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A comparative Pharmacokinetic Study of Two Marketed Ciprofloxacin Tablet Formulations Using Microbiological Assay

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Abstract: A comparative pharmacokinetics study of two ciprofloxacin conventional formulation was carried out on ten healthy male volunteers. The study was randomized, with two way crossover design. The agar well diffusion technique was applied for the assay of ciprofloxacin where plasma drug level was used as a parameter. *In vitro* studies, it was illustrated that the two tablet formulation, were pharmaceutically equivalent. *In vivo* studies, pharmacokinetic parameters, such as C_{max}, median T_{max}, and $AUC_{0-\infty}$, K, Cl/F, V_d/F and plasma half time $T_{½}$ were determined and subjected to statistical analysis . No significant difference in the rate or extent of absorption was observed between the two products. Therefore, the two marketed ciprofloxacin tablet formulations delivered equal amounts of the drug to the systemic circulation.

الملخص: تمت دراسة مقارنة الإتاحة الحيوية لعقارين من السيبروفلوكساسين (محلي و مستورد) على 10 متطوعين أصحاء ذكور. تم دراسة الإتاحة الحيوية لكل دواء بواسطة استخدام بلازما الدم للمتطوعين عن طريق استخدام طريقة التحليل الميكروبيولوجي حيث تم حساب جميع الثوابت الحركية للدواء من حيث معدل سرعة الامتصاص ومعدل سرعة التخلص وحجم التوزيع وفترة نصف العمر وكذلك تعيين قيم ووقت ذروة التركيز داخل الجسم وذلك في بلازما الدم للمتطوعين الأصحاء. وتم التوصل إلى أن كلا من العقارين (محلي ومستورد) متكافئان من جهة معدل الإذابة طبقا لدستور الأدوية (USP27-NF22) وكذلك من جهة التكافؤ الحيوي.

Introduction

Ciprofloxacin, a quinoline carboxylic acid derivative, is highly active *in vitro* against a broad spectrum of gram- negative and gram- positive organisms, including these resistant to aminoglycosides and ß-lactam antibiotics [1]. It is rapidly and well absorbed from the gastro-intestinal tract [2]. Oral bioavailability is 70% and a peak plasma concentration at about 2.5 μgml^{-1} is achieved 1 to 2 hours after a dose of 500 mg by oral administration [3-5]. The plasma half life time is about 3.5 to 4.5 hours, plasma protein binding ranges from 20 to 40 % and about 40 to 50 % excretion is unchanged in the urine [3-5].

Pharmacokinetics of ciprofloxacin in humans was found to be affected by its formulation [6-8], drug-drug interaction [9], gender [10-12] and different environmental conditions [13]. Concentration of ciprofloxacin in plasma was determined by microbiological assay by the agar well diffusion method with Escherichia coli ATCC25922 as the test organism [14-17]. Marco et al. [17] was found an excellent correlation between the results of the microbiological assay and that of the HPLC assay. The sensitivity and coefficient of variation of assay were 0.02 μgm^{-1} and 9.49%, respectively [17].

This study was performed to compare the bioavailability of locally manufactured ciprofloxacin tablet brand (Cipro. M.E. batch No.4409, Megapharm) relative to imported tablet brand (Cipro. Teva. Batch No.492005, Teva) in normal male volunteers receiving 500mg of each formulation as a single dose based upon ciprofloxacin absorption. Relative bioavailability was assessed through a comparison of the area under the plasma concentration $\mathbf{AUC_{0-\infty}}$, the average plasma peak level C_{max}, the elimination rate constant (K), oral clearance (Cl/F), apparent volume of distribution (V_d/F) and the mean peak time (T_{max}). The plasma half life of ciprofloxacin during each treatment period was also compared.

