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CEFIXIME;

DISPOSITION KINETICS AND BIOAVAILABILITY COMPARISON OF TWO FORMULATIONS OF CEFIXIME IN HEALTHY ADULT MALE SUBJECTS.

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ABSTRACT… Cefixime is a third generation and orally acting cephalosporin. It is a cell wall synthesis inhibitor and is well stable to presence of beta lactamase enzymes. Environmental and genetic differences play a greater role in disposition kinetics of a drug. **Objectives:** To determine disposition kinetics of cefixime in local population and to evaluate the bioequivalence of multinational and national brands of cefixime. **Period:** 2013-2014. **Setting:** Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad. **Methods:** In present study disposition kinetics and bioequivalence of two brands of cefixime, cefspan and ceforal-3, were investigated in 10 adult healthy male subjects after a single oral dose of 400 mg capsule of each with a 7 days washout period. After blood sampling, plasma concentration of cefixime was determined by HPLC method. For computing disposition kinetic parameters, one compartment open model was applied. **Results:** Mean values of disposition kinetic parameters; $t_{1/2}\beta$ 5.01 and 4.72 hours, Vd 1.10 and 1.29 L/kg and Cl_B 0.16 and 0.21 L/hr/kg of cefspan and ceforal-3, respectively, were found non significantly (P 0.05) different. Similarly mean values of bioavailability parameters; AUC 36.58 and 32.99 μg.hr/mL, AUMC 282.95 and 264.13 μg.hr^2/mL and MRT 7.79 and 7.83 hours of cefspan and ceforal-3, respectively, remained non significantly ($P > 0.05$) different. All the parameters were compared by paired t-test. Relative bioavailability was found to be within the range 80-125% which is acceptable for bioequivalence. **Conclusions:** The test formulation, ceforal-3, was found bioequivalent to the reference formulation, cefspan.

Key words: Disposition-kinetic; Bioavailability; Human; Cefspan; Ceforal-3; Bioequivalence.

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INTRODUCTION

Pakistan, like other developing countries, imports raw material and/or finished drugs for its human and veterinary health programs. It has been reported that genetic makeup in local human & animals and environmental conditions are different than those of their foreign counterparts in drug manufacturing countries.[1] These differences are manifested through the variation in disposition kinetic and bioavailability parameters suggesting that disposition kinetic and bioavailability of these drugs should be investigated in species and the environment where drug is to be employed clinically.[2]

Cefixime is a third generation semi-synthetic orally active cephalosporin and is well stable to inactivation by beta-lactamase enzyme. It is effective against different human ailments including drug resistant enteric fever. Cefixime is a bactericidal drug causing its action by inhibiting synthesis of the bacterial cell wall.[3] Several brands and dosage forms of cefixime are available in Pakistan. The literature available on disposition kinetics and bioavailability of these brands in local population of Pakistan is scanty. Moreover, the investigations regarding disposition kinetic and bioavailability of different brands of cefixime in local population may help to choose low cost brand to make the treatment more economical in case both the brands are bioequivalent.

Keeping in view the facts mentioned in preceding lines, disposition kinetic and bioavailability of two brands of cefixime i.e. ceforal-3 and cefspan were investigated in adult healthy male volunteers.

MATERIALS AND METHODS

Subjects

Disposition kinetic and bioequivalence of two brands of cefixime i.e. cefspan and ceforal-3 were investigated in ten adult healthy male volunteers, 25-30 years of age. Each volunteer was non allergic to cefixime or any other β-lactam antibiotic. Volunteers were declared healthy and fit for the study after clinical history and laboratory investigations. No medication was taken by any volunteer at least 7 days before initiation of the study. Drugs were administered orally in fasting condition and breakfast was offered at half an hour, post medication.

Study Protocol

Two brands of cefixime, cefspan®, Barrett Hodgson Pakistan (Pvt) Ltd., F/423, Karachi-75700, Pakistan and ceforal-3®, Zafa Pharmaceuticals Laboratories (Pvt) Ltd., L-1/B, industrial area, Karachi-75950, Pakistan were used in present study. Cefspan and ceforal-3 were administered orally with a wash out period of 7 days. Volunteers might be ambulatory during the study but were prohibited from strenuous activity on sampling days. In each individual a blank blood sample was collected prior to drug administration. More blood samples were taken with hourly interval up to 6 hours and then on 12 and 24 hours, post medication. Samples were collected aseptically in centrifuge tubes from forearm vein through IV cannula of 20G needle and then centrifuged at 3000 rpm for 5 minutes for serum separation. The collected serum was decanted in polypropylene tubes and stored frozen at -20°C until assayed.

