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COMPARATIVE PHARMACOKINETIC STUDIES OF TWO BRANDS OF CEFADROXIL SUSPENSION USING IMPROVED HPLC ASSAY PROCEDURE

F. A. Mohammed[1] and M. K. Quato[2]

[1]*Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt*
[2]*Department of Pharmacology, College of Pharmacy, Al-Isra University, Amman, Jordan*

تم فى هذا البحث دراسة مقارنة عوامل حركية عقار السيفادروكسيل (وتشمل أعلى تركيز للعقار فى بلازما الدم ، والوقت اللازم للوصول لأعلى تركيز والمساحة تحت المنحنى ومعدل اخراج العقار من الجسم وفترة نصف العمر للعقار فى الجسم) لمستحضرين من المعلقات المحتوية على السيفادروكسيل وهما مستحضر دروكسيل إنتاج الشركة المتحدة للأدوية - عمان - الأردن ، ومستحضر التراسيف إنتاج شركة برستول - الولايات المتحدة الأمريكية.

وقد أعطى كلا من المستحضرين لعدد ٢٤ متطوع على أسبوعين وقد أستخدمت طريقة محسنة لتعيين تركيز السيفادروكسيل فى بلازما الدم. وبعد تحليل النتائج لوحظ عدم وجود فروقات جوهرية فى عوامل حركية السيفادروكسيل للمستحضرين وكذلك كانت نسب الإتاحة البيولوجية المقارنة للعقارين ٩٥,٠٨٪ مما يعنى أن الإتاحة البيولوجية للعقار من المستحضرين واحدة وبذلك يمكن إعطاء أى منهما كان الآخر.

The pharmacokinetic parameters (C_{max}, t_{max}, AUC_{0-10h}, $AUC_{0-\infty}$, $AUMC_{0-10h}$, $AUMC_{0-\infty}$, MRT, $t_{0.5}$ and K_{el}) of cefadroxil were calculated following single oral administration of 10 ml (equivalent to 500 mg cefadroxil) of a local generic product (Droxil suspension, The United Pharmaceutical Co., Jordan) and a reference product (Ultracef suspension, Bristol, USA) to 24 healthy volunteers in a two-way cross-over study. After drug administration, serial blood samples were collected over a period of 10 hours. The concentration of cefadroxil in the separated plasma was determined by using an improved HPLC procedure. The mean values of C_{max}, t_{max}, AUC_{0-10h} and $t_{0.5}$ were 12.87±2.71 μg/ml, 1.18±0.31 h, 39.19±5.82 μgh/ml and 1.73±0.31 h, respectively for Droxil suspension, compared to 13.62±2.62 μg/ml, 1.27±0.41 h, 41.44±5.56 μgh/ml and 1.96±0.23 h respectively for Ultracef suspension. The overall mean relative bioavailability as assesed by the $AUC_{0-\infty}$ (Droxil / Ultracef) was 0.95±0.08. There was no statistical significant difference ($P < 0.05$) in the plasma concentrations or the pharmacokinetic parameters of cefadroxil after administration of the two products indicating that the two products are bioequivalent, and thus the two products are interchangeable.

INTRODUCTION

Cefadroxil is an oral cephalosporin which is similar to cephalexin and cephradine in structure and spectrum of antimicrobial activity, but has low frequency of mild side effects as well as different pharmacokinetic parameters. The plasma half-life of cefadroxil is about twice that of cephalexin (1.5 hours and 0.7 hours, respectively).[1] Thus, there appear to be 2 major advantages of cefadroxil over short acting cephalosporins (cephalexin and cephradine); first, the longer serum half-life results in higher, more extended concentrations of cefadroxil in blood, urine and tissue; and second, it can be administered with or without food and still achieve similar blood concentrations. In patients, especially the young and elderly, these features of cefadroxil may result in greater patient acceptability of the drug and thus increased compliance which can be of crucial importance in the successful management of infections in

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outpatient.[1]