Experimental

Subjects: The 10 male volunteers under investigation in this study were between 20 and 45 years old (mean, 30.0 years) and weighed between 65 and 95 kg (mean, 78.2 kg); their heights ranged from 170 to 185 cm (mean, 177.3cm). All volunteers were reported to be in good health as determined by physical examination. None of the subjects had a history of serious systemic illness, drug, smoker or alcohol abuse or hypersensitivity to any food or drug. None of the subjects was taking any medication one week

before or during the study any ailment. All subjects gave written informed consent before entry into the study. The protocol was approved by the General Director of Pharmacy, Ministry of Health, and Palestinian National Authority.

Materials

Brand M.E. was Cipro M.E., 500mg , B.N.4409, Megapharm and brand Teva was Cipro Teva, 500mg ,B.N.492005. Ciprofloxacin HCL working standard was supplied by the Middle East Pharmaceutical and cosmetics laboratories, Palestine. The purity was found to be 100.1± 0.98% according to reference methods [18]. All chemicals were of spectroquality or analytical grade.

***In vitro* studies**

The *in vivo* investigations were carried out at the Middle East pharmaceutical and cosmetics laboratories, Gaza, Palestine. The performed tests were appearance, friability, disintegration time, mean tablet weight, content uniformity and dissolution rate as specified by USP27-NF22 monograph for ciprofloxacin tablets. For assay, ten tablets from each formulation were powdered and the average weight of one tablet was determined by UV spectrophotometer method according to USP27-NF22 monograph. Dissolution rate (Apparatus 2: 50 rpm) was determined using a tablet dissolution tester (USP24), Electro lab. TDT, India. The dissolution medium was 900 ml water. Dissolution samples were analyzed using UV spectrophotometer at 277 nm.

Pharmacokinetic study

In vivo study was executed in two–way randomized crossover design. Each brand was administrated orally as a single dose (one tablet, 500 mg) in the sequence described in Table 1. Seven days washout period separated the study days. Volunteers fasted over night before each drug administration and food was not allowed for 4 hours thereafter. Each tablet was administrated with approximately 200ml of water. Venous blood samples (6ml) were drown at 0 , 0.5 , 1 , 1.5 , 2 , 2.5 , 3 , 4 , 6 , 8 and 12 hours from the antecubital vein. The samples were collected in heparinized centrifuge tubes. All the samples were centrifuged and plasma was separated and frozen at (-4 ^{o}C) until running the analysis.

Ciprofloxacin in plasma samples was determined by microbiological assay using agar well diffusion method. Zone of inhibition and concentration were plotted on semi logarithmic graph. Simple linear

regression equation was fitted by taking concentration as (*X*) and circular inhibition zone as (*Y*) which is as follows:

$$Y = 4.6X + 19$$

Tablet 1. Demographic data and sequence of administration (500mg) of Cipro M.E and Cipro Teva to 10 healthy male volunteers

Volunteers	Age(year)	Weight(kg)	Height(cm)	Period	
				1	2
1	32	75	174	M.E.	Teva
2	20	65	170	M.E.	Teva
3	26	68	177	M.E.	Teva
4	40	84	180	Teva	M.E.
5	30	78	182	Teva	M.E.
6	22	65	172	Teva	M.E.
7	27	90	176	M.E.	Teva
8	45	95	185	M.E.	Teva
9	32	87	183	Teva	M.E.
10	26	75	174	Teva	M.E.
Mean	30.0	78.2	177.3		

Pharmacokinetic parameter values were calculated from the curve of plasma concentration of the drug versus the real time of sampling from each volunteer. The maximum plasma concentration (C_{max}) and time to maximum plasma concentration (T_{max}), were assigned by visual inspection of the data. The terminal elimination rate constant (K) was determined by log-linear regression. The terminal elimination half-life ($T_{½}$) was determined by the following relationship: $T_{½}$= 0.693/K. The area under the ciprofloxacin concentration-time curve up to the last quantified data point [i.e. AUC_{0-12}] was calculated by the linear trapezoidal rule, and the $AUC_{12-\infty}$ was calculated with extrapolation to infinity by dividing the last measured concentration by K. The sum of these areas identified $AUC_{0-\infty}$. Oral clearance was calculated from [dose/ $AUC_{0-\infty}$] and reported as Cl/F. The apparent volume of distribution was determined from [dose/ $AUC_{0-\infty}$*K] and reported as V_d/F.