Analysis of cefixime

The concentration of cefixime in plasma was measured with a High Performance Liquid Chromatographic (HPLC) method having thermo hypersil-Keystone C18 column and UV/Visible detector set at 275 nm with flow rate of 1 mL/min. Equal volume (about 20 μl) of the Standard Preparation and the Sample Preparation was injected separately into the chromatograph. Chromatogram was recorded, and the areas for the major peaks for both were measured to calculate plasma concentrations of cefixime.[4]

Disposition kinetic analysis

Concentration of cefixime versus time data were used for calculating parameters of disposition kinetic and bioavailability. For disposition kinetic parameters one compartment open model was applied. Parameters of bioavailability were calculated by using method as followed by Gibaldi.[5]

Bioequivalence

Relative bioavailability of cefixime brands was calculated using method as followed by Aboulenein et al.[6] Estimates of extent and rate of absorption comparison in terms of AUC and C_{max} between the test (ceforal-3) and reference (cefspan) brands were calculated.

Statistical analysis

Mean ± SE for each concentration and parameter was calculated. Concentration of cefixime versus time data were used for calculating parameters of disposition kinetic and bioavailability. For disposition kinetic parameters one compartment open model was applied using a software APO PC-Computer Program MW/PHARM version 3.02 by F. Rombout, in cooperation with University Centre for Pharmacy, Department of Pharmacology and Therapeutics, University of Gronigen & Medi/Ware, copy right 1987-1991. Parameters of bioavailability were calculated by using method as followed by Gibaldi[5]. Data was statistically analyzed by applying student's t-test for significance ($P \leq 0.05$).

RESULTS

The results of plasma concentration, disposition kinetics and bioavailability parameters of two formulations have been presented below:

Plasma concentration

The mean ± SE values for plasma concentrations of cefspan and ceforal-3 in 10 healthy adult male subjects have been presented versus time in Table-I. The mean ± SE values of same data have been plotted on a semi-logarithmic scale in Figure-I. The Table and Figure indicate that mean ± SE concentrations of cefspan and ceforal-3 increased from 1.26 ± 0.12 and 0.93 ± 0.11 μg/

mL, respectively, at 1 hour to 4.05 ± 0.42 and 3.47 ± 0.56 μg/mL at 4 hours and then decline was progressive to 0.33 ± 0.05 and 0.28 ± 0.06 μg/mL, respectively, at 24 hours, post medication. The mean ± SE plasma concentrations of cefspan and ceforal-3 at all time intervals, from 1 hour to 24 hours, after drug administration, were found to be non-significantly (P > 0.05) different (Table-I).

Time (hours)	Cefspan	Ceforal-3
1	1.26±0.12 NS	0.93±0.11
2	2.02±0.20 NS	1.96±0.18

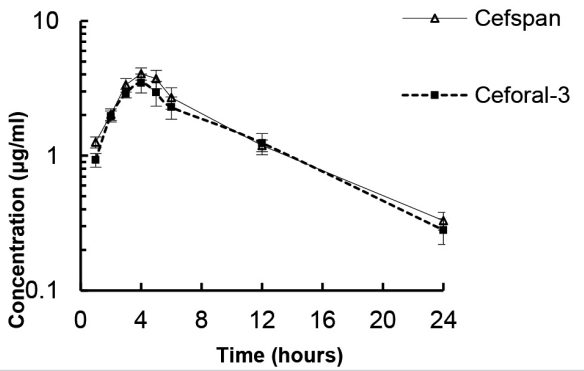

Figure-1. Mean±SE plasma concentrations (μg/mL) of Cefspan and ceforal-3 on a semi logarithmic scale versus time following a single oral administration 400 mg in healthy adult male subjects.

