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RESEARCH ARTICLES / BİLİMSEL ARAŞTIRMALAR

Bioequivalence Determination of Two Ofloxacin (400 mg) Tablets Manufactured in Turkey and in Germany

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Summary : *This study was conducted as a single dose, open-label, randomised, two-period cross-over study in order to determine the bioequivalence of Oflocide® 400mg fort tablet (Abdi İbrahim İlaç San. ve Tic. A.Ş., Istanbul /Turkey) as compared to Tarivid® 400mg tablet (Hoechst Marion Roussel, Bad Soden am Ts,Germany). The study was conducted at the Harrison Clinical Research GmbH, Munich Germany during 2000. The study included 16 healthy volunteers and there was a wash-out period of 14 days between the phases. Blood samples were collected at t=0 and at 0.33, 0.66, 1, 1.33, 1.66, 2, 2.5, 3.5, 6, 9, 14, 24, 31, 38 and 48 hours.The ofloxacin concentration in plasma was analysed by HPLC. Mean pharmacokinetic parameters for test and reference drugs were C_{max} 6,00 and 5,86 (µg/mL), AUC_{last} 32,90 and 32,90 (µg*h/mL), AUC_{inf} 34,20 and 34,20 (µg*h/mL), t_{max} 1,13 and 1,00 (h), $t_{1/2}$ 5,69 and 6,40 (h), respectively. Primary variables tested were Cmax 102 % (90-117 %), AUC_{last} 100 % (96-104 %) and AUC_{inf} 100 % (96 - 105 %) within a confidence interval of 90 %. Both test and reference drugs were well tolerated and the test product was found bioequivalent to the reference product.*

Key Words: *ofloxacin, bioequivalence, HPLC, AUC, C_{max}, t_{max}, $t_{1/2}$*

Türkiye'de ve Almanya'da üretilmiş olan iki ofloksasin tabletin (400mg) biyoeşdeğerliklerinin saptanması

Özet : *Bu çalışma Oflocide® 400mg fort tabletin (Abdi İbrahim İlaç San ve Tic A.Ş., İstanbul/ Türkiye) Tarivid® 400 mg tablete (Hoechst Marion Roussel, Bad Soden am Ts, Almanya) kıyasla biyoeşdeğerliğinin belirlenmesi amacıyla tek doz, açık, randomize, çift zamanlı çapraz olarak yürütülmüştür. Çalışma 2000 yılında Almanya Münih'de Harrison Clinical Research GmbH'de yapılmıştır. Çalışmaya 16 gönüllü dahil edilmiş ve fazlar arasında 14 günlük temizlenme süresi bırakılmıştır. Kan örnekleri t=0 anında ve 0.33, 0.66, 1, 1.33, 1.66, 2, 2.5, 3.5, 6, 9, 14, 24, 31, 38 ve 48.saatlerde alınmıştır. Plazma ofloksasin konsantrasyonu HPLC ile incelenmiştir. Test ve referans ilaçlar için ortalama farmakokinetik parametreler sırasıyla C_{maks} 6,00 ve 5,86 (µg/mL), AUC_{son} 32,90 ve 32,90 (µg*h/mL), AUC_{inf} 34,20 ve 34,20 (µg*h/mL), t_{maks} 1,13 ve 1,00 (h), $t_{1/2}$ 5,69 ve 6,40 (h) olmuştur. Test edilen primer değişkenler %90 güven aralığında Cmaks %102 (% 90-117), AUC_{son} % 100 (% 96-104) ve AUC_{inf} %100 (% 96-105) değerlerindedir. Gerek test gerekse referans ürünler iyi tolere edilmiş olup, test edilen ürün referans ürün ile biyoeşdeğer bulunmuştur.*

Anahtar kelimeler : *ofloksasin, biyoeşdeğerlik, HPLC, AUC, C_{maks}, t_{maks}, $t_{1/2}$*

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INTRODUCTION

Ofloxacin is chemically (±) - 9 - fluoro - 2,3 - dihydro - 3 - methyl - 10 - (4 - methyl - 1 - piperazinyl) - 7 - oxo - 7H - pyrido [1,2,3 - de] - 1,4 - benzoxazine - 6 - carboxylic acid and its empirical formula is $C_{18}H_{20}FN_3O_4$. Being an antimicrobial agent with broad spectrum activity derived from a functional fluoroquinolone group, ofloxacin exerts its bactericidal activity by inhibiting DNA-girase of sensitive pathogens similar to other quinolones[1-3]. Ofloxacin is therefore active against many gram (+) and gram (-) aerobic and anaerobic bacteria.