Differences between plasma concentration and other parameters were compared using paired t-test and the variance ratio f-test. A $p<0.05$ was considered to be level of significance. For T_{max}, a non-parametric

comparison was made using Wilcoxon test at a level of significance p = 0.05.

Results and Discussion:

Demographic data and sequence of administration of ciprofloxacin dose to 10 healthy male volunteers are shown in Table 1. The *in vitro* data for the two ciprofloxacin formulations are show in Table 2. Each brand convene all the specifications as stipulated by USP77-NF22 for ciprofloxacin. Therefore *in vitro* studies indicated that the two product, Cipro M.E. and Cipro Teva were pharmaceutically equivalent.

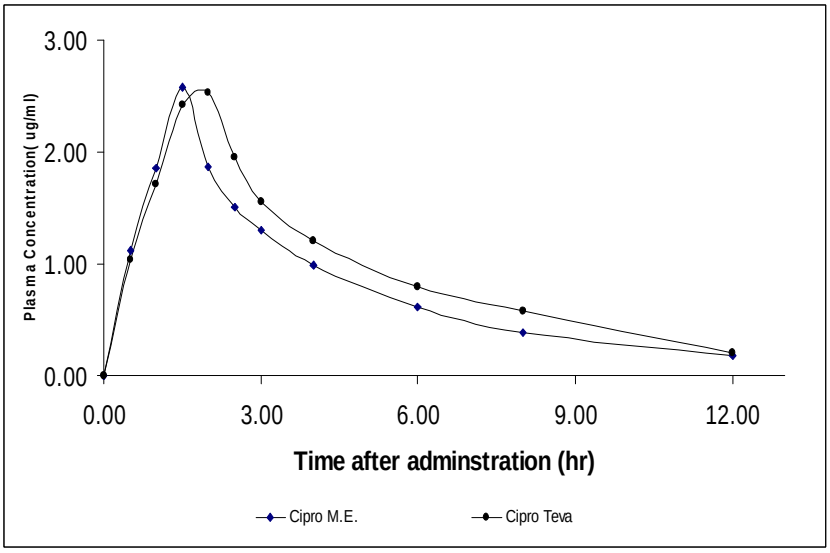
Table 2. Bioavailability and in vitro data for ciprofloxacin formulations under study

Parameters	Cipro M.E.	Cipro Teva	F-test	t-test	W-test
C_{max} μgml^{-1}	2.58±0.48	2.74±0.39	1.23	1.41	-
T_{max} (hr)	1.50	1.75	-	-	0.89
$T_{½}$ (hr)	3.70	3.62	1.017	-	-
AUC_{0-12} $\mu gml^{-1}hr$	10.74	11.11	1.10	1.16	-
$AUC_{0-\infty}$ $\mu gml^{-1}hr$	11.64	12.13	1.10	1.38	-
%AUC	96.0	100.0	-	-	-
K (hr^{-1})	0.20	0.21	1.20	0.50	-
Cl/F (L hr^{-1})	42.87	41.42	1.10	-	-
V_d/F (L)	215.9	197.3	1.16	-	-
Tablet Weight (mg)	547	565	-	-	-
Assay (%), UV	98.8	99.6		-	-
Disintegration Time (min.)	6.0	10.0	-	-	-
%dissolution At 30 min	98.0	96.0	-	-	-

$f_{(10,10)} = 3.18$ ($p<0.05$) , $t_{(10)} = 2.262$ ($p<0.05$) , $w_{(10)} = 1.0$ ($p=0.05$).

A calibration curve was constructed by plotting the zoned inhibition of ciprofloxacin versus the concentration, with linearity over a range of 0.1-3.0 µg/ml. The correlation coefficient and coefficient of variation of assay were 0.9994 and 6.1%. In Figure 1 and Tables 3 and 4, the mean plasma concentrations of ciprofloxacin at each sampling time following oral administration of the two brands to 10 healthy male volunteers were summarized.

