelines of Korea Food and Drug Administration (KFDA). Twenty-four normal subjects, 23.5±3.72 years in age and 68.3±8.89 kg in body weight, were divided into two groups and a randomized 2×2 cross-over study was employed. There was one week washout period between the doses. After one capsule containing 100 mg of cefixime was orally administered, plasma was taken at predetermined time intervals and the concentrations of cefixime in plasma were determined using HPLC with UV detector. The pharmacokinetic parameters such as AUC_t, C_{max} and T_{max} were calculated and ANOVA test was utilized for the statistical analysis of the parameters. The results showed that the differences in AUC_t, C_{max} and T_{max} between two products were -3.91%, -2.23% and -3.18%, respectively, when calculated against the reference drug. The 90% confidence intervals using logarithmically transformed data were within the acceptance range of $\log 0.8 \le \delta \le \log 1.25$ (e.g., $\log 0.8786 \le \delta \le \log 1.0523$ and $\log 0.8889 \le \delta \le \log 1.0512$ for AUC_t and C_{max}, respectively). The 90% confidence intervals using untransformed data was within ±20% (e.g., $-10.37\% \le \delta \le 6.73\%$ for T_{max}). All parameters met the criteria of KFDA for bioequivalence, indicating that Alpha-Cefixime capsule (Alpha Pharmaceutical Co.) is bioequivalent to Suprax capsule (Dong-A Pharmaceutical Co.).

Key words □ bioequivalence, cefixime, suprax, cross-over study

INTRODUCTION

Cefixime is an oral third-generation cephalosporin antibiotic. The drug has been clinical effective in treating otitis media, respiratory tract infections and uncomplicated urinary tract infections due to susceptible organisms (Lacy *et al*., 1993; Sweetman, 2002). Cefixime has bioavailability of about 50%. Following oral administration of cefixime with food, the total amount absorbed is not altered but the time to maximal concentration is increased by 0.8 hours (Faulkner *et al*., 1988; Montay *et al*., 1991). Therapeutic plasma concentrations for cefixime have not been defined, but depending on the dose, peak plasma concentrations of 1-4 μg/mL or 1.9-7.7 μg/mL were measured with single or multiple doses of 200 mg and 400 mg, respectively (Faulkner *et al*., 1988; Montay *et al*., 1991). Peak plasma concentrations normally occur within 2-6 hours after single oral doses (Faulkner *et al*., 1988; Montay *et al*., 1991). The drug has a half-life of 3~4 hours, and a relatively low proportion (15~20%) of a dose is excreted by the renal route (Faulkner *et al*., 1988; Mamzoridi *et al*., 1996).

The purpose of this study was to evaluate the bioequivalence of two cefixime capsules, Suprax capsule (reference drug) and Alpha-Cefixime capsule (test drug) in 24 Korean healthy volunteers, according to the guidelines of Korea Food and Drug Administration (Korea Food and Drug Administration, 2005). Typical bioavailability, including AUC_t (the area under the plasma concentration-time curve from 0 until the last sampling time, 12 hr), C_{max} (the maximum plasma concentration) and T_{max} (time to reach C_{max}) parameters were compared.

MATERIALS AND METHODS

Test and reference products

The test product was Alpha-Cefixime capsule (100 mg of

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cefixime, lots no. 338501, Alpha Pharmaceutical Co., Seoul, Korea). The reference product was Suprax capsule (100mg of cefixime, lots no. 5285, Dong-A Pharmaceutical Co., Seoul, Korea).

Subjects

This bioequivalence study involved 24 Korean healthy subjects. The mean age of subjects was 23.5±3.72 years, with a range of 21~30 years, mean body weight was 68.3±8.89 kg, with a range of 52~91 kg, mean height was 173.0±19.4 cm, with a range of 159~183 cm. All the subjects were enrolled after passing a clinical examination, including a physical examination and laboratory tests (plasma analysis: hemoglobin, hematocrit, WBC, platelets, WBC differential, blood urea nitrogen, total bilirubin, cholesterol, total protein, albumin, alkaline phosphatase, glucose fasting, ALT, and AST, and urine analysis: specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC, and casts). Any with potential hypersensitivity to this type of medication, a history of the hepatic, renal, or cardiovascular disease, of chronic alcohol consumption or other medications was excluded. This criteria was applied to elimination the source of variation which can influence the pharmacokinetics of the drug. All the subjects were restricted not to take using other drugs from at least one week before the study and until the completion of the study. They also refrained from alcoholic beverages and xanthine-containing foods and beverages 48 h before the study, until the last sampling time.

