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Pharmacokinetic Study of Two Macrolide Antibiotic Oral Suspensions Using an Optimized Bioassay Procedure

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Abstract

Purpose: Erythromycin (ERY) (CAS 114-07-8) is a macrolide antibacterial with a broad and essentially bacteriostatic action and Azithromycin (AZI) (CAS 83905-01-5) is a semi-synthetic, acid stable erythromycin derivative with an expanded spectrum of activity and improved tissue pharmacokinetic characteristics relative to ERY. The aim of the present study was to develop an optimized procedure for determination of macrolide antibiotics in human serum as well as to compare the bioequivalence of two commercial brands of Erythromycin and Azithromycin Suspensions in healthy Iranian volunteers.

Methods: Two brands of erythromycin ethylsuccinate and azithromycin oral suspensions were used. An equivalent 400-mg ERY suspension and 500-mg AZI suspension were given orally to each subject in two separate studies as a single dose with 200 ml of water. ERY and AZI concentrations in serum were determined using an optimized agar well diffusion technique with Sarcina lutea (Micrococcus Luteus, ATCC 9341).

Results: Following administration of test and reference ERY products the mean values for C_{max} (1168.5, 1115.0 ng/ml), T_{max} (1.4, 1.38 hr), AUC_0^t (4021.4, 4010.0 ngh/ml), AUC_0^∞ (4852.6, 4787.4 ngh/ml) and $T_{1/2}$ (3.64, 3.61hr) were obtained. For AZI mean values for C_{max} (468.4, 488.1 ng/ml) ,T_{max} (1.96, 2.08 hr) , AUC_0^t (7575.4, 8046.6 ngh/ml), AUC_0^∞ (7990.7,8436.7 ngh/ml) and $T_{1/2}$ (24.98,25.18 hr) were reported. A two-compartment model best described the disposition of ERY and AZI after oral administration in human. The analytical method was validated in terms of linearity, accuracy and precision. The limit of quantitation for ERY and AZI were 50 and 40 ng/ml respectively.

Conclusion: From the results obtained it can be concluded that the optimized method introduced in this study could be successfully applied for the evaluation of pharmacokinetic parameters of both AZI and ERY. Moreover the results revealed that test preparations were equivalent with respective innovator products in terms of rate and extent of absorption.

Keywords: Erythromycin; Macrolide; Azithromycin; Bioequivalence; Sarcina lutea

Introduction

Erythromycin (ERY) (CAS 114-07-8) is a complex macrolide antibiotic consisting mainly of erythromycin A, a 14- membered lactone ring with a 9-keto group, carrying a neutral and an amino sugar (Bryskier et al., 1993; Mazzei et al., 1993). It is mainly active against Gram-positive bacteria and possesses only limited activity against Gram-negative bacteria (Zuckerman et al., 2009). The mechanism of action of ERY is inhibition of protein synthesis via interference of the translocation step (Brisson-Noël et al., 1988). Peak plasma concentrations generally occur between 1 and 4 hours after a dose and have been reported to range between about 0.3 and 1.0 micrograms/mL after 250 mg of ERY base , and from 0.3 to 1.9 micrograms/mL after 500 mg. Around 70 to 75% of the base is protein bound. ERY crosses the placenta: fetal plasma concentrations are variously stated to be 5 to 20% of those in the mother (Sweetman, 2006). Azithromycin (AZI) (CAS 83905-01-5) is a semi-synthetic, acid stable ERY derivative with an expanded spectrum of activity and improved tissue pharmacokinetic characteristics relative to erythromycin (Mazzei et al., 1993; Dunn and Barradell, 1996).On peroral administration, AZI is absorbed rapidly and follows a two-compartment model. It has a narrow therapeutic range (0.1–0.4 g/ml) and a long elimination half-life (Sweetman, 2006). The incidence or severity of adverse effects is mainly because of high peak plasma concentrations of AZI that are seen within 2–3 h after peroral administration of immediate release (IR) dosage forms (Dunn and Barradell, 1996; Foulds et al., 1990). Compared with ERY, AZI exhibits an extensive spectrum of activity, improved acid stability, more favorable pharmacokinetic parameters, and a lower prevalence of adverse events and drug interactions (Kanfer et al., 1998). Both of ERY and AZI suspensions are multisource drug products and are marketed by more than one pharmaceutical manufacturer. Since the formulation and method of manufacture of drug products can affect the bioavailability and stability of drugs, the generic drug manufacturer must demonstrate its bioequivalence to the brand name or innovator drug product. Therefore in this work the bioequivalence of two generic suspension formulations of ERY (Erythroxir, batch no. EAN13) and AZI (Azithroxir, batch no. 12-84 Experimental) intended for marketing by Exir Pharmaceutical Company, Boroujerd, Iran, and the respective reference formulations in healthy Iranian volunteers were investigated.

