Bioequivalence of Two Oral Formulations of Gatifloxacin Tablets in Healthy Male Volunteers

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KEY WORDS: Gatifloxacin, pharmacokinetics, bioavailability, bioequivalence

ABSTRACT

Objective: The purpose of this randomized, crossover study was to compare the bioavailability of a generic and innovator formulation of gatifloxacin 400 mg tablets under fasting conditions.

Methods: Seventeen blood samples per period were collected from 24 healthy, Arab male volunteers over 36 hours, plasma gatifloxacin concentrations were determined by HPLC assay, and pharmacokinetic parameters were determined by the non-compartmental method.

Results: Mean ± SD C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and $t_{1/2}$ were 4.88 ± 1.02 and 4.88 ± 0.99 µg/mL, 1.19 ± 0.62 and 1.14 ± 0.79 h, 37.81 ± 5.54 and 39.12 ± 5.19 µg.h/mL, 39.68 ± 5.86 and 38.00 ± 5.43 µg.h/mL, and 8.07 ± 0.62 and 8.08 ± 0.75 h for the generic and innovator formulation, respectively. ANOVA revealed significant formulation effect for AUC_{0-t} and $AUC_{0-\infty}$. However, the parametric 90% confidence intervals on the mean of the difference between log-transformed values were within the bioequivalence accepted range of 80 to 125%, namely: 102.06% to 107.34%, 101.69% to 107.10%, and 94.00% to 106.10%, for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}, respectively.

Conclusion: The results indicate that the two formulations are both statistically different and equivalent, in the rate and extent of absorption. This may be due to a large sample size in relation to sample variance.

INTRODUCTION

Gatifloxacin is a fluoroquinolone broad spectrum antibacterial agent that is active against both gram-positive and gram-negative organisms, anaerobes, as well as, *Mycoplasma* and *Chlamydia*.[1] Gatifloxacin is well absorbed after oral administration with a bioavailability of 96%, which is not affected by concomitant food intake.[2-6] Peak plasma concentrations occur between 1 and 2 hours post-dose.[2]

The aim of this study was to compare, under fasting conditions, the rate and extent of absorption of two 400 mg tablet formulations of gatifloxacin, a generic (test) formulation: Tymer, manufactured by Jamjoom Pharma, Jeddah,

Saudi Arabia and an innovator (reference) formulation: Tequin, manufactured by Bristol-Myers Squibb, Egypt.

SUBJECTS AND METHODS

Subjects

Twenty-four healthy, non-smoking, adult Arab male volunteers were enrolled in the study. Their mean age ± SD was 30 ± 4.7 (range, 22 to 38) years. Their mean body weight was 71.1 ± 10.6 (range, 50.9 to 93.9) kg and their mean height was 1.74 ± 0.06 (1.61 to 1.84) m, giving a mean body mass index (BMI) of 23.4 ± 3.1 (18.3 to 29.6) kg/m^2.

Based on medical history, clinical examination, and routine laboratory investigations (CBC and differential, fasting glucose, creatinine, sodium, potassium, chloride, CO_2, ALT, ALP, total bilirubin, and urinalysis), none of the volunteers was found to have a history, or current evidence, of hepatic, renal, gastrointestinal, or hematological illness, or allergy to gatifloxacin or related compounds.

The volunteers were asked to abstain from taking any medication (including over-the-counter drugs) for at least 2 weeks prior to, and throughout the study; and from smoking and taking alcohol or caffeine or consuming related xanthenes-containing beverages or food for at least 48 hours prior to, and throughout the study. They were informed about the risks, benefits, procedures, and aims of the study, as well as their rights as research subjects. Each volunteer signed an informed consent document before entering the study. The study protocol and the consent form were approved by the Research Ethics Committee of the King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia. The study was conducted according to the Declaration of Helsinki, Good Clinical Practice (GCP) Guidelines, and Good Laboratory Practice (GLP) Guidelines.