Each subject received an oral dose of 100mg of cefixime in a standard 2×2 cross-over design with one-week washout period between each treatment phase. Subjects were randomly divided into two equal groups and assigned to one of the two sequences of drug administration. The study was approved by a local ethics committee. All the subjects signed a written informed consent, in accordance with the Korea Guidelines for Bioequivalence Tests.

Drug administration and sample collection

The subjects were hospitalized (Chosun University Hospital, Gwangju, Korea) at 7:00 p.m. the day before drug administration. At 7:00 a.m., the median cubital vein was cannulated and 1 mL of heparinized injectable normal saline solution was flushed into the cannula to prevent blood clotting. The doses were taken at 8:00 a.m. on each dosing day with 240 mL of drinking water. Four hours after oral administration, all the subjects were given standard meals. The subjects were not allowed to take a supine position or to sleep until 4 h after oral administration. Approximately 5 mL blood samples were collected before and 1, 2, 3, 3.5, 4, 4.5, 5, 6, 8, 10, and 12 h after drug administration. The cannula was flushed with 1 mL of heparinized injectable normal saline solution after each blood sampling. The blood sample was centrifuged immediately, and the plasma was frozen at -70°C until the HPLC analysis. After one week period washout, the study was repeated in the same way to complete cross-over study

HPLC analysis of cefixime in plasma

The concentrations of cefixime in plasma were performed using a reported HPLC method (Falkowski *et al*., 1987) on a Shimadzu chromatographic system (Kyoto, Japan). The mobile phase involved a mixture of phosphate buffer (10 mmol/L), pH=2.6 and acetonitrile (86:14 v/v), pumped at a flow rate of 1.0 mL/min through the column (Capcell Pak C18 MG, 4.6× 100mm, Shiseido, Tokyo, Japan) at room temperature. Peaks were monitored by UV absorbance at 280 nm. Cefixime stock solution (1 mg/mL in methanol) was serially diluted with methanol and added at drug free plasma to obtain concentration of 100, 200, 500, 1000, 2000, and 5000 ng/mL. These standard solutions were employed for the preparation of calibration graphs. In order to assess the intra-day coefficient of variation (CV) and accuracy for plasma samples, samples of cefixime were spiked into human plasma at final concentrations of 100~ 5000 ng/mL. Limit of detection (LOD) was determined from signal to noise ratio (S/N)=3 and lower limit of quantitation (LLOQ) from S/N=10. The precision and accuracy for inter-day assay were assessed at the same concentration and repeated for five different days. After thawing at room temperature, an aliquot of each sample (500 μL) was pipetted into an eppendorf tube and 20 μL of internal standard solution (7-hydroxycoumarin, 100 μg/mL in water) was added. After vortexing briefly, 500 μL of 6% trichloroacetic acid was added to each sample. The mixture was shaken and centrifuged at 13,000 rpm for 5 min. A 50 μL of the supernatant was injected into the HPLC system.

Pharmacokinetic analysis

Each subject received an oral dose of 100 mg of cefixime in a standard 2×2 cross-over model in a randomized order. Pharmacokinetic parameters such as AUC_t, C_{max} and T_{max} were calculated from plasma concentration-time curve. C_{max} and T_{max} were recorded actual measurement value and AUC_t was calculated by trapezoidal formula in 0-12 h using Excel program.

Statistical analysis

Their ratios (test/reference) using log-transformed data, together with their means and 90.0% confidence intervals, were analyzed with analysis two-way analysis of variance (ANOVA) that performed with the Equiv Test version 1.0 (Statistical Solution Ltd., 1998) at a significant level of 0.05. The bioequivalence of two cefixime capsules estimated by AUC_t, C_{max} and T_{max}. The ANOVA was performed using logarithmic transformed AUC_t and C_{max}. The ANOVA of T_{max} was carried out on the untransformed data. The range of bioequivalence for the parametric analysis was set to the 80-125%,

Table I. Precision and accuracy for the HPLC analysis of cefixime in plasma

Concentration (ng/mL)	Precision C.V. %		Accuracy %	
	Intra-day (n=5)	Inter-day (n=5)	Intra-day (n=5)	Inter-day (n=5)
100	2.68	10.42	115.1	109.5
200	2.69	12.33	113.5	112.4
500	4.69	7.95	100.0	102.4
1000	2.28	6.29	96.4	96.7
2000	4.89	5.44	99.1	99.8
5000	2.66	5.36	100.3	105.6