Suspensions are highly effective dosage forms in terms of bioavailability, ranking second only to solutions when administered orally. Suspensions are normally more effective than tablets or capsules, even though the particle size of the material used in both may have been identical at the start. With poorly absorbed drugs it is reasonable to assume on the basis of available surface area that deflocculated dispersions might be better absorbed than flocculated ones. However, one must be careful to consider the physical state (i.e. flocculated versus deflocculated) of the suspension *in-vivo*. The presence of endogenous materials in the gastrointestinal tract may markedly change the *in-vitro* physical state of the suspension following its administration. Particle size and shape, the presence of polymorphic forms, and the addition of complexing agents are some of the factors that can be expected to affect the dissolution and bioavailability of drugs whose absorption is dissolution-rate limited. It is well known that pharmaceutical equivalent products are sometimes not bioequivalent and that the bioavailability of oral suspensions are affected by many factors including: 1) Physico-chemical factors of the drug, 2) Anatomical and physiological factors, 3) Formulation factors and 4) Chemical stability in gastrointestinal tract (G.I.T.).[2]

The objective of this study was to compare the pharmacokinetic parameters of cefadroxil for a local (test) commercial oral suspension, Droxil (a dry suspension containing 250 mg cefadroxil per 5 ml) that manufactured in Jordan by the United Pharmaceutical Manufacturing Company with a standard reference oral suspension, Ultracef (a dry suspension containing 250 mg cefadroxil per 5 ml) produced by Bristol Laboratories, USA, by using high-performance liquid chromatographic assay for cefadroxil.

EXPERIMENTAL

Subjects and methods

Subjects

Twenty-four healthy male volunteers [age 24.37 ± 0.449 years (SE), weight 80.75 ± 2.46 Kg (SE) and height 175.91 ± 1.50 cm (SE)] were selected among university students and employees after passing a screening procedure including a physical examination and laboratory investigation.

Subjects were excluded from the study if they had a history of serious illness, drug or alcohol abuse, were taking any medications including over the counter products (OTC) within 1 weak prior to the start of the study. Also, subjects having serious illness on physical examination were excluded from the study. A written and informed consent was obtained from each volunteer. The protocol of the study was approved by the Ethical Committee at Al-Isra University, College of Pharmacy, Amman, Jordan.

Commercial products

Droxil suspension (a local generic product), a dry suspension containing 250 mg cefadroxil per 5 ml produced by the United Pharmaceutical Manufacturing Company, Amman, Jordan (Lot No. 50103) and Ultracef suspension (reference product), a dry suspension containing 250 mg cefadroxil per 5 ml produced by Bristol Laboratories, USA (Lot No. K5W26C).

Study design

The study design was a balanced, randamized, open-label, two-period cross-over with one week washout evaluation of the bioavailability of the two suspension preparations of cefadroxil, Droxil (test product) and Ultracef (reference product). Prior to the day of dosing the volunteers were asked to fast overnight (12 hours) and were randomly assigned to receive either a single dose of 500 mg cefadroxil (10 ml) of Droxil (test suspension) or Ultracef (reference suspension). There was a one week washout period between the two phases. At 7.30 a.m on the day of dosing an indwelling canula was placed in the forearm antecubital vein of each subject and the zero-hour blood samples was drawn. At 8.00 a.m., the participants were asked to swallow 10 ml (equivalent to 500 mg cefadroxil) of the

suspension. The participants were asked to take 250 ml water with the medications and were then asked to remain fasting for 4 hours during the study. Venous blood samples (10 ml) were collected at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 600, 8.00 and 10.00 hours after drug administration. The volunteers were permitted to have breakfast after the two-hour blood collection and lunch after four-hour collection. The food and fluid intake in all study periods were identical. No drugs, alcohol, or caffeine containing beverages were allowed within 24 hours perior to and during the study. The plasma was separated from the blood samples by centrifugation at 2000 revolutions per minute for 10 minutes and was then frozen at -20° until analysis within one week by high-performance liquid chromatography (HPLC).