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Materials and Methods

Clinical protocol and study design

The study was an open-label, single-dose, single-blind, randomized study with a cross over design carried out in accordance with the guidelines of the Declaration of Helsinki (World Medical Assembly 1964) as revised in Edinburgh (2000). The protocol of this study was approved by the ethical committee of the Tabriz University of Medical Sciences. For each drug twenty four male healthy volunteers were enrolled in the study. They were all Iranians, aged between 21 and 31 years (23.2 ± 1.7 years) and weight from 56 to 90kg (70.0 ± 9.3kg). The volunteers were informed about possible risks and adverse effects of taking the drug, and written consent was obtained. The test products and reference were randomized and given to the volunteers. One-week and two-week washout periods were included between each administration of ERY and AZI preparations respectively. An equivalent 400 mg ERY suspension was given orally as a single dose with 200 ml of water. Five milliliters of blood were drawn at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 10 hours after each administration. In the second study an equivalent 500 mg AZI suspension was administered orally to each subject as a single dose with 200 ml of water. Five milliliters of blood were drawn at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 hours after administration. The blood samples were taken from subject's forearm veins. All samples were centrifuged and the serum samples were separated and kept frozen at the temperature below -20°C for subsequent analysis.

Bio-analysis

Optimized analytical procedures were used for determination of ERY and AZI in plasma. Microbiological assay of ERY and AZI was performed by an agar well diffusion procedure with Sarcina lutea (Micrococcus Luteus, ATCC 9341). Briefly the assay plate composed of two layer of antibiotic agar: the first one called base layer contained 21 ml of antibiotic agar I (P^H 8.6 for AZI and 8.3 for ERY) and the second layer known as the seed layer made of 4 ml of antibiotic agar I (P^H 6.7) inoculated with the bacterial inoculums. After 24 h of incubation in 35°C, the diameter of inhibition zone was measured. The method was validated by determination of the following operational characteristics: linearity, precision and accuracy. The linearity was evaluated by linear regression analysis, which was calculated by the least squares regression method in concentration level of 50, 100, 200, 400, 800 and 1600 ng/ml of spiked plasma. The precision of the assay was determined by repeatability (intra-assay) and intermediate precision (inter-assay) and was expressed as the relative standard deviation (RSD) of four quality control samples. The accuracy was determined by adding known amounts of ERY and AZI reference substance (Quality control samples) at the beginning of the process. For ERY one milliliter of plasma and one hundred micro liter of concentrated phosphate buffer P^H 8.3 were incubated for two hours at 40°C to hydrolyze the ester linkage of the ERY ethylsuccinate. Two hundred micro liter of the resulting plasma samples were applied to the bioassay plates, duplicate of six clinical and standard samples (range 50-1600 and 40-800 ng/ml for ERY and AZI, respectively) were distributed over the sample plate. The plates were incubated at 35°C for 24 h. Calibration graphs were constructed by plotting the diameters of the inhibition zones against the logarithm of the ERY or AZI concentrations.