RESULTS AND DISCUSSION

Analysis of cefixime in plasma

Cefixime and internal standard were well separated from the biological background under the described chromatographic conditions at retention times of 7.4 min and 13.2 min, respectively (Fig. 1). No interference with constituents from plasma matrix was observed. The mobile phase used guaranteed good repeatability of retention times. The calibration curves were obtained by analyzing five samples. The curve was linear in whole range tested (100~5000 ng/mL) and described by following equation: ratio of peak areas = 0.000651 × cefixime concentration-0.0198 with a correlation coefficient of 0.999. The intra-day precision (C.V) of the method for cefixime ranged from 2.28% to 4.69%, while the intra-day accuracy ranged from 96.4% to 115.18%. The inter-day precision ranged from 5.36% to 12.33%, while the inter-day accuracy ranged from 96.7% to 112.4% (Table I).

Pharmacokinetic analysis of cefixime in plasma

The two products were well-tolerated in all subjects, unexpected incidents that could have influenced the outcome of the study did not occur. There was no drop-out and all subjects who started the study continued to the end and were discharged in good health. The plasma cefixime (reference and test products) concentration-time profiles in 24 healthy subjects were determined up to 12 h after the oral administration of 100 mg of cefixime are shown in Fig. 2. Table II shows the pharmacokinetic parameters of cefixime for each subject. In our study, AUC_t, C_{max} and T_{max} for reference and test products were 12397.0±3585.6 ng·hr/mL and 11912.6±3129.9 ng·hr/mL, 1894.5±502.2 ng/mL and 1852.3±544.2 ng/mL, and 4.58±0.56 h and 4.44±0.92 h, respectively (Table II). The difference of mean of the test product/mean of the reference product for AUC_t, C_{max} and T_{max} were -3.91%, -2.23% and -3.18%. Pharmacokinetic studies of cefixime have been performed (Yom, 2002; Asiri *et al*., 2005). Yom (2002) reported the following

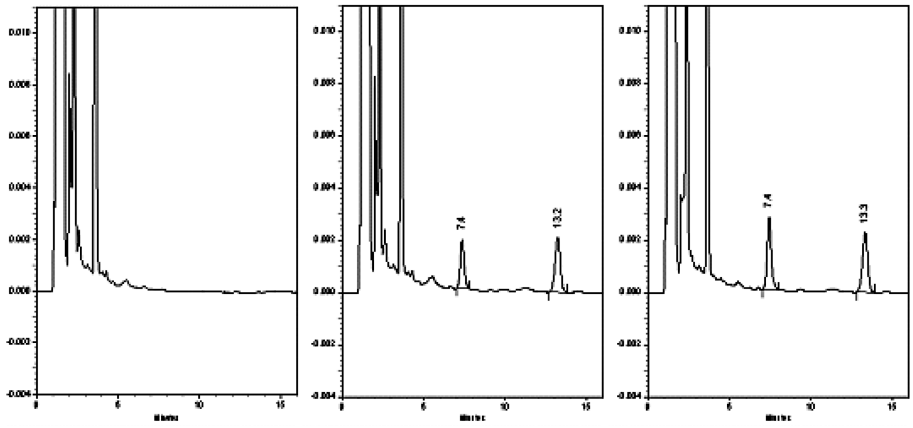

Fig. 1. Chromatogram of (left) blank human plasma, (middle) blank human plasma spiked with cefixime (1000 ng/mL, 7.4 min) and internal standard (2 ng/mL, 13.2 min) and (right) plasma sample at 3.5 h after oral administration of 100 mg cefixime capsule.