Analysis of cefadroxil in human plasma

Cefadroxil was determined in the separated plasma samples using a modified HPLC method of assay.[3] In screw-capped centrifuge tubes, 1 ml of the separated plasma was mixed with 0.5 ml of amoxycillin solution (internal standard, 5 μg/ml) and 500 μL of perchloric acid (5%). The mixture was then vortex-mixed and centrifuged for 10 minutes at 3500 rpm. The supernatant was then separated and mixed with 5 ml methylene chloride. The tubes were vortex mixed for 10 sec and then centrifuged at 3500 rpm for 10 minutes. One hundred microliteres of the supernatant were injected into the chromatograph.

Chromatographic system

The HPLC apparatus was composed of a computerized Hitachi System.[4] A Hitachi pump (model L-6200 A) operating at a flow rate of 1.5 ml/min, a variable UV detector (L-400 A) set at 254 nm, an interface (D-6000 A) and a 100 μL fixed volume autosampler (AS-2000). The separation was achieved on a stainless-steel reversed-phase Lichrosphere RP-18 column (25 x 4.0 mm, 5 μm) with a C18 precolumn (30-40 μm). The mobile phase consisted of a mixture of 0.1 M phosphate buffer (pH 3) and acetonitrile at a ratio of 92:8 V/V. Cefadroxil concentrations of the plasma samples were calculated by comparing the peak height ratio of cefadroxil /amoxycillin in plasma samples to those prepared by spiking blank plasma with various known concentrations of cefadroxil (0.5 to 16 μg/ml) and amoxycillin (5 μg/ml). All solvents used in the analysis of the plasma samples were of HPLC grade and were purchased from Lab. Scan (UK), and reagents were of analytical grade and were obtained from GCC (UK).

Pharmacokinetics data and statistical analysis

A computer program (PNC) was used to estimate the pharmacokinetic parameters of cefadroxil from the individuals plasma concentration versus time profiles following oral administration of Droxil suspension (test product) and Ultracef suspension (reference product).

The pharmacokinetic parameters estimated include: C_{max}, t_{max}, $AUC_{0\text{-}10h}$, $AUC_{0\text{-}\infty}$, $AUMC_{0\text{-}10h}$, $AUMC_{0\text{-}\infty}$, MRT, $t_{0.5}$ and K_{el}. The C_{max} (maximum concentration) was calculated from the plasma concentration versus time, and the time to reach the maximum concentration is t_{max}. The area under the curve $AUC_{0\text{-}10h}$ was calculated by the trapezoidal rule and extrapolated to infinity ($AUC_{0\text{-}\infty}$) by adding the area approximated by dividing the last measured concentration by the elimination rate constant which was calculated from the log transformation of plasma concentration versus time curves. The mean residence time (MRT) was calculated from the $AUMC_{0\text{-}\infty}$ and $AUC_{0\text{-}\infty}$ by the equation 1:[6,7]

$$MRT = AUMC_{0\text{-}\infty} / AUC_{0\text{-}\infty} \quad \text{Eq. (1)}$$

Where $AUMC_{0\text{-}\infty}$ is the area under the (first) momentum time curve from t= 0 to infinity, and $AUC_{0\text{-}\infty}$ is the area under the plasma concentraion versus time curve from t= 0 to infinity.[5,6]

A two-way statestical analysis of variance of the plasma concentrations and pharmacokinetic purameters was performed using ANOVA at a 5% significance level ($P < 0.05$). A p-value less than or equal to 0.05 was considered significant. Data reported as the means $\pm$ SD (n= 24). The relative bio-availability (F_R) was calculated by dividing the

$AUC_{0-\infty}$ obtained for each individual after administration of Droxil suspension (test product) by that obtained for the same individual after administration of Ultracef suspension (reference product), using equation 2:

$$F_R = AUC_{0-\infty} \text{ (Droxil)} / AUC_{0-\infty} \text{ (Ultracef)} \quad \text{Eq. (2)}$$

RESULTS AND DISCUSSION

The analytical technique used to determine concentration of cefadroxil in plasma samples was based on a published method[3] with some improvement. The retention times for amoxycillin (internal standard) and cefadroxil in the utlized HPLC procedure were 3.7 and 4.8 min respectively (Figure 1) compared to 15 min for cefadroxil in the published method.[3] There was no interference due to the endogenous plasma components at the retention times of the drug and the intrnal stndared (Figure 1) indicating the selectivity of the assay.[3] Thus, the utlized HPLC method for determination of cefadroxil in plasma resulted in analysis time less than 5 minutes for each plasma sample. A typical standard calibration plot of cefadroxil in spiked blank plasma is shown in Figure 2 and can be described by the equation: $Y = 0.77x + 0.027$ with a correlation coefficient (r) of 0.996. The limit of detection for the utilized procedure was 0.05 μg cefadroxil/ml plasma. The within-day and between-day precisions of the assay are shown in Table 1.