Pharmacokinetic parameters and statistical analysis

The pharmacokinetic parameters for investigated macrolide antibiotics consisting of maximum plasma concentration (C_{max}) and the time at which this concentration is reached (T_{max}) were determined from the individual subject plasma concentration-time profiles by visual inspection and used as criteria of the rate of absorption. The area under the plasma concentration - time curve from time zero to t was calculated using linear trapezoidal rule. The apparent elimination rate constant (K_{el}) was determined by linear regression of log-transformed data in the terminal phase of plasma concentration-time profile. The constant K_{el} was used to extrapolate AUC_{0-t} and was calculated by the quotient of $0.693/k_{el}$ (Zakeri-Milani et al., 2009; Zakeri-Milani et al., 2008; Valizadeh et al., 2009). The $AUC_{0-\infty}$ which means the area under the serum concentration-time curve extrapolated to infinity was calculated according to the following equation:

$$AUC_{0-\infty} = AUC_{0-t} + Ct/K_e$$

Bioequivalence between the products was determined by calculating 90% confidence intervals (90% C.I.) for the ratio of C_{max}, AUC_0^t and AUC_0^∞ values for the test and reference products. Analysis of variance (ANOVA) was used to assess formulation, sequence, subjects and period effects (Zakeri-Milani et al., 2009; Zakeri-Milani et al., 2008; Valizadeh et al., 2009). Moreover a computer program (kinetic 5.0 PK/PD Analysis) was used for pharmacokinetic analyses. Peroral data from each volunteer were fitted to one, two and three compartment models with first order absorption, with lag time and elimination from the central compartment.

Results and Discussion

Validation of bioanalytical method

In the present work the bioequivalence of two commercial brands of Erythromycin and Azithromycin suspensions in healthy Iranian volunteers was investigated using optimized microbiological assay method. Several methods have been reported to detect and quantify ERY and AZI concentrations in biological fluids, majority of them have used high-performance liquid chromatography (HPLC) with fluorescence detection after derivatization with 9-fluorenylmethyloxycarbonyl chloride. Liquid chromatography-tandem mass spectrometry was also used to determine plasma drug concentrations (Xue-Min et al., 2007; Xu et al., 2008; Setiawati et al., 2009; Bahrami et al., 2005). However there are some evidences

Added concentration (ng/ml)		Measured concentration (ng/ml) series 1	2	3	4	Mean (ng/ml)	SD (ng/ml)	CV (%)	Accuracy (%)
Intra assay	50	53.8	51.6	47.8	50.8	51.0	2.5	4.9	102.0
	200	188.7	198.4	193.4	195.4	194.0	4.1	2.1	97.0
	800	790.9	785.9	792.1	792.3	790.3	3.0	0.4	98.8
	1600	1542.4	1603.2	1587.8	1577.0	1577.6	25.8	1.6	98.6
Inter assay	50	51.4	49.8	55.4	48.3	51.2	3.1	6.0	102.5
	200	206.1	201.8	192.3	190.8	197.8	7.4	3.7	98.9
	800	803.0	841.6	786.0	795.0	806.4	24.5	3.0	100.8
	1600	1683.4	1629.1	1619.9	1608.3	1623.9	12.9	0.8	101.5

Table 1: Intra and inter-assay precision obtained from 4 levels of erythromycin QC sample.

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Added concentration (ng/ml)		Measured concentration (ng/ml) series 1	2	3	4	Mean (ng/ml)	SD (ng/ml)	CV (%)	Accuracy (%)
Intra-assay	50	50.6	49.8	53.5	51.4	51.3	1.6	3.1	102.7
	100	98.1	99.4	97.5	95.3	97.6	1.7	1.7	97.6
	200	203.4	195.5	199.3	194.9	198.3	4.0	2.0	99.1
	800	801.5	811.0	807.1	812.3	808.0	4.9	0.6	101.0
Inter-assay	50	53.5	51.4	48.3	50.2	50.9	2.2	4.3	101.7
	100	97.5	103.2	98.7	95.8	98.8	3.1	3.2	98.8
	200	199.3	203.6	200.5	195.8	199.8	3.3	1.6	99.9
	800	807.1	818.6	812.0	805.2	810.7	6.0	0.7	101.3

Table 2: Intra and inter-assay precision obtained from 4 levels of azithromycin QC sample.