Figure1. Mean plasma concentration-time curves after administration (500mg) Of Cipro M.E. and Cipro Teva. to 10 healthy male volunteers



Ciprofloxacin plasma concentration has no any significant difference between the two brands at each sampling since the calculated t-value indicated insignificant difference between the two compared brands (Table 2).

The mean peak plasma concentration, mean C_{max} ± SD was 2.58±0.48 μgml^{-1} for Cipro M.E. (Table 5) and 2.74± 0.39 μgml^{-1} for Cipro Teva (Table 6). The ratio of the C_{max} mean values (Cipro M.E. relative to Cipro Teva) is 0.942. The median peak time, T_{max} were 1.52 hr for Cipro M.E. (Table 5) and 1.75 hr (range 1.50-2.00 hr) for Cipro Teva (Table 6). Values of T_{max} for both formulations fall within the specified reported values for ciprofloxacin [3-5].

Table 3. Concentration of ciprofloxacin (M.E.) in plasma in 10 volunteers

Volunteer	Ciprofloxacin concentration (μgml^{-1} ± SD) at the following time (hr) after dosing									
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	6.0	8.0	12.0
1	1.30	2.17	3.17	1.96	1.74	1.52	1.02	0.87	0.65	0.18
2	0.65	2.17	2.61	2.39	1.74	1.74	1.09	0.87	0.19	0.18
3	0.87	1.52	2.96	1.52	1.30	1.09	1.09	0.53	0.21	0.15
4	1.96	2.17	2.83	1.74	1.30	1.52	1.30	0.89	0.21	0.18
5	0.17	1.22	1.96	1.74	1.52	1.30	1.09	0.65	0.20	0.18
6	1.21	1.52	1.96	1.52	1.09	0.21	0.20	0.39	0.17	0.12
7	0.72	2.39	2.87	2.63	1.91	1.91	0.98	0.72	0.42	0.20
8	0.96	1.67	2.16	1.67	1.43	1.20	0.98	0.63	0.35	0.17
9	2.16	2.39	3.11	1.91	1.43	1.67	1.17	0.72	0.54	0.20
10	1.19	1.24	2.16	1.91	1.67	1.43	0.90	0.78	0.34	0.20
Mean ± SD	1.12 ± 0.49	1.85 ± 0.44	2.58 ± 0.48	1.87 ± 0.36	1.51 ± 0.25	1.36 ± 0.47	0.99 ± 0.30	0.71 ± 0.16	0.33 ± 0.17	0.18 ± 0.03

T_{max} was not altered significantly between the two brands (Table 2). The $AUC_{0-\infty}$ values averaged 11.64 μgml^{-1} hr for Cipro M.E. and 12.13 μgml^{-1} hr for Cipro Teva. The ratio of the mean area under the curve of Cipro M.E. relative to Cipro Teva was 0.96, the ratio fall within the range of 0.80 – 1.20 for bioequivalence .

Table 4. Concentration of ciprofloxacin (Teva) in plasma in 10 volunteers

Volunteers	Ciprofloxacin concentration (μgml^{-1} ± SD) at the following time (hr) after dosing									
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	6.0	8.0	12.0
1	1.52	1.96	2.39	3.26	2.39	1.74	1.52	1.00	1.09	0.20
2	1.52	2.39	2.83	2.39	1.96	1.74	1.30	0.87	0.21	0.19
3	1.09	1.52	1.96	2.83	1.96	1.30	1.09	0.87	0.65	0.18
4	1.30	1.74	2.61	2.17	1.74	1.52	1.30	0.43	0.22	0.20
5	0.19	1.09	1.96	2.17	1.74	1.52	1.30	0.87	0.65	0.20
6	0.22	1.96	2.17	1.96	1.52	0.43	0.21	0.50	0.17	0.17
7	1.67	2.63	3.11	2.63	2.16	1.91	1.40	0.48	0.23	0.19
8	1.20	1.67	2.16	3.11	2.16	1.43	1.20	0.78	0.72	0.20
9	1.43	1.91	2.87	2.39	1.91	1.67	1.40	0.73	0.24	0.22
10	0.24	0.26	2.16	2.39	1.91	1.67	1.40	0.78	0.72	0.22
Mean ± SD	1.04 ± 0.60	1.71 ± 0.67	2.42 ± 0.43	2.53 ± 0.41	1.95 ± 0.27	1.49 ± 0.41	1.21 ± 0.37	0.73 ± 0.19	0.58 ± 0.33	0.2 ± 0.02