Absolute bioavailability of ofloxacin tablet formulations is in general approximately 98 % after oral intake with maximal serum concentration reached within 1-2 hours. The drug quantity absorbed after

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single or multiple dose administration of 200 and 400 mg shows a proportional increase in a dose dependent manner.

Ofloxacin half-life is approximately 8 hours. Following therapeutic dose administration, cervical, pulmonary, ovarian, prostatic fluid and tissue, skin and saliva concentrations of ofloxacin are 0,8 - 1,5 fold higher than plasma concentrations. Its elimination is mainly via the kidneys. 65 - 80 % of the dose administered is eliminated from the kidneys in an unchanged form within 48 hours. Less than 5 % is found in the urine as desmethyl or N-oxide metabolites. 4 - 8 % and is eliminated via the feces.

MATERIALS AND METHOD

Volunteer selection

16 healthy caucasian volunteers (11 females and 5 males) were included in the study. Volunteer age was 20-39 (mean, 29) height 163 - 189 cm (mean, 172 cm) and weight 55 - 85 kg (mean 65 kg). The demographics of the volunteers are shown in Table 1.

Inclusion criteria

Healthy, male or female volunteers between 18 to 45 years of age, with a body weight in the range of 50 - 110 kg (no deviation greater than 20 % according to Broca index), blood pressure and heart rate within normal limits in a sitting position with no ECG abnormality, without any systemic disease past or present were included in the study. Females were required to practice reliable contraception from 3 weeks before the start of the study until 3 weeks following the termination of the study.

Exclusion criteria

Volunteers were excluded from the study for the following reasons: history of hypersensitivity to ofloxacin or related drugs; hepatitis, gall-bladder or liver diseases; patients with diseases which could interfere with the pharmacokinetic parameters of the drugs; receipt of medical therapy including OTC drugs within 2 weeks before the initial period; participation in another clinical trial 4 weeks before the study; blood loss of 450 mL or more within 1 month prior to the study; regular smokers (> 10 cigarettes/day); more than 3 cups of caffeine containing beverages daily; alcohol or drug abuse; HIV-seropositive or clinical/laboratory finding of Hepatitis B and C; pregnancy or breast-feeding.

Study protocol

Study protocol and amendments were approved by

Table 1. Demographic data

Sequence	Subjects	Age (year)	Sex	Height (cm)	Weight (kg)	Ethnic group
T-R	1	20	Female	167	60.0	caucasian
	4	39	Male	181	72.0	caucasian
	6	29	Male	181	85.0	caucasian
	8	30	Female	163	55.0	caucasian
	9	35	Female	167	56.0	caucasian
	12	35	Female	165	60.0	caucasian
	14	23	Female	167	57.0	caucasian
	15	24	Male	170	65.0	caucasian
R-T	2	23	Female	165	57.0	caucasian
	3	20	Female	174	59.0	caucasian
	5	38	Male	174	72.0	caucasian
	7	38	Female	167	70.0	caucasian
	10	24	Female	173	61.0	caucasian
	11	23	Female	179	63.5	caucasian
	13	35	Female	175	65.0	caucasian
	16	24	Male	189	83.0	caucasian

the Independent Ethics Committee of the Bayerische Landesarztekammer, and the German Federal Authority (Bundesinstitut für Arzneimittel und Medizinprodukte) and the local authority (Regierung von Oberbayern) were informed according to law.

Test medicines

Both test (lot no. of 01,00,001 manufactured by Abdi İbrahim İlaç Sanayi) and reference (lot no. of 40U609, manufactured by Hoechst Marion Roussel) products were tested physically and chemically (appearance, tablet weight, disintegration, ofloxacin identification and assay, dissolution profiles, etc) before starting the clinical trial and both were found to be appropriate[4].

Dosage

Volunteers were admitted to the clinic on the evening prior to treatment in each test period. After fasting through one night (approx. 8 hours), the test or reference drug containing ofloxacin 400 mg was administered by mouth with 250 mL water in the phase I clinic. After intake, a mouth check was made to confirm that the tablet was ingested.

After the dose administration, volunteers remained in bed for 4 hours and fasted. At the end of this 4 hours, all volunteers received 250 mL water and were served a standardised meal.

Blood samples

For the determination of plasma ofloxacin level, 16 blood samples (9 mL of each) were taken from each volunteer. Blood samples were taken in disposable syringes containing lithium - heparin.

Blood samples were taken at the following times:

t=0 (just before the dosage administration) and at 0.33h, 0.66h, 1h, 1.33h, 1.66h, 2.00h, 2.50h, 3.50h, 6.00h, 9.00h,14.00h, 24.00h, 31.00h, 38.00h and 48.00h post-dosage.