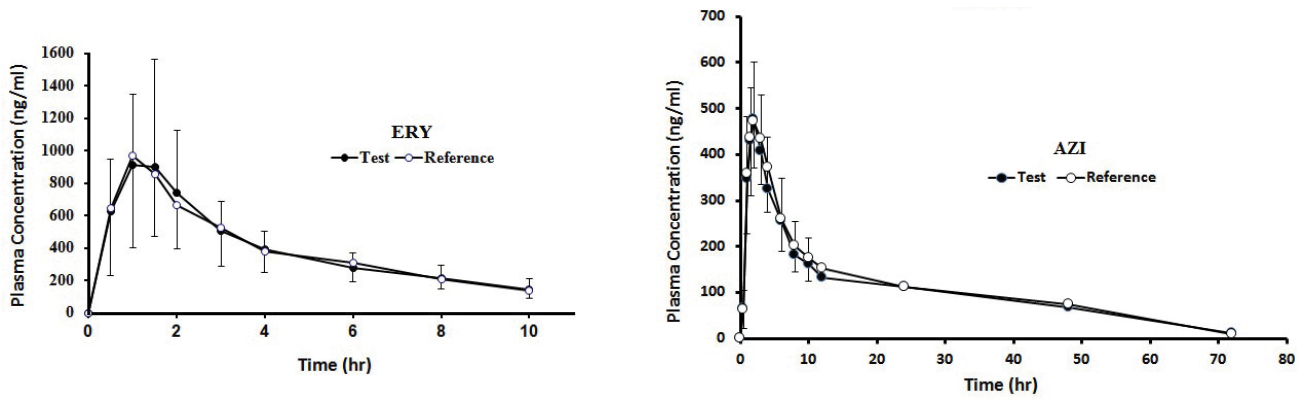

Figure 1: Average (±SD) plasma concentration versus time plots of 24 subjects after administration of 400 mg ERY suspension of test and reference products (Top) and 500 mg AZI suspension of test and reference products (Bottom).

Subject	C_{max} (ng/ml)		T_{max} (hr)		AUC_0^{∞} ngh/ml)		AUC_0^{∞}(ngh/ml)		$T_{1/2}$ (hr)	
	T	R	T	R	T	R	T	R	T	R
Mean	1168.5	1115.0	1.40	1.38	4021.4	4010.0	4852.6	4787.4	3.64	3.61
SD	569.7	476.1	0.59	0.61	1159.2	1292.4	1374.6	1409.3	1.22	1.01
RSD	48.8	42.7	42.22	44.54	28.8	32.2	28.3	29.4	33.57	28.04
Lower CI (90%)	977.2	955.1	1.20	1.17	3632.2	3576.1	4391.1	4314.2	3.23	3.27
Upper CI (90%)	1359.8	1274.8	1.59	1.58	4410.6	4443.9	5314.1	5260.6	4.06	3.95

Table 3: $C_{max,}$ T_{max}, AUC_0^t, AUC_0^{∞}, $T_{1/2}$ mean values following administration of ERY Test (T) and Reference (R) products.

Subject	C_{max} (ng/ml)		T_{max} (hr)		AUC_0^t (ngh/ml)		AUC_0^{∞} (ngh/ml)		$T_{1/2}$ (hr)	
	T	R	T	R	T	R	T	R	T	R
Mean	486.4	488.1	1.96	2.08	7575.4	8046.6	7990.7	8436.8	24.98	25.18
SD	130.0	108.6	0.29	0.60	2630.5	2620.0	3290.3	3299.3	6.87	5.10
RSD	26.7	22.2	14.90	28.89	34.7	32.6	41.2	39.1	27.52	20.27
Lower CI (90%)	442.7	451.7	1.86	1.88	6692.2	7167.0	6886.0	7329.0	22.67	23.46
Upper CI (90%)	530.0	524.6	2.06	2.29	8458.6	8926.3	9095.5	9544.5	27.29	26.89