Study Drugs

The test formulation was Tymer 400 mg tablets (Batch # 410715, manufactured by Jamjoom Pharma, Saudi Arabia), whereas the reference formulation was Tequin 400 mg tablets (Batch # F21944, manufactured by Bristol-Myers Squibb, Egypt).

Study Design

The administration of the two formulations was carried out in a two-way crossover design with a seven-day washout period. The subjects were randomly assigned to one of the two sequences: Tequin → Tymer or Tymer → Tequin. Each volunteer received a single 400 mg tablet of either formulation with 250 mL of water after an overnight fast.

The volunteers were ambulatory during the study but were prohibited from strenuous activity. Standardized breakfast and dinner were given at 4 and 10 hours after drug administration, and were identical in the two periods of the study. For the period of 12 hours following drug administration, the volunteers were under direct medical supervision. No volunteer vomited after the administration of the drug and no adverse events were identified.

Blood Sampling

The timing of blood collection was planned according to the previously reported value of time to peak plasma concentration (T_{max}) and plasma elimination half-life ($t_{½}$).[2-6] Venous blood samples were obtained before, and at 0.33, 0.66, 1.00, 1.25, 1.5, 1.75, 2, 2.25, 3, 4, 6, 8, 10, 12, 24 and 36 hours after, drug administration for a total of 17 samples. An intravenous cannula was placed into the volunteers' forearm vein before drug administration and left in place until the 12-hour blood sample was collected. The blood samples were collected in coded, evacuated, heparinized tubes,

mildly shaken, and centrifuged at room temperature (3000 rpm for 10 minutes). The plasma was decanted in coded polypropylene tubes and stored at –20˚C for 1 to 7 days at the clinical study site and then at –80˚C until analyzed.

Gatifloxacin Level Determination by HPLC

Gatifloxacin and ciprofloxacin (internal standard, IS) were separated at room temperature on a 5-µm (particle-size), 3 X 50-mm Xterra MS C18 column with Guard Pak pre-column module and Nova-Pak C18 4-µm insert. The compounds of interest were detected using a 996 photo-diode array detector set at 293 □m. The mobile phase consisted of 0.02 M disodium hydrogen phosphate (phosphate buffer, pH adjusted to 3.0 with phosphoric acid) and acetonitrile (80:20 v/v) and was delivered at a flow rate of 1.0 mL/min. Samples were processed as follows: 10 µg of the IS in 200 µL of phosphate buffer was added to 500 µL plasma sample; and the mixture was vortexed and passed through an Amicon Centrifee-MS filter (Millipore Corporation, Bedford, Mass). 100 µL of the ultrafiltrate was injected in the HPLC system by an autosampler. The retention times of gatifloxacin and the IS were around 5.8 and 3.8 minutes, respectively. The relationship between gatifloxacin concentration in plasma and the peak height ratio (gatifloxacin/IS) was linear ($R^2 \geq 0.999$) in the range of 0.1 to 6 µg/mL, and the intra- and inter-day coefficient of variations were □ 2.77% and □ 4.59%, respectively. The lower quantification limit of gatifloxacin in plasma was 0.1 µg/mL and recovery was 85%. Gatifloxacin in plasma was stable (> 99%) for at least 7 weeks when stored at –20˚C. All the blood samples were analyzed blindly within one month of collection, and after a single cycle of freeze and thaw.