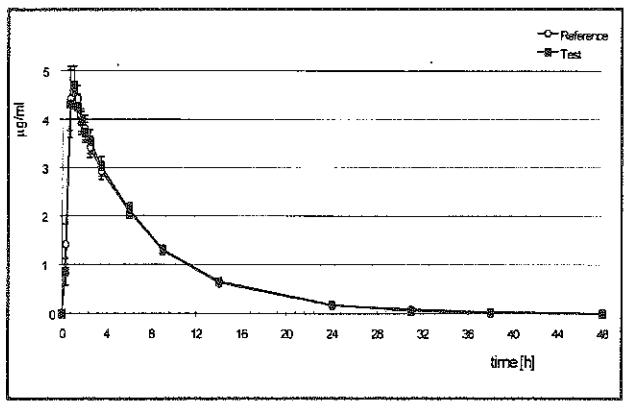
Immediately after the collection of blood samples, these samples were centrifuged for 10 minutes at 3000 rpm. Plasma supernatants were divided into two equal aliquots and stored at -20°C in absolute darkness.

HPLC assay

Plasma ofloxacin determination was done by the HPLC-UV method according to the published literature[5-7]. The limit of quantitation (LOQ) was 100 ng/mL and all samples taken before dose administration (t=0) were below the LOQ, whereas in all samples taken after administration the measured ofloxacin plasma concentrations were above the LOQ, with the exception of nine samples taken at t=0.33 h and 77 additional samples at t=24, 31, 38 or 48 h.

Results

The mean ofloxacin plasma concentrations versus time curves of both products are illustrated in Figure 1. All pharmacokinetic parameters for both products are presented in Table 2. The summary of statistical results are presented in Table 3.



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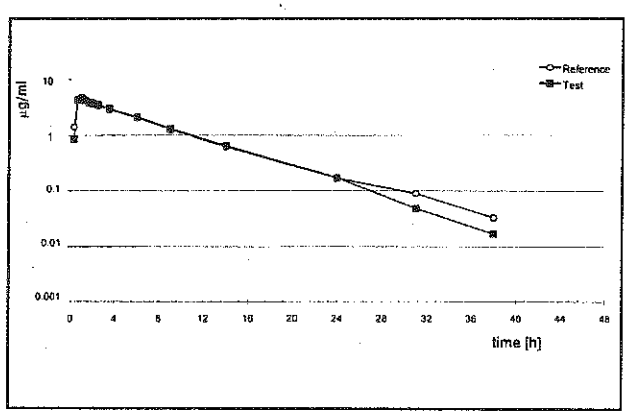

Figure 1. Ofloxacin plasma concentrations, mean ± SEM, n=16

Table 2. Main pharmacokinetic parameters of both products

		C_{max} (µg/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{last} (µg*h/mL)	AUC_{linf} (µg*h/mL)	AUC_{ext} (%)
mean	R	5.86	1.00	6.40	32.9	34.20	3.90
	T	6.00	1.13	5.69	32.9	34.20	3.90
geo.mean	R	5.67	0.93	6.10	32.1	33.40	3.70
	T	5.81	1.04	5.51	32.1	33.40	3.70
median	R	5.48	0.84	6.49	31.8	33.40	3.40
	T	5.46	1.00	5.16	33.4	34.40	3.40
SEM	R	0.40	0.11	0.52	1.93	1.97	0.40
	T	0.42	0.13	0.40	1.84	1.86	0.40
SD	R	1.59	0.42	2.08	7.70	7.86	1.50
	T	1.70	0.52	1.60	7.38	7.42	1.70
CV %	R	27.10	42.00	32.50	23.40	23.00	39.40
	T	28.30	45.90	28.10	22.40	21.70	42.30
min	R	3.62	0.67	3.78	22.30	23.50	2.30
	T	4.11	0.67	3.97	20.30	22.10	2.20
max	R	9.79	2.00	10.5	48.50	50.20	8.00
	T	10.80	2.50	9.90	47.30	49.40	8.30
n	R	16.00	16.00	16.00	16.00	16.00	16.00
	T	16.00	16.00	16.00	16.00	16.00	16.00

SEM= Standard Error of the mean , SD= Standard deviation, CV= Coefficient of variation, n= Number of subjects, R = Reference product , T= Test product, AUC =Area under the plasma concentration time curve, C_{max}=Observed maximum plasma concentration, t_{max}=Observed sampling time of C_{max}, $t_{1/2}$=Apparent terminal elimination half life.