Table 4: $C_{max,}$ T_{max}, AUC_0^t, AUC_0^{∞}, $T_{1/2}$ mean values following administration of AZI Test (T) and Reference (R) products.

that the microbiological assay can be considered as an alternative method to HPLC (Ambros et al., 2007; Cárceles et al., 2005). This assay can reveal subtle changes not demonstrable by conventional chemical methods. Moreover, microbiological assay requires not only no specialized equipment but also no toxic solvents (Mendez et al., 2005). Furthermore the presented method has lower limit of quantitation compared to previously reported bioassays. A linear relationship between diameters of the inhibition zones and the logarithm of the ERY concentrations was found between 50 and 1600 ng/ml (R^2= 0.991). The limit of quantitation for ERY was 50 ng/ml, with a within-day reproducibility of ±0.4% for 800 ng/ml and a day-to-day reproducibility of ±3.0% for the same concentration. For AZI, a linear calibration curve was found between 40 and 800 ng/ml (R^2=0.997). The limit of quantitation for AZI was 40 ng/ml, with a within-day reproducibility of ±2% for 200 ng/ml and a day-to-day reproducibility of ±1.6% for the same concentration (Table1 and Table 2).

Pharmacokinetic analysis

The plasma concentration–time profile of each individual treatment was constructed (Figure 1). Tables 3 and 4 show the individual as well as mean pharmacokinetic parameters after administration of investigated drugs. The results presented in Tables 3 and 4 show that the percentage of total area under curve ($AUC_{t-\infty}$) that was extrapolated is below 20% for AZI (17.12% and 16.23% for test and reference formulations respectively) and below 10% for ERY (5.19% and 4.62% for test and reference formulations respectively). This indicates that the overall sampling time was long enough to describe the pharmacokinetic profile of investigated drugs after oral administration of formulations. A two-compartment model best described the decline in AZI and ERY plasma concentrations following oral administration in each subject (Figure 2). The elimination parameters estimated by the model, k_{10}, k_{12}, k_{21} for investigated drugs were as follow: 0.090 (/h), 0.419 (/h), 0.084 (/h) for AZI test formulation, 0.098 (/h), 0.405 (/h), 0.103 (/h) for AZI reference formulation, 0.825 (/h), 2.26 (/h), 0.415(/h) for ERY test formulation, and 0.443 (/h), 1.34

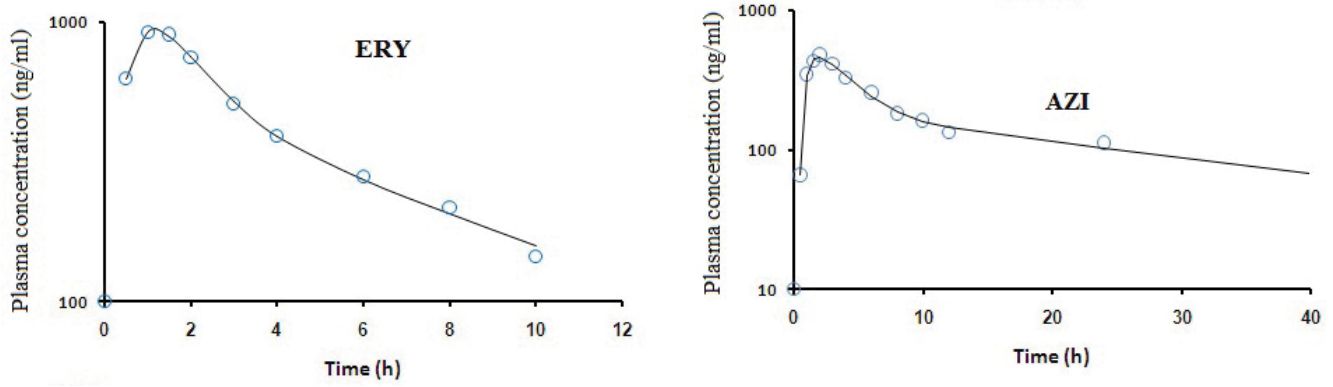

Figure 2: Observed (open circles) and predicted (solid line) plasma concentrations according to a two-compartment model, following oral administration (top: ERY, bottom: AZI) .

