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Bioavailability Evaluation of Two Ceftriaxone Formulations Using Two Way Crossover Design in Volunteers

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For the bioequivalence study of two ceftriaxone injection formulations (Rocephin® ; Roche, and Triaxone® ; Hanmi), the HPLC analytical method for the analysis of ceftriaxone in plasma was used. Fourteen healthy volunteers completed the study and each subject were IM injected single doses (1 g) of the test and the reference formulations in a two-way crossover design with an one week drug free interval between doses. Following each administration, plasma concentrations of ceftriaxone were monitored over a period of 24 h. Bioequivalence parameters AUC_{24h}, T_{max}, C_{max} and MRT determined from the data obtained for the two formulations were examined by analyses of variance (ANOVA) and other criteria and tests for bioequivalence. Results of ANOVA and confidence limits of test/reference ratios of AUC_{24h}, T_{max}, C_{max} and MRT, and statistical tests indicated the bioequivalence of the two formulations (i. e., test/reference ratio was within $100\pm20\%$) except for T_{max}. The mean of T_{max} showed only 6. 9% difference from the reference but the detection limit was 22.5% which is slightly over the 20% criteria. No pharmacokinetic parameters including Ka, Kel, Vd and Cl indicated sigificant difference in between the two formulations. It was concluded that the data yielded for the two ceftriaxone formulations demonstrated that they were bioequivalent.

Key words : Ceftriaxone, Pharmacokinetics, Bioequivalence, HPLC, ANOVA

INTRODUCTION

Ceftriaxone is a semisynthetic cephalosporin antibiotic drug with a long serum half-life. Ceftriaxone exhibits nonlinear dose-dependent pharmacokinetics. Serum concentrations, the area under the serum concentration-time curve (AUC) and most of pharmacokinetic parameters (except elimination half-life and the fraction excreted unchanged in urine) of ceftriaxone are dose dependent (Findlay *et al.*, 1993). Following IM administration of a single ceftriaxone dose of 0.5~1 g in healthy adults, the drug appears to be completely absorbed and peak serum concentrations are attained 1.5~4 hours after the dose (McEvoy *et al.*, 1994).

Ceftriaxone is used for the treatment of lower respiratory tract infections, skin and skin structure infections, bone and joint infections, intra-abdominal infections, urinary tract infections, meningitis, septicemia, and gonorrhea caused by susceptible organisms (McEvoy *et al.*, 1994). Dosage of ceftriaxone sodium is expressed in terms of ceftriaxone and is identical for IM or IV administration. The usual adult dosage of ceftriaxone for the treatment of most infections caused by susceptible organisms is 1~2 g given once daily or in equally divided doses twice daily, depending on the type and severity of the infection (Lacy *et al.*, 1993).

The new ceftriaxone was synthesized by Hanmi Pharm Co. using the improved synthetic pathway having process patent, and formulation as Triaxone®. Since the material synthesized by the different method, may show different physical and pharmacokinetic properties, the present study was designed to assess the performance of the single dose (1 g IM) bioequivalence of two different injection formulations of ceftriaxone (Rocephin® and Triaxone®) in a group of strictly controlled volunteers.

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MATERIALS AND METHODS

Drugs - Ceftriaxone 1 g (potency) vial.

Test formulation : Triaxone 1g vial, Lot No 78019 (1994. 4. 11.)

Reference formulation : Rocephin 1g vial, Lot No F40212 (1994. 2. 14.)

Informed Consent

All subjects participating in this study were provided with detailed information on the possible side effects of ceftriaxone and other hazards that might be encountered. Each subject submitted a written informed consent prior to his/her entry in the study.

Subject

The subjects were selected from healthy male volunteers, aged 21~24 years, with standard weight-to-height ratio. In order to be eligible to participate in the study, each volunteer was required to be an abstainer from drug or alcohol abuse and to be free of cardiovascular, hepatic, renal, or gastrointestinal diseases, as assessed by physical examination and review of medical history. They were also required to have their blood pressure and results of clinical laboratory tests (blood chemistry, hematology, and urinalysis) within the normal ranges. Fourteen subjects were initially entered and completed the study. All subjects avoided using other drugs for at least one week prior to the study and until after its completion. They also refrained from alcoholic beverages and food and beverages containing caffeine 48 h prior to each dosing and until the collection of the last blood sample.