The half life $T_{½}$ average was 3.70 hr for Cipro M.E. and 3.62 hr for Cipro Teva. Finally the terminal elimination rate constant (K), oral clearance (Cl/F), apparent volume of distribution (V_d/F) and AUC_{0-12} were calculated

for each brand and compared. The differences are not statistically significant (Table 2).

Table5. Pharmacokinetic parameters of Cipro.M.E.

Volunteer	C_{max} μgml^{-1}	T_{max} (hr)	T½ (hr)	K (hr^{-1})	AUC_{0-12} ($\mu gml^{-1}hr$)	$AUC_{0-\infty}$ ($\mu gml^{-1}hr$)
1	3.17	1.5	2.62	0.26	11.97	12.66
2	2.61	1.5	2.62	0.26	10.86	11.55
3	2.96	1.5	4.75	0.15	11.23	12.23
4	2.83	1.5	2.54	0.27	10.65	11.32
5	1.96	1.5	3.84	0.18	10.21	11.21
6	1.96	1.5	6.68	0.10	10.35	11.55
7	2.87	1.5	3.47	0.20	11.27	12.27
8	2.16	1.5	3.92	0.18	9.34	10.28
9	3.11	1.5	3.47	0.20	11.84	12.84
10	2.16	1.5	3.11	0.22	9.56	10.47
Mean ±SD	2.58 ± 0.48	1.5	3.70 ±1.15	0.20 ±0.05	10.74 ± 0.82	11.64 ±0.87

Table 6. Pharmacokinetic parameters of Cipro Teva

Volunteer	C_{max} μgml^{-1}	T_{max} (hr)	T½ (hr)	K (hr^{-1})	AUC_{0-12} ($\mu gml^{-1}hr$)	$AUC_{0-\infty}$ ($\mu gml^{-1}hr$)
1	3.26	2.0	2.26	0.30	12.79	13.46
2	2.83	1.5	2.66	0.26	10.82	11.55
3	2.83	2.0	2.66	0.26	10.71	11.40
4	2.61	1.5	4.48	0.15	10.54	11.87
5	2.17	2.0	2.66	0.26	10.57	11.34
6	2.17	1.5	5.47	0.13	10.25	11.56
7	3.11	1.5	6.23	0.11	12.31	14.04
8	3.11	2.0	3.11	0.22	11.97	12.88
9	2.87	1.5	3.54	0.20	10.47	11.57
10	2.39	2.0	3.11	0.22	10.66	11.66
Mean ±SD	2.74 ± 0.39	Range(1.5-2) Median 1.75	3.62 ±1.17	0.21 ±0.06	11.11 ± 0.89	12.13 ± 0.96

The two formulations Cipro M.E. and Cipro Teva for the active ingredient ciprofloxacin exhibited all the specifications as stipulated by USP77-NF22 for ciprofloxacin tablet. Therefore *in vitro* study results show that both brands of ciprofloxacin were found to be equivalent.

Relative bioavailability of locally manufactured Cipro M.E. compared to imported Cipro Teva tablets average were 96 %. C_{max}, $AUC_{0-\infty}$, K, Cl/F, V_d/F and $T_{½}$ were not significantly different from each other. T_{max} for both formulations were within specified reported value. In conclusion, these results revealed that, No difference in the rate or extent of absorption between the two products. Therefore, the two marketed ciprofloxacin tablet formulations delivered equal amounts of the drug to the systemic circulation.

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