Subject	C_{max} ratio (T/R)		AUC_0^t ratio (T/R)		AUC_0^t ratio (T/R)		T_{max} difference (T-R)	
	ERY	AZI	ERY	AZI	ERY	AZI	ERY	AZI
Mean	1.07	1.00	1.03	0.95	1.04	0.96	0.02	-0.13
SD	0.23	0.14	0.20	0.15	0.20	0.15	0.8	0.6
RSD (%)	21.8	14.3	19.1	15.7	19.5	16.1	3776.8	-475.5
Lower CI (90%)	0.99	0.95	0.96	0.90	0.97	0.91	-0.24	-0.32
Upper CI (90%)	1.14	1.05	1.09	1.00	1.10	1.01	0.29	0.07

Table 5: Ratios of C_{max}, AUC_0^t , AUC_0^∞ and difference of the T_{max} values of test (Exir) and Reference product, and test (Exir) and Reference products.

Pharmacokinetic parameter		ANOVA (P-value) Variation source			C.I. 90% for the ratios
		Product	Group	Period	
Azithromycin	C_{max}	0.78	0.52	0.11	95.2-104.8
	AUC_0^∞	0.06	0.51	0.19	90.0-100.1
	AUC_0^∞	0.12	0.48	0.23	90.6-101.0
Erythromycin	C_{max}	0.37	0.42	0.28	98.7-114.3
	AUC_0^t	0.83	0.82	0.72	96.1-109.2
	AUC_0^∞	0.47	0.54	0.79	96.8-110.3

Table 6: .Analysis of variance (ANOVA) for the assessment of the product, group and period effects, and 90% confidence intervals (90% C.I.) for the ratio of C_{max}, AUC_0^t and AUC_0^∞ values for the test and reference products of Azithromycin and Erythromycin, (α =0.05).

(/h), 1.23 (/h) for ERY reference formulation. The estimated clearance and volume of distribution for average plasma concentration of respective formulations were 8.32 (L/h/Kg) and 32.2 (L/Kg), 6.15 (L/h/Kg) and 36.8 (L/Kg), 13.5 (L/h/Kg) and 10.4 (L/Kg), 1.4 (L/h/Kg) and 8.8 (L/Kg). The results indicate that peak serum concentrations of AZI are lower than those of ERY. This is because AZI accumulates to a greater degree in various host cells, which is reflected by its significantly larger volume of distribution reported in this study. As a consequence, AZI has a lower serum area under the curve. The test over reference ratios of pharmacokinetic parameters are shown in Table 5. Bioequivalence between the products was determined by calculating 90% confidence intervals (90% C.I.) for the ratio of C_{max}, AUC_0^t and AUC_0^∞ values for the test and reference products. Analysis of variance (ANOVA) was used to assess group and period effects (Table 6). The currently accepted criterion for bioequivalence for most dosage forms requires that the mean pharmacokinetic parameters of the test dosage form should be within 80% to 120% of the reference dosage form using the 90% confidence interval. The multivariate analysis, accomplished through analysis of variance (ANOVA) for assessment of period, group and product effects, revealed the absence of any of these effects in the present study. For erythromycin the 90% confidence intervals for the ratio of C_{max}, AUC_0^t and AUC_0^∞ (98.7-114.3%, 96.1-109.2%, 96.8-110.3%) values for the test and reference products were within the 80–120% interval proposed by FDA and EMEA (Zakeri-Milani et al., 2009; Zakeri-Milani et al., 2008; Valizadeh et al., 2009). In the case of AZI the 90% confidence intervals for the ratio of C_{max}, AUC_0^t and AUC_0^∞ (95.2-104.8%, 90.0-100.1%, 90.6-101.0%) values for the test and reference products were within the 80–120% interval proposed by FDA and EMEA. Thus, from the results obtained it can be concluded that the AZI (Zimexir, Exir) and ERY (Erythroxir, Exir) test formulations are bioequivalent with respective reference formulations in terms of rate and extent of absorption.

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