Study design and plasma samples

The administration of the two formulations of ceftriaxone to the subjects followed a balanced two-way crossover design with a week drug free interval between the two administrations. Subjects were assigned randomly to receive the test (Test; Triaxone 1000 mg IM) or reference (Reference; Rocephin 1000 mg IM) for the first dose and then the respective alternate formulation for the second dose. After an overnight fast, each subject was injected with 1000 mg ceftriaxone in vial which was dissolved in 3.5 ml of 1% lidocaine.

Fluid and food intakes were controlled for 10 h following each dose. Beverage and standard lunch was supplied at 1.0 and 3.0 h, respectively, after each dose. Beverages and foods containing caffeine were not permitted over the entire course of the study. Blood samples (6 ml each) were collected by venipuncture using the three way cock equiped with 10 ml of 20 IU/ml heparin solution, and stored in heparinized glass tubes at 0(predose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 24 h postdose. The plasma was immediately separated by centrifugation and stored at -20°C until analysis.

Analysis of plasma samples

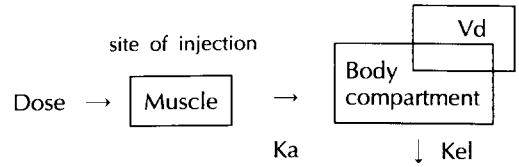
HPLC of ceftriaxone were carried out according to the reported method (Kwon and Bourne, 1986). The mobile phase consisted of pH 7 phosphate buffer 48 ml (dissolved 13.6 g of dibasic potassium phosphate and 4.0 g of monobasic potassium phosphate in 1L of distilled water), acetonitrile 600 ml, tetraheptylammonium bromide 3.2 g and distilled water to adjust the volume to 1000 ml. The samples were separated on a reverse phase column (μ-Bondapak C 18, Waters Associates, MA, U.S.A.) with a flow rate of 1.0 ml/min and detected using a UV detector (model 441, 280 nm, Waters Associate). Other parts of HPLC system consisted of the pump (model 510, Waters Associates), the injector (U6K, Waters), and the integrator (Data module 730, Waters). Standard curves, generated daily for the determination of test samples, remained linear ($r>0.998$) in the concentration range 5-100 μg/ml of plasma. The measured retention time for ceftriaxone was 5.2 min, and the sensitivity of the assay was 1 μg/ml.

Analysis of data and statistics

The basic pharmacokinetic parameters for ceftriaxone (AUC, C_{max}, T_{max}) were determined from the plasma concentration-time data obtained by HPLC method. For each set of data, the maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were obtained directly from the plasma concentration-time data. The area under the plasma concentration-time curve of the analyte (AUC^t_o) up to the last time (t_{last}=24 h), showing a 24 h plasma concentration (C_{last}) was determined by using the trapezoidal rule. The AUC_{inf} values were determined by adding the quotient of C_{last} and the appropriate kel to the corresponding , that is (Gibaldi and Perrier, 1982; Shargel and Yu, 1992) :

$$AUC_{inf} = AUC_{24h} + C_{last}/Kel$$

For the calculation of pharmacokinetic parameters including Ka, Kel, and Vd, the plasma concentration-time curve was fitted to the model below.