The plasma concentrations (mean $\pm$ SEM) of cefadroxil after oral administration of Droxil suspension and Ultracef suspension (500 mg/10 ml) into 24 volunteers are shown in Figure 3.

Table 2 shows the pharmacokinetic parameters (mean $\pm$ SD) of cefadroxil obtained from the plasma data. The pharmacokinetic data indicated that cefadroxil was rapidly absorbed from the two products. The mean values of the C_{max}, t_{max} and AUC_{0-10h} were 12.87 ± 2.71 μg/ml, 1.18 ± 0.31 h and 39.19 ± 5.82 μgh/ml respectively for Droxil suspension compared to 39.19 ± 5.82 μgh/ml, 1.27 ± 0.41 h and 41.44 ± 5.79 μgh/ml respectively for Ultracef suspension (Table 2). The plasma levels of cefadroxil declined with a first-order elimination rate constants (K_{el}) of 0.35 ± 0.04 h^{-1} and 0.40 ± 0.08 hr^{-1} for Ultracef and Droxil suspensions respectively. The biological half-lives values ($t_{0.5}$) were 1.96 ± 0.23 h and 1.73 ± 0.31 h for Ultracef and Droxil suspensions respectively. No significant difference ($P < 0.05$) was observed for the plasma concentrations or the pharmacokinetic parameters of cefadroxil after administration of the two products (Figure 3 and Table 2). In addition, the observed plasma concentrations of cefadroxil and the pharmacokinetic parameters (C_{max}, t_{max} and $t_{0.5}$) were in the same order of magnitude as the reported values.[1,6-9] The overall mean relative bioavailability as assesed by the $AUC_{0-\infty}$ (Droxil to Ultracef) was 0.95 ± 0.08). These data was in the acceptable range (0.80-1.25) for bioequivalence products.[10] The obtained results revealed that there was no statistical difference ($P < 0.05$) in the plasma concentrations and pharmacokinetic parameters of the two cefadroxil products. This indicates that the two products which are pharmaceutical equivalent (contain identical amounts of the same therapeutic moiety, cefadroxil) are bioequivalent and thus are interexchangeable.

Conclusion

An improved and specific HPLC method of assay of cefadroxil in plasma was adopted and used to follow the plasma drug concentration after oral administration of cefadroxil as suspension (once as a generic brand and second as a reference leading brand). The rates and extent of bioavailability as measured by the maximum concentration (C_{max}), area under the curve (AUC) and elimination half-life ($t_{0.5}$) showed that the two suspension formulations are bioequivalent. It is concluded that the two products are both pharmaceutical equivalent and bioequivalent and thus they are interexchangeable.

Table 1: Within-day and between-day precision of cefadroxil in human plasma.

Added Conc. (μg/ml)	Within-day[a]				Between-day[e]			
	Measured concentration			% Biase[d]	Measured concentration			% Biase[d]
	mean (μg/ml)	SD[b]	CV%[c]		mean (μg/ml)	SD[b]	CV%[c]	
0.50	0.491	0.020	4.07	-1.80	0.489	0.025	5.11	-2.20
1.00	0.984	0.050	5.08	-1.60	0.967	0.045	4.65	-3.30
8.00	7.69	0.37	4.81	-3.87	7.92	0.41	5.17	-1.00
16.00	10.07	0.73	4.54	0.437	15.83	0.88	5.55	-1.06

a: means for 5 determinations for each concentration carried out in the same day.
b: standard deviation
c: coefficient of variation
d: % biase = [(measured concentration – added concentration) / added concentration] x 100
e: mean for 5 determinations for each concentration carried out over a period of two weeks.

