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COMPARATIVE BIOAVAILABILITY OF FOUR ORAL FORMULATION OF CEPHRADINE CAPSULES

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ABSTRACT

A crossover study was utilised to compare the bioavailability of four different brands of cephradine capsules in eight normal human volunteers. Relative bioavailability of three local generic dosage forms was compared with a commercial cephradine capsule. The plasma and urine cephradine concentrations were determined by a microbiological assay (disk diffusion) using *Staphylococcus aureus* (ATCC 29737) as the test organism. Relative bioavailability and pharmacokinetic parameters of local generic dosage forms of cephradine were calculated using the plasma and urine data. Statistical analysis of the data indicated no significant differences (P=0.05) between brands of cephradine capsules. Results of this study showed that the extent and rate of absorption of various tested capsules are comparable and all tested brands are compatible and bioequivalent.

Key Words: Bioavailability, Cephradine, Microbiological assay, Bioequivalent, Relative bioavailability

INTRODUCTION

Cephradine, [(7R)-7-α-D-Cyclohexa-1,4-dienyl-glcylamino)-3-methyl-3-cephem-4-carboxylic acid] is a semisynthetic derivative of cephalosporine C. Cephradine is bactericide and has a broad antibacterial spectrum against both gram positive and gram negative bacteria. It is rapidly absorbed from gastrointestinal tract and has low plasma protein binding (6-20%). Cephradine is excreted unchanged by kidneys and almost the entire dose is recovered within six hours (1, 2). Since commercially available products may not demonstrate equivalent bioavailability, evaluation of the bioavailability of various solid dosage forms is necessary. This assessment is more valuable where only generic products are considered. This study was conducted to determine the relative bioavailability of three different generic cephradine capsules in comparison with a brand name cephradine capsule (Volesef®, Squibb, England).

MATERIALS AND METHODS

Subjects: Eight normal healthy male volunteers 20 to 29 years of age weighing 62 to 75 kg were employed in this investigation. All subjects were selected after passing clinical pathologic screening for the liver, kidney and hematology functions. All subjects had no known history of hypersensitivity to penicillin and/or cephalosporines, had no history of acute or chronic disease, had not donated blood within two months before beginning of the study, and had not received any medication two weeks prior to the study. Informed written consent was obtained from each subject.

Experimental Design: The study was designed as a randomised double blind complete crossover investigation. All subjects were fasted overnight for 8 hours before each experiment. Each volunteer received 500 mg of cephradine in four different capsule formulations (A, B, C, D) on four separate occasions. Each dosing sequence was separated by a one-week washout period. Formulation, C, (Volesef® Squibb, Pharmaceutical Company, England), a commercial cephradine capsule was used as a standard for comparison with three local generic formulation, labelled as A, B and D, (Jaber Ibn-Hayyan, Pharmaceutical Company Tehran, Iran).

Sampling: Blood samples were collected into heparinized glass tubes just prior and at 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 hours after administration of the drug. Plasma was separated immediately after collection from heparinized blood and kept frozen at -20°C until analysis. Urine samples were collected hourly for the first four hours after administration of the drug and then every two hours for the remaining period up to eight hours. Urine volume was measured and an appropriate sample was frozen for analysis.

Assay: Plasma and urine samples were analysed for cephradine by a disk diffusion microbiological

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assay using *Staph. aureus* 29737 as the test organism (3,4,5). Antibiotic medium 1 (Difco) seeded with a 0.1% suspension of *Staph. aureus* was poured on 100 x 15 glass petri dishes. Twenty microliter samples of plasma were placed on 6.25mm disks (Aboryhan Pharmaceutical Company) on the surface of agar plates. The plates were incubated at 37°C for 16 hours and the zone of growth inhibition was measured to the nearest 0.1mm. Total drug concentration in plasma was then determined by a standard curve. All assays were performed in triplicate. Standard curves for each biological fluid samples which were freshly prepared on each day of analysis, using human plasma or a phosphate buffer as the diluent. The lower limit of sensitivity for the cephradine assay was 0.25 μ/ml.