3	3.35±0.38 NS	2.89±0.21
4	4.05±0.42 NS	3.47±0.56
5	3.72±0.58 NS	2.94±0.61
6	2.70±0.49 NS	2.29±0.43
12	1.19±0.12 NS	1.24±0.22
24	0.33±0.05 NS	0.28±0.06

Table-I. Mean±SE plasma concentrations (μg/mL) of cefspan and ceforal-3 following a single oral administration 400 mg in healthy adult male subjects.

NS: Non significantly (P > 0.05) different from the respective value.

Disposition kinetics

Mean ± SE results of various disposition kinetic parameters in 10 healthy adult male subjects are shown in Table-II. It can be seen from the Table that mean ± SE values of disposition kinetic parameters; extrapolated zero time concentration (B) 5.94 ± 0.78 and 5.45 ± 0.86 μg/mL, elimination rate constant (β) 0.16 ± 0.02 and 0.18 ± 0.03 hr-1, elimination half life ($t_{1/2}\beta$) 5.01 ± 0.61 and 4.72 ± 0.72 hr, volume of distribution (V_d) 1.10 ± 0.15 and 1.29 ± 0.21 L/kg and total body clearance (Cl_B) 0.16 ± 0.02 and 0.21 ± 0.04 L/hr/kg of cefspan and ceforal-3, respectively, have been found statistically non significantly (P > 0.05) different from each other.

Parameters	Units	Cefspan	Ceforal-3
B	μg/mL	5.94±0.78 NS	5.45±0.86
B	hr^{-1}	0.16±0.02 NS	0.18±0.03
$t_{1/2}\beta$	hr	5.01±0.61 NS	4.72±0.72
V_d	L/kg	1.10±0.15 NS	1.29±0.21
Cl_B	L/hr/kg	0.16±0.02 NS	0.21±0.04

Table-II. Mean±SE disposition kinetic parameters of cefspan and ceforal-3 following a single oral administration 400 mg in healthy adult male subjects

NS: Non significantly (P□0.05) different from the respective value.

Bioavailability

Mean ± SE results of bioavailability parameters in ten healthy adult male subjects have been shown in Table-III. It can be seen from the Table that mean ± SE values of bioavailability parameters; maximum plasma concentration (C_{max}) 2.93 ± 0.24 and 2.53 ± 0.31 μg/mL, time to reach peak plasma concentration (T_{max}) 4.11 ± 0.16 and 3.95 ± 0.26 hr, total area under curve (AUC) 36.58 ± 3.14 and 32.99 ± 5.01 μg.hr/mL, total area under the first moment curve (AUMC) 282.95 ± 23.11 and 264.13 ± 43.42 μg.hr^2/mL and mean residence time (MRT) 7.79 ± 0.28 and 7.83 ± 0.28 hours of cefspan and ceforal-3, respectively, remained statistically non significantly (P > 0.05) different from each other.

Parameters	Units	Cefspan	Ceforal-3
C_{max}	μg/mL	2.93±0.24 NS	2.53±0.31
T_{max}	hr	4.11±0.16 NS	3.95±0.26
AUC	μg.hr/mL	36.58±3.14 NS	32.99±5.01
AUMC	$\mu g.hr^2$/mL	282.95±23.11 NS	264.13±43.42
MRT	hr	7.79±0.28 NS	7.83±0.28

Table-III. Mean±SE parameters for bioavailability of cefspan and ceforal-3 following a single oral administration 400 mg in healthy adult male subjects

NS: Non significantly (P>0.05) different from the respective value

Bioequivalence

The parameters of AUC and C_{max} of cefixime brands, cefspan (reference) and ceforal-3 (test), were used to calculate bioequivalence by measuring their relative bioavailability (Table-IV). The ratios of the mean AUC and C_{max} obtained were 0.9021 and 0.8664, respectively. The relative bioavailability was within range of 80%-125% which is acceptable for bioequivalence.