Table 2: Mean pharmacokinetic parameters of cefadroxil after oral administration of Ultracef and Droxil suspensions (500 mg cefadroxil /10 ml) into 24 Volunteers.

Pharmacokinetic Parameters	Ultracef Mean ± SD	Droxil Mean ± SD	ANOVA test (P <0.05)*
C_{max} (μg/ml)	13.62 ± 2.62	12.87 ± 2.71	N.S.
T_{max} (h)	1.27 ± 0.41	1.18 ± 0.31	N.S.
$AUC_{0\text{-}10\,h}$ (μgh/ml)	41.44 ± 5.56	39.19 ± 5.82	N.S.
$AUC_{0\text{-}\infty}$ (μg h/ml)	42.79 ± 7.97	39.19 ± 5.82	N.S.
$AUMC_{0\text{-}10\,h}$ (μg h^2/ml)	110.04 ± 22.98	92.06 ± 25.91	N.S.
$AUMC_{0\text{-}\infty}$ (μg h^2/ml)	127.56 ± 31.14	101.85 ± 31.81	S.
MRT (h)	2.98 ± 0.31	2.78 ± 0.37	N.S.
$T_{0.5}$ (h)	1.96 ± 0.23	1.73 ± 0.31	N.S.
K_{el} (h^{-1})	0.35 ± 0.04	0.40 ± 0.08	N.S.

*A two-way analysis of variance (ANOVA) was performed for statistical analysis.
N.S. = non significant difference.
S. = significant difference.