Pharmacokinetic analysis: Plasma and urine data were analysed for appropriate pharmacokinetic parameters using a one-compartment open model with first-order absorption (6, 7). The area under the plasma concentration-time curves (AUC) was estimated using the trapezoidal method and extrapolated to infinity. The elimination half-life values were determined by the method of least squares. The maximum plasma concentration (C_{max}) and the time of C_{max} (t_{max}), were also calculated (8). The urinary data were used to calculate the elimination half-life of cephradine using the method of least squares of urinary excretion rate against time (mid-point method). The relative bioavailability of various dosage forms was compared using urine and plasma data.

RESULTS AND DISCUSSION

Plasma pharmacokinetic of cephradine: The mean plasma levels of cephradine in eight healthy volunteers following oral administration of 500mg cephradine of four different formulations (A,B,C,D) are shown in figure 1. These results indicate that the plasma concentration versus time profile of various cephradine dosage forms are very similar. Peak plasma concentration (C_{max}) and the time necessary to reach peak plasma concentration (t_{max}) are the two pharmacokinetic parameters which have been utilised for the rate of drug absorption (9).

Average peak plasma concentration (C_{max}) for four formulations and subjects were 12.11 μg/ml (table 1). This value is in agreement with the data (18 μg/ml) reported in the literatures (10, 11). Statistical analysis of the data indicated no significant differences (P=0.05) between different formulations of cephradine capsules. The average time of peak plasma level (t_{max}) for all formulations and subjects was 1.13 hrs (table 1) which agrees with the previous finding (0.83-1.12 hrs). Analysis of variance for the time of peak plasma concentration of cephradine showed no significant differences (P=0.05) between different brands of cephradine capsules (11,12). A comparison of the mean of other pharmacokinetic parameters such as the area under the plasma concentration time curve (AUC) and elimination half-life are also shown in table 1.

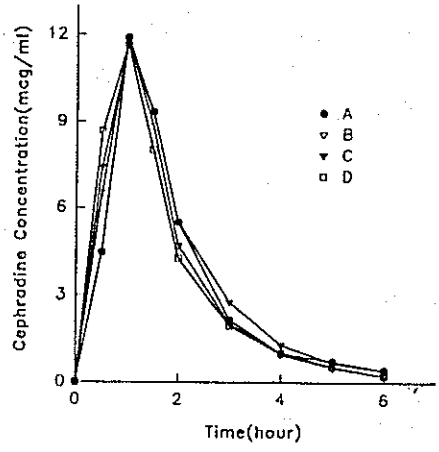

Figure 1: Mean plasma cephradine concentration in eight healthy volunteers following oral administration of 500 mg of four different formulations: A, B, C, D.

All the pharmacokinetic parameter values are in agreement with the data reported in the literatures (10-13). Statistical analysis of these data showed no significant differences (P=0.05) between the pharmacokinetic parameters of four different formulations.

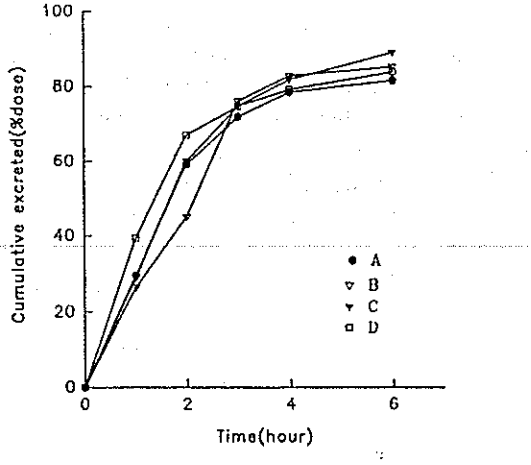

Figure 2: Average cumulative amount of cephradine excreted (% dose) into urine following oral administration of 500mg of cephradine capsule as four different formulations to (A, B, C, D) eight subjects.