Table 3. Summary of statistical results, n=16 (all subjects included)

ln-transformed data - ratio analysis

Variable	Geometric mean			90%-Confidence Interval for the ratio of means
	Test	Reference	Ratio	
C_{max} [µg/mL]	5.81	5.67	1.03	(90.10; 117)
AUC (0-tlast) [µg * h /mL]	32.10	32.10	1.00	(95.80; 104)
AUC (0-∞) [µg * h /mL]	33.40	33.40	1.00	(95.60; 105)

Variable	ANOVA p-values			CV%	d.f.
	Carry-over	Treatment	Period		
C_{max}	0.98	0.73	0.24	21.10	14.00
AUC (0-tlast)	0.98	0.99	0.62	7.00	14.00
AUC (0-∞)	0.95	0.98	0.58	7.30	14.00

untransformed data, non-parametric analysis

Variable	Arithmetic mean				90% Confidence Interval for the difference (ratio) of means	30%-acceptance range for the difference (ratio)
	Test	Reference	Difference	Hodges Lehmann-Estimator		
t_{max} [h]	1.13	1.00	0.13	0.03	(-0.17; 0.50); (83.40; 150%)	(-0.30; 0.30); (70.00; 130%)

Variable	p-values		
	Carry-over	Treatment	Period
t_{max}	1.00	0.79	0.53

All data collected in the case report forms of this study were entered into a MS-Access 2.0 database. The data were validated by double-data entry procedures.

Analysis of varience (ANOVA) was performed with SAS GLM with a factorial design protocol. The calculation of 90%-confidence intervals was performed with SAS. For the parameters AUC_{last}, AUC_{inf}, and C_{max} the raw data were ln-transformed. For t_{max} a non-parametric analysis was chosen. Maximum ofloxacin concentrations (C_{max}) as well as times of maximum concentrations (t_{max}) were determined from the data. The areas under the plasma versus time curves (AUC) from zero to infinity were calculated by the linear trapezoidal rule.

The ANOVAs for the primary variables indicated no significant treatment, carry-over or period effects. The 90%-confidence intervals were well within the equivalence ranges specified in the protocol, i.e. 80-125% for AUC and 70-143% for C_{max}.

For t_{max}, the 90%-confidence interval for differences of means was only partially included in the 30%-difference acceptance range. The Wilcoxon-Mann-Whitney tests indicated no significant treatment, carry-over or period effects for t_{max}.

Adverse effects

No adverse effects necessitating subject withdrawal were noted during the study.

A total of 8 adverse events were seen, 4 with the test product, 3 with the reference product and 1 in the wash-out phase between periods. All symptoms disappeared later. The following adverse events were reported: headache (3 cases), migraine (2), diarrheea (11), flatulence (1), increased sGPT (1).

DISCUSSION

This study was conducted with healthy subjects (20 - 39 years, 11 females, 5 males) according to the Study Protocol. The study medication appeared to be safe and well tolerated. All subjects received the same dosages of medication, i.e. one single dose of 400 mg ofloxacin in the form of the test product and one single dose of 400 mg ofloxacin in the form of the reference product with a 14-day washout period between administrations.

For the reference product, the AUC_{inf} was 34.20 ± 7.90 (SD) µg*h/mL, AUC_{last} was 32.90 ± 7.70 (SD) µg*h/mL, C_{max} 5.86 ± 1.59 (SD) µg/mL and t_{max} 1.00 ± 0.42 (SD) h.

For the test product, AUC_{inf} was 34.2 ± 7.40 (SD) µg*h/mL , AUC_{last} was 32.9 ± 7.40 (SD) µg*h/mL, C_{max} 6.00 ± 1.70 (SD) µg/mL and t_{max} 1.13 ± 0.52 (SD) h.

For AUC_{inf}, AUC_{last} and C_{max}, the 90%-confidence intervals (C.I.) for the ratios test/reference were well within the pre-defined acceptance ranges of 80 - 125% for AUC-ratios and of 70 - 143% for C_{max}, respectively. The 90%-confidence interval for AUC_{inf} ranged from 95.60% to 104% (point estimate: 100%), for AUC_{last} the 90%-CI ranged from 95.80% to 104% (point estimate: 100%), and for C_{max} the 90%-CI ranged from 90% to 116% (point estimate: 103%).

The 90%-confidence interval for the difference in t_{max} - to be evaluated only descriptively - ranged from -0.167 h to 0.500 h (point estimate: 0.029 h) and was only partially included in the relevant acceptance range of (30% of the reference mean (-0.30 h; 0.30 h).

Thus, bioequivalence of the test product with the reference product can be concluded with regard to the pharmacokinetic parameters evaluated.

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