Parameter	Ceforal-3 (Test)	Cefspan (Reference)	Ratio (T/R)	Relative Bioavail-ability
AUC (μg. hr/ml)	32.99	36.58	0.9021	90.21%
C_{max}(μg/ml)	2.54	2.93	0.8664	86.64%

Table-IV. Relative bioavailability for AUC and C_{max} of two cefixime formulations

DISCUSSIONS

Disposition kinetic and bioequivalence of two formulations of cefixime were investigated following single 400 mg oral administration in healthy male subjects. The plasma concentrations of cefixime at various time intervals were used for describing disposition kinetic and bioavailability parameters. Besides plasma concentrations results of disposition kinetic parameters; $t_{1/2}\beta$, V_d and Cl_B and bioavailability parameter; Cmax, Tmax and AUC have been discussed as under:

Plasma concentration

During the course of antimicrobial therapy an antibiotic must maintain a certain therapeutic level or minimum inhibitory concentration (MIC) in plasma. The recommended MIC for cefixime has been reported in the range of 0.06-1.00 μg/mL[7]. In males an upper limit of cefixime MIC was maintained in plasma for more than 12 hours (Table-I). However plasma levels of the drug did not fall below the lower limit of MIC even after 24 hours of drug administration (Table-I). So both brands, after a single oral administration, maintained their therapeutic levels till last sampling time of 24 hours. Once maximum concentration was achieved in plasma, drug level declined progressively, thereafter.

Disposition kinetics

Table-II showed that disposition kinetic parameters of two formulations of cefixime were found to be non significantly (P > 0.05) different from each other. The elimination half life is a measure of rate of drug elimination and is time taken for the plasma/serum concentration of drug to decline by 50% during the elimination phase of disposition curve. The mean ± SE elimination half life values, 5.01 ± 0.61 and 4.72 ± 0.72 hours, for cefspan and ceforal-3, respectively, were longer than that of 3 hours after administration of 400 mg cefixime[7] and in another study after its multiple doses of 50, 100, 200 and 400 mg in healthy male subjects.[8] However, the biological half life values of present study were corresponding to 4 hours after 400 mg cefixime oral administration in adult male volunteers.[9] The longer elimination half life, 6.9 hours, was reported in rats administered with 17.8 mg/kg intravenous cefixime.[10]

The apparent volume of distribution relates the drug concentration in plasma to the total amount of the drug in the body after distribution equilibrium has been attained. The more than unity mean ± SE values of volume of distribution recorded in male adults of present investigations (1.10 ± 0.15 L/kg for cefspan and 1.29 ± 0.21 L/kg for ceforal-3) reflect excellent tissue penetration of drug. These values were found to be higher than the value, 0.3 L/kg, reported in their foreign counterparts following intravenous administration of 200 mg cefixime.[11] The values of V_d in present study were similar to 1.1 L/kg after 400 mg oral[12] and 1.71 L/kg after 200 mg intravenous[13] cefixime

in adult male subjects. Further, higher values of 2.2 and 2.8 L/kg were recorded in dogs following oral doses of 6.25 and 25 mg/kg cefixime, respectively.[14]

Total body clearance represents the sum of metabolic and excretory processes and is the volume of blood completely cleared of a drug in a unit time. The mean ± SE total body clearance in adult male subjects of present study (0.16 ± 0.02 and 0.21 ± 0.04 L/hr/kg for cefspan and ceforal-3, respectively, were comparable to 0.141 L/hr/kg12in healthy adult subjects receiving 400 mg cefixime orally. Higher values were observed in healthy humen as 0.39, 0.41, 0.43 and 0.45 mL/min/kg for respective multiple doses of 50, 100, 200 and 400 mg cefixime.[8] The values of Cl_B of both brands of cefixime in present study were far lower than 3.55 L/hr/kg observed after 200 mg intravenous injection of cefixime[13]. These differences may be subjected to environmental as well as species variations.[1]

The half life of a drug is a derived parameter that changes as a function of both clearance and volume of distribution. The apparent volume of distribution of a drug is a function of the volume of tissues in which drug distributes, partition coefficient of drug between tissues and circulatory blood, the blood flow to the tissues and binding of drugs to the plasma and tissue proteins. The total body clearance depends upon blood flow to the organ, fraction of unbound drug in blood and maximal ability of the organ to remove the drug. Most of these factors are under environmental and genetic control.[5,2]

Bioavailability

Bioavailability refers both to the rate of the drug absorption and to the extent of absorption. It is the function of C_{max}, T_{max} and AUC. In present study bioavailability parameters of two formulations of cefixime were found to be non significantly ($P > 0.05$) different from each other (Table-III).