PHARMACOKINETIC ANALYSIS

The following pharmacokinetic parameters were directly determined or calculated by the standard non-compartmental method: (1) Maximum plasma concentration (C_{max}) and time to peak plasma concentration (T_{max}). Both were directly obtained from the data. (2) The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = (\ln 2)/K_{el}$, where K_{el} is the apparent elimination rate constant. K_{el} was, in turn, calculated as the slope of the linear regression line of natural log-transformed plasma concentrations. The last seven quantifiable levels were used to determine K_{el}. (3) The area under the plasma concentration-time curve ($AUC_{0\text{□}\,t}$) was calculated from the measured levels, from time zero to the time of last quantifiable level, by the linear trapezoidal rule. (4) The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0\text{□}}$) was calculated according to the following formula: $AUC_{0\text{□}} = AUC_{0\text{□}\,t} + C_t/K_{el}$, where C_t is the last quantifiable plasma level. (5) The rate of absorption was evaluated by means of the ratio: $C_{max}/ AUC_{0\text{□}}$ (6) The ratio $AUC_{0\text{□}\,t} / AUC_{0\text{□}}$ was determined as an indicator of the adequacy of the blood sampling period.[7]

Pharmacokinetic calculations were performed on a personal computer using Microsoft Excel (Version 2000) with relevant add-ins (PK Functions for Microsoft Excel, JI Usansky, A Desai, and D Tang-liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, Calif).

STATISTICAL ANALYSIS

Factorial analysis of variance (ANOVA) for crossover design was used to assess the effect of formulation, period, sequence, and subject nested in sequence, on natural log-transformed data of $AUC_{0\text{□}\,t}$, $AUC_{0\text{□}}$, C_{max}, K_{el}, $t_{1/2}$,

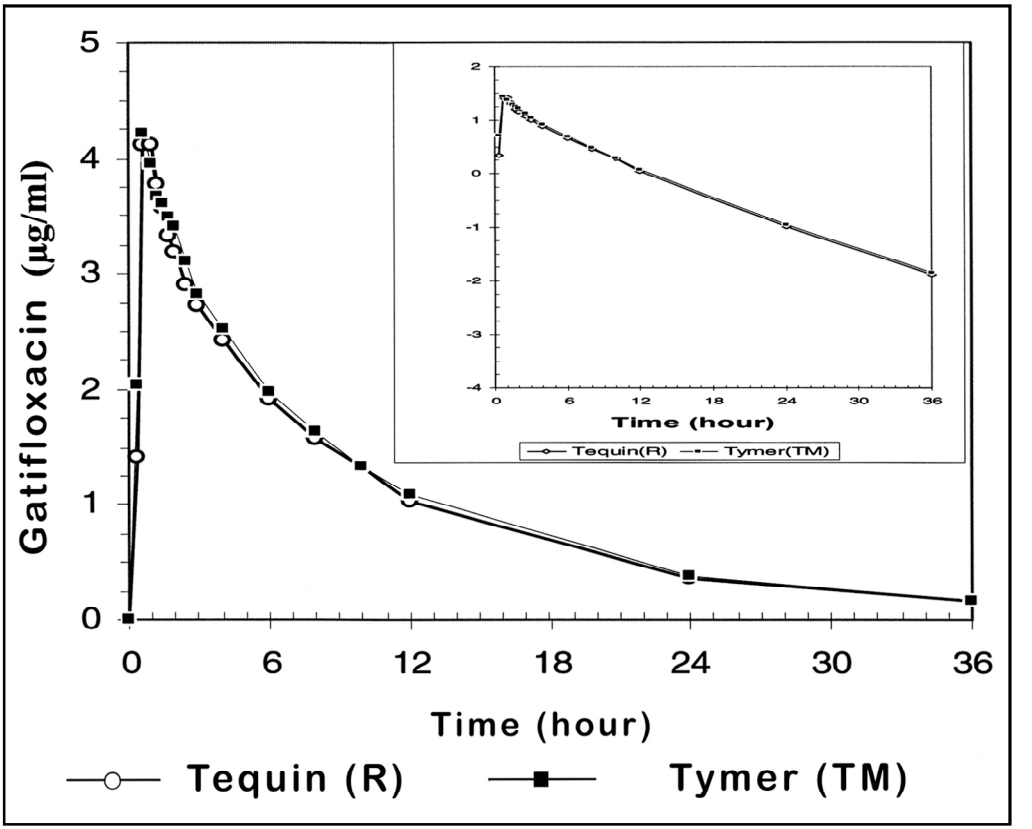

Figure. Mean timed plasma concentrations of gatifloxacin after single oral administration of 400 mg tablet of Tequin and Tymer to 24 male volunteers, before and after natural logarithm transformation (insert).

and C_{max}/ AUC_{0} . The ANOVA of T_{max} was carried out on untransformed data. Sequence effect was tested against the mean squares term of subject nested in sequence. All other effects were tested against mean residual error.