The parameters of Ka (absorption rate constant), Kel (elimination rate constant) and Vd (volume of distribution) were calculated using the computer program "Boomer" by the numerical integration method of Fehlberg RKF 45 and fitting method of Damping Gauss Newton (Bourne *et al.*, 1986). As there is no elimination function in the muscle, fraction absorbed (F) was regarded as 1 for the estimation of Vd. An elimination half-life ($t_{1/2}$) for ceftriaxone in plasma was calculated from the quotient of ln2 and Kel. Total clearance (Cl_{total}) was calculated by dose/AUC. The

mean residence time (MRT) was calculated from the AUMC(area under the moment curve) using the equation below (Gibaldi and Perrier; 1982, Shargel and Yu, 1992).

$$MRT_{24h} = \frac{AUMC_{24}h}{AUC_{24}h}$$

$$AUMC_{24h} = \int_0^{24h} t \cdot C\, dt$$

For bioequivalence purposes, pharmacokinetic parameters were examined by three-way analysis of variance (ANOVA) in which the effects of formulation, period, and subjects were examined. For this, the computer program of bioequivalence which was developed by Korea National Research Institute of Health Safety was utilized. The ANOVA of AUC_{24h}, T_{max}, C_{max} and MRT_{24h} values were carried out by three-way ANOVA (Midha *et al.*, 1990).

The power of each ANOVA to detect a 20% difference between Test and Reference was calculated by the method of Lamda test. The confidence limits of Test : Reference ratios were also computed. Comparison of the pharmacokinetic parameters between the formulations was performed by paired Student t-test. The level of confidence was set at 95% (α=0.05) for all the statistical test (Midha *et al.*, 1990).

RESULTS AND DISCUSSION

Ceftriaxone was well tolerated by the subjects and other than the commonly encountered effects such as dull pain on injection site, no clinical side effect was observed in any subject during the entire study.

Both of the formulations of ceftriaxone were readily absorbed from the injection site as indicated by the data obtained. The mean T_{max} values for Test (Triaxone) and Reference (Rocephin) were 1.93 and 2.07 h, respectively. After reaching C_{max}, plasma concentrations of ceftriaxone declined monoexponentially in most of the subjects. The plasma concentration versus time profiles of some subjects showed a second peak after C_{max} in the decline phase between 3 and 8 h which may be due to biliary recycling of ceftriaxone in these subjects (Midha *et al.*, 1990). The HPLC procedure employed in this study was sensitive enough to monitor ceftriaxone concentrations in plasma over the entire 24 h study period in all volunteers after each administration.

Mean plasma concentration of ceftriaxone

Fig. 1. and 2. show the semilogarithmic plot of the arithmetic means of the plasma concentrations of ceftriaxone over time after separate administration of the two formulations. It can be seen that the mean plasma concentration-time profiles from Test and Reference are almost superimposable during the study period. The means and SDs of the plasma concentrations at each sampling time following administration of Test and Reference are given in Table I. No plasma concentrations at each sampling time showed any significant effect of the formulation as indicated by paired Student t-test.

Pharmacokinetic parameters

Table II shows the pharmacokinetic parameters of

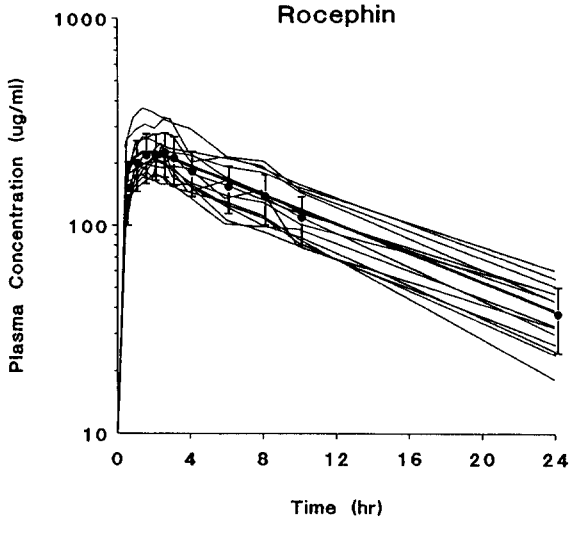


Fig. 1. Plasma concentrations of the reference formulation in each volunteer (n=14; —) following administration of 1 g IM injection, as determined by HPLC. (—; plasma concentration fitted by computer grogram, ●; mean plasma concentration)