Area under the plasma concentration time curve (AUC) was used to evaluate the extent of absorption of various cephradine capsules (table 1). Taking formulations C as a standard (100% availability assumed), the relative bioavailability of the tested formulations are shown in table 2. No statistically significant differences (P=0.05) between the different brands of cephradine capsules were observed. However significant inter-subject variation was observed (table 2). The 90% confidence interval for the ratio (the test to the

Table 1. Average pharmacokinetic parameters per formulation, following oral administration of 500mg of cephradine capsules as four different formulations to eight subjects

Formulations	C_{max} (mg/l)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ $mg.l^{-1}.h$
C *	12.11 ± 2.70**	1.25 ± 0.35	0.95 ± 0.17	23.14 ± 2.28
B	12.16 ± 1.67	1.06 ± 0.17	0.87 ± 0.08	20.58 ± 1.66
A	12.18 ± 2.52	1.12 ± 0.23	1.02 ± 0.12	21.16 ± 1.87
D	12.01 ± 2.57	1.12 ± 0.23	0.88 ± 0.08	20.85 ± 2.09

*Used as standard

** Mean ± standard deviation

Table 2. Relative bioavailability of various formulations of cephradine capsules following oral administration to eight subjects

Subject / Formulation	RK	FT	AH	MH	AK	HJ	MB	KG	Mean±SD
C*	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
A	83.7	110.3	100.8	92.8	81.1	88.4	87.6	90.5	91.9 (9.5)
B	81.8	87.1	91.7	98.7	83.9	82.8	89.1	98.3	89.2 (6.6)
D	79.8	78.5	92.5	98.8	89.5	86.3	95.3	102.1	90.4 (8.5)

* Used as standard

Table 3. Comparison of $A_{e\infty}$, $AUC_{0-\infty}$ and relative bioavailability (obtained from plasma or urine data) of various formulations of cephradine capsules

Formulation	$AUC_{0-\infty}$	$A_{e\infty}$ (%dose)	F_r (plasma)	F_r (urine)
C*	23.1	89.4	100.0	100.0
A	21.2	82.3	91.9	92.7
B	20.6	85.8	89.2	97.9
D	20.8	84.3	90.4	96.3

- Used as standard

standard formulation) of means of the rate (C_{max}, t_{max}) and extent (AUC) of drug bioavailability were within the WHO requirements (80-125%).

Urinary excretion of cephradine: The cumulative amount of unchanged drug excreted in the urine is a valuable parameter for bioavailability (9). Since the concentration of cephradine in the urine sample collected after 8 hours was negligible, therefore the cumulative amount excreted after 6 hours would be a proper indication of the extent of cephradine absorption. The average cumulative amount of cephradine excreted over a period of 6 hours after administration of various formulations is shown in figure 2. The average amount of drug excreted over the same period of time after administration (6 hours) to eight subject is 85.47± 2.99 (range from 82.23 to 89.40, % dose). This amount is in agreement with the previous study (82 ± 0.11, %dose), (12, 13). Analysis of variance of these data indicates that there are no significant statistical differences (P=0.05) between different formulations and different subjects. The relative bioavailability was calculated from the cumulative amount of unchanged cephradine excreted over a period of 6 hours after drug administration. Using formulations C as a standard (100% availability assumed) the relative bioavailability of all tested formulations showed no significant differences (P=0.05). These results support the information obtained from plasma data.

Comparison of plasma and urinary data: The elimination half-life at 0.86 ± 0.09 hours calculated from urine data, is in reasonable agreement with values of 0.93 ± 0.07 hours derived from the plasma data. Comparison of the area under the plasma concentration time curve (AUC), total recovery of drug (A_{e_∞}, %dose) and other pharmacokinetic parameters for different formulations are in good agreement and are shown in Table 3. These results clearly shows that results obtained from urinary data support the data obtained from plasma.

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