During present investigations, after oral administration of cefspan and ceforal-3 in healthy adult male subjects, the respective mean ± SE values of peak plasma concentrations (C_{max}) were 2.93 ± 0.24 and 2.53 ± 0.31 μg/mL. The values of C_{max} observed in thepresent study were found corresponding to 2.63 and 3.85 μg/mL for 200 and 400 mg cefixime Tablets, respectively[8] and 2.17 μg/mL for oral cefixime 400 mg[15] in healthy male subjects. However, values of C_{max} of both brands of cefixime used in present study were higher than earlier investigations, 0.7, 1.2 and 2.1 μg/mL following twice a day oral administration of 50, 100 and 200 mg cefixime, respectively, in healthy volunteers.[16] The peak plasma concentrations observed for cefspan and ceforal-3 were found to be lesser than some earlier recorded values i.e. 4.74 μg/ml and 4.96 μg/mL for loprax and reference cefixime 400 mg capsules, respectively[17], given orally in healthy male volunteers.

The mean ± SE values of T_{max} for both brands of cefixime, cefspan 4.11 ± 0.16 hours and ceforal-3 3.95 ± 0.26 hours, were comparable with the values, 3.7 and 3.3 hours for cefixime 200 mg administered in fasting and non fasting conditions, respectively[18] and 3.7 hours after administration of 200 mg cefixime Tablets in healthy human volunteers.[13] The lesser T_{max}, of 2 hour, was reported after administration of cefixime 400 mg in healthy males.[7] The value of 6.7 hours for cefixime 400 mg administered in healthy male volunteers[19] was found higher than the values observed in present study. T_{max} is a function of the absorption rate from a given dosage form and thus effected by the formulation efficiency. Earlier T_{max} might produce unwanted effects on prolonged use of the drug.

The mean ± SE AUC of cefspan (36.58 ± 3.14 μg.hr/mL) and of ceforal-3 (32.99 ± 5.01 μg.hr/mL) were comparable to the value, 36.4 μg.hr/mL[8] following oral administration of cefixime 400 mg in healthy volunteers. The AUC values calculated in healthy male subjects involved in present investigations were found to be lesser than that of 45.01 and 45.22 μg.hr/mL of 400 mg loprax and reference cefixime capsules, respectively[17], in adult males. Present AUC values were higher than 26 μg.hr/mL[15] after oral administration of cefixime

400 in healthy volunteers. These differences may be attributed to the genetic or environmental variation.

Bioequivalence

Relative bioavailability, 90.21 and 86.64%, based on the ratio of AUC and ratio of C_{max} values of test brand, ceforal-3, to reference brand, cefspan, was found in the acceptable range of bioequivalence (80% to 125%). Earlier bioequivalence of two brands of 400 mg cefixime Tablets[17] have been reported in healthy male subjects following oral administration. Further, Ming-Hui[20] observed that 200 mg cefixime in Tablet form was bioequivalent to 200 mg cefixime in capsule form after oral administration in healthy volunteers. Consequently formulations of cefixime, ceforal-3 as test (local brand) and cefspan as reference (international brand), are therapeutically bioequivalent and interchangeable and therefore can be considered equally effective in medical practice.

CONCLUSIONS

Based on disposition kinetic and relative bioavailability data, it may be suggested that ceforal-3 and cefspan are bioequivalent following oral administration of 400 mg capsules in adult healthy male subjects.

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"Patience is not simply the ability to wait - it's how we behave while we're waiting."

Joyce Meyer

AUTHORSHIP AND CONTRIBUTION DECLARATION

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1	Dr. Muhammad Mudassar Ashraf	Research work, data collection, data entry paper writing	
2	Prof. Dr. Ijaz Javed	Research project design, study parameter calculations, supervision, proof reading and final correction of paper before submission.	
3	Dr. Bilal Aslam	Statistical analysis of data, sample collection and storage monitoring research supervision and proof reading.	
4	Prof. Dr. Tanweer Khaliq	HPLC analysis of drug, supervision of research work and proof reading.	