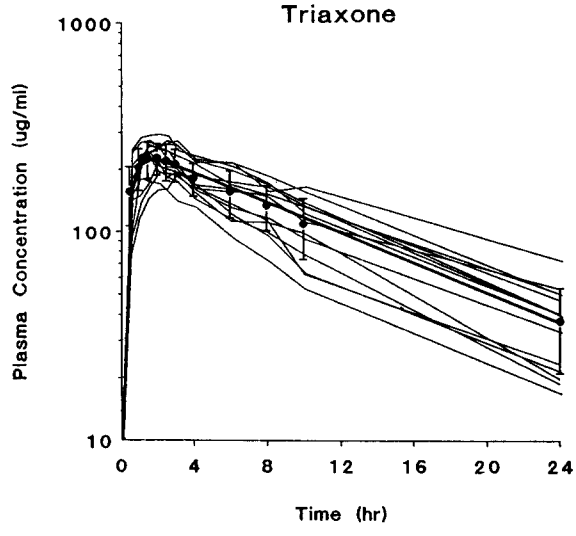


Fig. 2. Plasma concentrations of the reference formulation in each volunteer (n=14; —) following administration of 1 g IM injection, as determined by HPLC. (—; plasma concentration fitted by computer grogram, ●; mean plasma concentration)

AUC, Ka, Kel and Vd, which were calculated by the computer program "Boomer". T_{max} and C_{max} values were obtained from the raw data. The relative bioavailability of Test on Reference was found to be 101.1% and 101.6% for AUC_{24h} and AUC_{inf} respectively. The difference of mean T_{max} were -6.8% and C_{max} were 3.7%, which is in the criteria of 20% difference. No other parameters including Ka, Kel and Vd shows the difference of more than 20% between the Test and Reference formulations. Bioequivalence between the two formulations based on each of the above pharmacokinetic parameters were separately assessed by paired *t-tests*. As can be seen in Table II, the differences of all the parameters were less than 20%, and no parameter showed significance in difference.

When the 75/75 rule was applied to the Test : Reference ratio of AUC_{24h} value, 78.6% (11/14) of the subjects were within 100±25%. The Test : Reference ratios of C_{max} were within these limit for 13/14 (92.9%) and T_{max} were slightly out ranged to be 71.4% (10/14).

Three-way ANOVA analysis

Table I. Mean plasma concentrations of ceftriaxone following test and reference formulations as determined by HPLC

Time	Reference (Rocephin)	Test (Triaxone)	% difference	t-test[a]
0.5	148.91±50.06[b]	155.65±49.06	4.5	NS[c]
1.0	196.70±53.97	203.58±46.30	3.5	NS
1.5	213.41±56.79	223.61±44.20	4.8	NS
2.0	215.64±53.01	225.31±38.51	4.5	NS
2.5	220.57±52.86	218.34±43.37	-1.0	NS
3.0	207.18±54.36	210.14±37.58	1.4	NS
4.0	179.56±43.77	179.79±32.48	0.1	NS
6.0	151.44±38.72	155.70±39.48	2.8	NS
8.0	136.54±36.70	133.39±32.55	2.3	NS
10.0	108.06±28.48	108.66±35.18	0.6	NS
24.0	37.06±12.83	37.46±16.25	1.1	NS

[a]paired Student t-test (α=0.05)
[b]mean±SD of 14 volunteers
[c]Not significantly different (P>0.05)

The results of three-way ANOVA analysis of the pharmacokinetic parameters indicated that there were no significant effects of formulation, period, or sequence on any pharmacokinetic parameter (Table III and Table IV). Table III shows an example of AUC_{24h} ANOVA table, for F-value between groups, between periods and between drugs. No case shows the significance in differences between Test and Reference, which shows that the study was designed properly, cotrolled well and the drugs are bioequivalent.