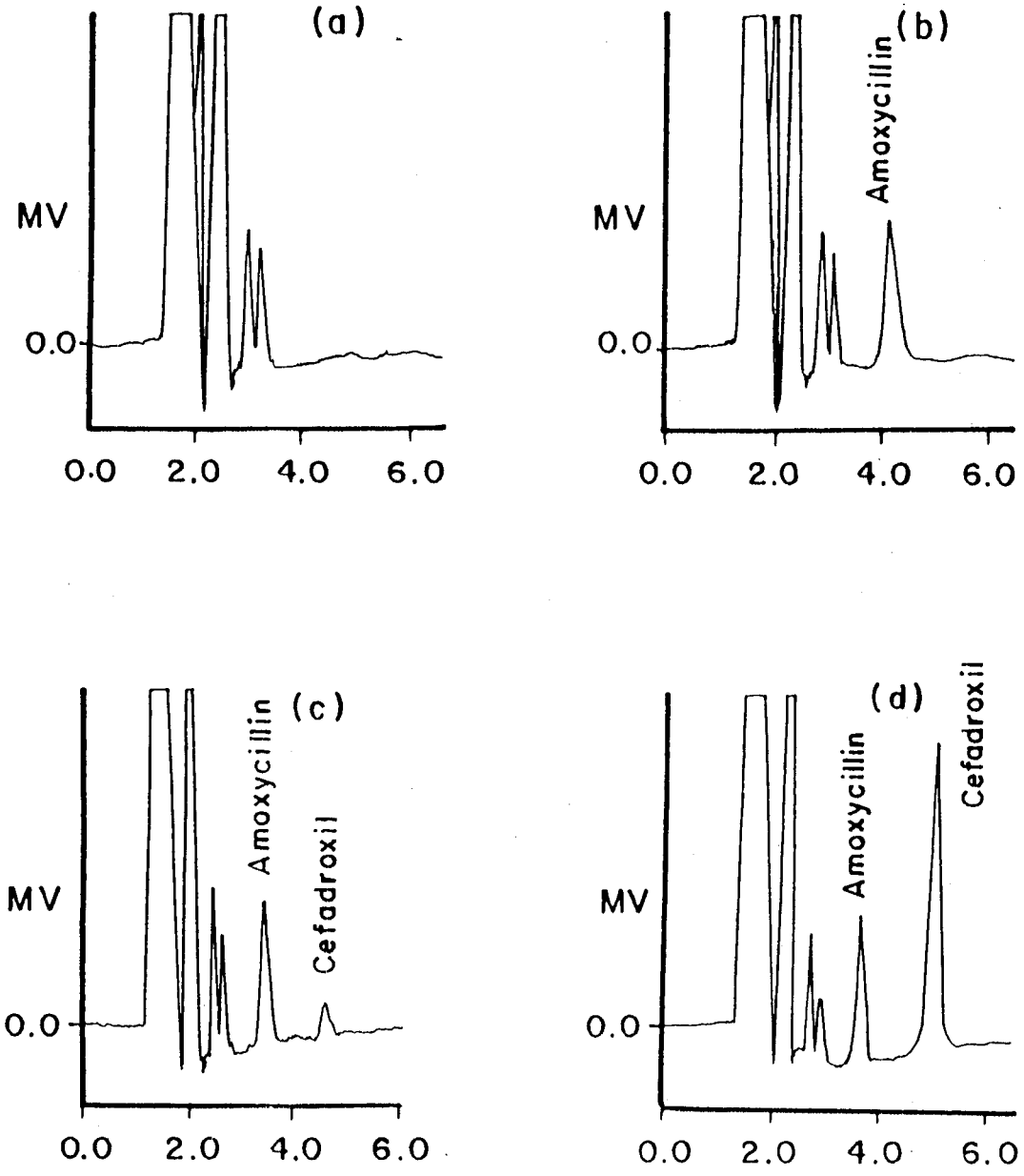

Fig. 1: Typical chromatograms of
(a) Blank plasma
(b) Blank plasma spiked with amoxycillin (5 μg/ml)
(c) Blank plasma spiked with amoxycillin (5 μg/ml) and cefadroxil (0.5 μg/ml)
(d) Blank plasma spiked with amoxycillin (5 μg/ml) and cefadroxil (5 μg/ml)

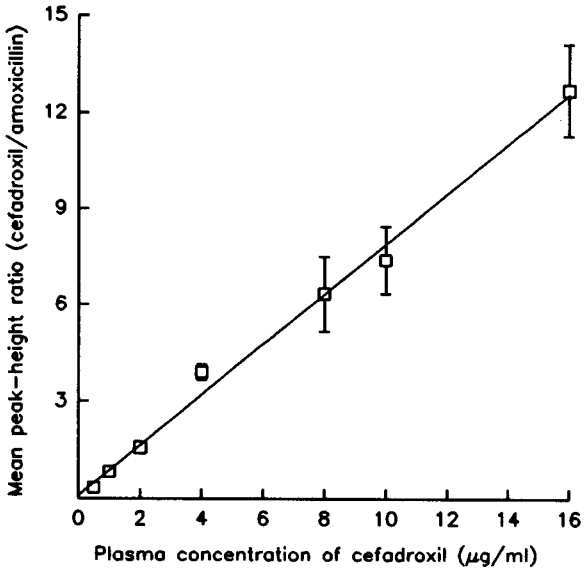

Fig. 2: Standard calibration curve of cefadroxil in plasma.

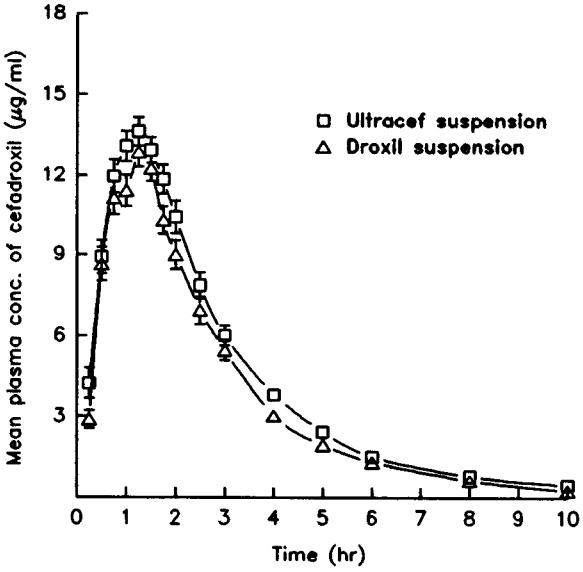

Fig. 3: Mean plasma concentration-time curves of cefadroxil after oral administration of Ultracef and Droxil suspensions (500 mg cefadroxil/10 ml) into 24 volunteers.

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