Table II. Pharmacokinetic parameters of cefixime capsules for each subject

Subjects	AUC_t (ng · hr/mL)				C_{max} (ng/mL)				T_{max} (h)	
	Reference		Test		Reference		Test		Reference	Test
	AUC_t	$logAUC_t$	AUC_t	$logAUC_t$	C_{max}	$logC_{max}$	C_{max}	$logC_{max}$	T_{max}	T_{max}
A1	10554.2	9.264	11678.9	9.366	1577.9	7.364	1799.0	7.495	5.0	5.0
A2	10297.3	9.240	11962.7	9.390	1447.3	7.277	1715.7	7.448	4.0	5.0
A3	6849.0	8.832	5232.4	8.563	1091.1	6.995	851.3	6.747	5.0	3.5
A4	12017.6	9.394	10040.5	9.214	1896.7	7.548	1464.9	7.290	6.0	4.5
A5	10485.5	9.258	11210.9	9.325	1491.7	7.308	1772.6	7.480	4.0	6.0
A6	10506.3	9.260	11797.1	9.376	1615.8	7.388	1690.6	7.433	3.5	5.0
A7	5786.0	8.663	5347.1	8.584	1020.4	6.928	869.4	6.768	5.0	5.0
A8	15663.4	9.659	9462.5	9.155	2226.1	7.708	1241.3	7.124	5.0	3.5
A9	13109.2	9.481	8910.3	9.095	1903.4	7.551	1299.1	7.169	4.5	3.5
A10	16906.8	9.735	11867.2	9.382	2319.4	7.749	1946.6	7.574	5.0	4.5
A11	11114.3	9.316	12742.0	9.453	1571.2	7.360	1937.2	7.569	4.0	4.5
A12	12371.4	9.423	13491.0	9.510	2027.4	7.615	1941.6	7.571	5.0	5.0
B1	9375.7	9.146	12661.4	9.446	1553.7	7.348	2078.4	7.639	5.0	4.5
B2	9777.7	9.188	13299.8	9.496	1746.2	7.465	2039.8	7.621	4.0	4.5
B3	11460.4	9.347	8609.4	9.061	1572.0	7.360	1296.5	7.167	4.5	2.0
B4	11892.8	9.384	13790.8	9.532	2023.9	7.613	2167.0	7.681	5.0	5.0
B5	14023.9	9.549	15934.6	9.676	2027.3	7.614	2322.6	7.750	4.5	5.0
B6	13419.6	9.504	15806.9	9.668	1870.5	7.534	2242.1	7.715	4.0	5.0
B7	10229.5	9.233	11031.3	9.308	1686.4	7.430	1847.1	7.521	5.0	5.0
B8	21623.0	9.982	13998.1	9.547	3010.3	8.010	1871.6	7.535	4.5	6.0
B9	19589.2	9.883	12027.5	9.395	2982.7	8.001	2491.1	7.820	4.0	3.5
B10	14784.7	9.601	15197.6	9.629	2546.1	7.842	2601.9	7.864	4.0	4.0
B11	11358.9	9.338	10824.7	9.290	1853.2	7.525	1677.5	7.425	5.0	3.0
B12	14331.2	9.570	18978.8	9.851	2407.4	7.786	3290.4	8.099	4.5	4.0
Mean	12397.0	9.385	11912.6	9.346	1894.5	7.513	1852.3	7.479	4.58	4.44
S.D.	3585.6	0.292	3129.9	0.302	502.22	0.266	544.22	0.316	0.56	0.92

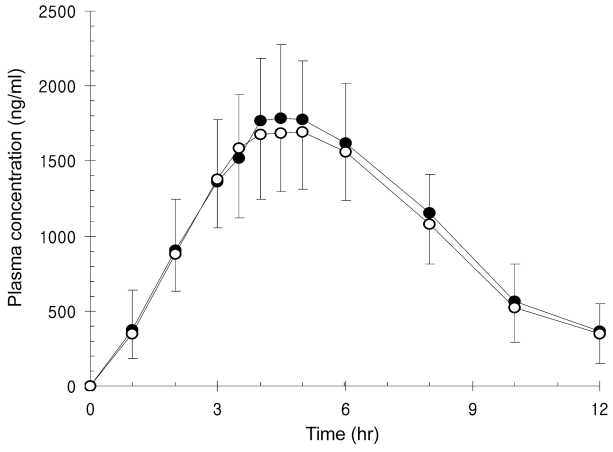

Fig. 2. Plasma concentration-time curves of cefixime following oral administration of reference (●) and test product (○) at the cefixime dose of 100 mg. Data are expressed as mean±S.D. (n=24).

pharmacokinetic parameters: AUC_t, 15.73~15.87 μg · hr/mL; C_{max}, 2.10~2.12 μg/mL; and T_{max}, 4.50~4.83 h after a single oral dose of 100 mg cefixime capsule. And also, in Asiri *et al* (2005) study, AUC_t, C_{max} and T_{max} were 18.65~34.29 μg · hr/mL, 2.37~4.38 μg/mL and 2.5~4.5 h after a single oral dose of 100 mg cefixime suspension.