Statistics of ceftriaxone bioequivalence

The statistical detection limit to have a 80% power between Test and Reference for AUC_{24h}, C_{max}, and T_{max} values were 19.5%, 13.5% and 22.5%, respectively (Table IV). Thus, the detection limit of T_{max} only outranged slightly over the limit of 20%. However, the accurate determination of T_{max} is more dependant on the frequency of blood sampling than on the formulation difference in this case. This is supported by the small difference of 6.8% between the formulations. Table IV includes the confidence limits for the percent Test : Reference ratios of mean AUC_{24h}, AUC_{inf}, C_{max}, T_{max}, and MRT_{24h}. The confidence limits for the Test : Reference ratios in all the parameters were narrow, encompassed the ideal relative bioavailability of 100%, and were within the bioequivalence limits of 100±20%, except Tmax which was -9.1%~22.9%, slightly over the limit.

In conclusion, the bioequivalence of two ceftriaxone formulations was established based on all three of the parameters (AUC_{24h}, C_{max}, T_{max}) as determined by HPLC. Bioequivalence was clearly demonstrated for AUC_{24h} and C_{max}. In case of T_{max}, it showed 6.8% difference only but the detection limit was 22.5%, which is slightly over the 20% limit. All other pharmacokinetic parameter showed the difference of less than 20%, and demonstrated non-significance in difference between two formulations.

We conclude that this study clearly demostrates the bioequivalence of Test (Triaxone) and Reference

Table II. Pharmacokinetic parameters of the ceftriaxone I.M. injection formulations in voluteers (does=1 g)

Parameters	Recephin	Triaxone	% difference	t-test
$AUC_{24\ hr}$ (μg/m/hr)	2621±635[a]	2650±614	1.1	NS[b]
AUC_{inf} (μg/m/hr)	3095±803	3146±888	1.6	NS
C_{max} (μg/ml)	203.0±53.4	238.6±35.4	3.7	NS
T_{max} (hr)	2.07±0.58	1.93±0.65	-6.8	NS
Ka (hr^{-1})	2.13±0.85	2.46±1.51	15.5	NS
Kel (hr^{-1})	0.084±0.012	0.089±0.017	6.0	NS
Cl (L/hr)	0.32±0.10	0.32±0.12	0.0	NS
$t_{1/2}$ (hr)	8.4±1.2	8.2±1.7	-2.4	NS
Vol (L)	4.23±0.83	3.99±0.66	-5.7	NS

[a]mean±SD (n=14)
[b]Significance by paired t-test, NS; not significant (p>0.05)

Table III. Example of ANOVA Table (AUC_{24hr})

Variation sources	dF	SS	MS	F	F(1, 12)
Between Subjects	13	44544.5	3426.5	4.69	
Between Groups	1	2.98	2.88	0.0008	4.75
Subject/Group	12	44541.6	3711.8	5.08	
Intra Subjects Variation					
Period	1	6.0	60.0	0.08	4.75
Drug	1	510.8	510.8	0.07	4.75
Residual	12	8762.1	730.2		
Total Variation	27	53877.5			

dF : defree of freedon, SS : Sum of squares,MS : Mean of squares, F : F-value of ANOVA.

Table IV. Statistics of ceftriaxone bioequivalence study between the (Triaxone) and the reference (Rocephin) formulations

Range of criteria	% difference	F-test[a]	Detection limit	Confidence limit[c]
Parameter	< 20%	$F < 4.75$[b]	< 20%	−20%~20%
$AUC_{24\ hr}$ (μg · hr/ml)	1.1%	0.003	19.5%	−12.8%~15.0%
$AUV_{inf.}$ (μg · hr/ml)	1.6%	0.006	21.5%	−13.6%~17.0%
C_{max} (μg/ml)	3.7%	0.001	13.5%	−6.0%~13.4%
T_{max} (hr)	6.9%	0.956	22.5%	−9.1%~22.9%
$MRT_{24\ hr}$ (hr)	1.5%	0.040	6.9%	−3.4%~ 6.4%

[a]F-value between test and reference formaulations by ANOVA
[b]F (1, 12) value at α=0.05
[c]Confidence limit of % difference. (delta%)

(Rocephin) formulations after 1 g IM injection in volunteers.

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