Bioequivalence test of cefixime products

No significant sequence effect was found for all of the bioavailability parameters indicating that the cross-over design was properly performed. Significant F test values were found between the subjects and subjects nested sequence (SEQ) for AUC_t and C_{max} indicating a substantial inter-subject variation in the pharmacokinetics of cefixime from the two preparations (Table III). Geometric means of the parameters (Table IV) are given for the test and reference products separately for each period and as combined estimates. The parametric 90% confidence intervals for AUC_t and C_{max} were $\log 0.8786 \leq \delta \leq \log 1.0523$ and $\log 0.8889 \leq \delta \leq \log 1.0512$, respectively, which were within the commonly accepted bioequivalence range of $\log 0.8 \leq \delta \leq$

Table III . ANOVA analysis of AUC_t and Cmax

	AUC_t					C_{max}				
	Df	SS	MS	F	P-value	Df	SS	MS	F	P-value
Inter-Subjects										
Carry-over	1	0.4125	0.4125	5.1363	0.0366	1	0.7411	0.7411	9.5340	0.0053
Residuals	22	1.7670	0.0803	2.7424	0.0109	22	1.7102	0.0777	3.4168	0.0028
Intra-Subjects										
Drug	1	0.0015	0.0015	0.0520	0.8216	1	0.0007	0.0007	0.0003	0.9860
Period	1	0.0080	0.0080	0.2732	0.6063	1	0.0075	0.0075	0.3300	0.5714
Residuals	22	0.6443	0.0292			22	0.5005	0.0227		
Total	47	2.8334				47	2.9595			

α=0.05, F(1, 22)=4.301

Table IV. Statistical results of bioequivalence test between two cefixime capsules

	Parameters[a)]		
	AUC_t	C_{max}	T_{max}
Difference	-3.91%	-2.23%	-3.18%
Test/Reference point estimate	0.9616	0.9667	-0.0833
Confidence interval[b)]	log0.8786≤δ≤log1.0523	log0.8889≤δ≤log1.0512	-10.37%≤δ≤6.73%

[a)]The AUC_t and C_{max} values were calculated on the basis of log-transformed data, and T_{max} values on the basis of untransformed data.
[b)]α= 0.05

log1.25. The parametric 90% confidence intervals for T_{max} was -10.37%≤δ≤6.73%, which was within the commonly accepted bioequivalence range of ±20%. Geometric means of the parameters such as AUC_t, C_{max} and T_{max} of the test product were similar to those of the reference product, which proved that there was no significant difference between the bioavailability of reference product and test product.

It was shown that this method is suitable for the analysis of cefixime in plasma samples for the bioequivalence studies. Based on the statistical analysis results of this study, we can assume that Suprax capsule (cefixime 100 mg) is bioequivalent to Alpha-Cefixime capsule (cefixime 100 mg) and can be used interchangeably in clinical practice.

REFERENCES

Asiri, Y. A., Al-Said, M. S., Al-Khamis, K. I., Niazy, E. M., El-Sayed, Y. M., Al-Rashood, K. A., Al-Yamani, M. J., Alsarra, I. A. and Al-Balla, S. A. (2005). Comparative bioavailability study of cefixime (equivalent to 100 mg/5 ml) suspension (Winex vs Suprax) in healthy male volunteers. *Int. J. Clin. Pharmacol. Ther.* **43**, 499-504.

Falkowski, A. J., Look, X. M., Noguchi, H. and Silber, B. M. (1987). Determination of cefixime in biological samples by reversed phase high performance liquid chromatography. *J. Chromatogr.* **422**, 145-152.

Faulkner, R. D., Fernandez, P., Lawrence, G., Sia, L. L, Falkowski A. J., Weiss, A. I., Yacobi, A. and Silber, B. M. (1988). Absolute bioavailability of cefixime in man. *J. Clin. Pharmacol.* **28**, 700-706.

Korea Food and Drug Administration. (2005). *Korea guidelines for bioequivalence test 2005-31*. Seoul, Korea.

Lacy, C. F., Armstrong, L. L., Lipsy, R. J. and Lance, L. L. (1993). *Drug information handbook.* 13th Ed. Lexi-Comp Inc, Atlanta, pp 161-162.

Mamzoridi, K., Kasteridou, N., Peonides, A. and Niopas, I. (1996). Pharmacokinetics of cefixime in children with urinary tract infections after a single oral dose. *Pharmacol. Toxicol.* **78**, 417-420.

Montay, G., Masala, F., Le Roux, Y., Le Liboux, A., Uhlrich, J., Chassard, D., Thebault, J. J., Roche, G. and Frydman, A. (1991). Comparative bioavailability study of cefixime administered as tablets or aqueous solution. *Drugs* **42**, 6-9.

Statistical solutions Ltd. (1998). Equiv Test 1.0. Sangus, MA, USA.

Sweetman, S. C. (2002). Martindale - The complete drug reference. 33rd Ed., Pharmaceutical Press, London, pp 166-167.

Yom, Y. K. (2002). Bioequivalencestudy of a cefixime capsule preparation. *J. Kor Soc. Hosp. Pharm.* **19**, 402-405.