Parametric 90% confidence intervals on the mean of the difference between the two formulations (Tymer - Tequin) of log-transformed values of AUC and C_{max} were computed under the assumption of multiplicative model (using mean residual error obtained from ANOVA). In addition, bioequivalence between the two formulations was assessed by Schurimann's two one-sided *t* tests.[8]

Analyses of the data were performed with the statistical software package, SAS, (Statistical Analysis System, SAS Institute Inc., Cary, NC), using the General Linear Model (GLM) Procedure.

RESULTS

Mean timed plasma concentrations of gatifloxacin obtained after the oral administration of the two formulations to the twenty-four volunteers are shown in the Figure before and after natural logarithm-transformation (insert). A summary of the pharmacokinetic parameters of gatifloxacin is shown in Table 1.

Table 2 presents a summary of ANOVA results of the pharmacokinetic parameters examined. There were significant effects for AUC (formulation, period, and subject), C_{max} (period and

Table 1. Pharmacokinetic Parameters of Tymer 400 mg Tablet Compared to Tequin 400 mg Tablet (n = 24)

Parameter	Mean* ± SD		CV (%)†	
	Tymer	Tequin	Intra-subject	Inter-subject
AUC_{0-t} (μg.h/mL)	37.81 ± 5.54	36.12 ± 5.19	5.1	14
$AUC_{0-\infty}$ (μg.h/mL)	39.69 ± 5.86	38.00 ± 5.43	5.2	14
C_{max} (μg/mL)	4.88 ± 1.02	4.88 ± 0.99	12.2	17.
1 T_{max} (h)	1.19 ± 0.62	1.14 ± 0.79	44.8	54.
6 K_{el} (h^{-1})	0.086 ± 0.007	0.086 ± 0.008	3.7	7.4
$t_{1/2}$ (h)	8.07 ± 0.62	8.08 ± 0.75	3.7	7.4
$C_{max}/AUC_{0-\infty}$ (h^{-1})	0.12 ± 0.021	0.13 ± 0.02		
AUC_{0-t} / $AUC_{0-\infty}$	0.95 ± 0.01	0.95 ± 0.01		

*Arithmetic mean of untransformed data
†CV, Coefficient of variation calculated from ANOVA tables of log transformed data (except for T_{max}) as follows:
Intra-subject CV = 100 x $(MSResidual)^{0.5}$.
Inter-subject CV = 100 x $\{(MSSubject(Seq)-MSResidual)/2\}^{0.5}$ (13)

Table 2. *P* values for Source of Variation Obtained from ANOVA*

Source of Variation	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}	T_{max}	K_{el}	$t_{1/2}$	$C_{max}/AUC_{0-\infty}$
Formulation	0.0052	0.0097	0.9707	0.6866	0.9818	0.9818	0.1599
Period	0.0041	0.0048	0.0033	0.9330	0.0204	0.0204	0.0332
Sequence	0.3845	0.4222	0.7804	0.4195	0.1457	0.1457	0.6608
Subject	< 0.0001	< 0.0001	0.0002	0.0010	< 0.0001	< 0.0001	< 0.0001

*All parameters were logarithmically transformed prior to data analysis (ie, assuming multiplicative model) except for T_{max} which was analyzed using untransformed data (ie, assuming additive model).

subject), T_{max} (subject), K_{el} and $t_{1/2}$ (period and subject) and C_{max}/ AUC_{0-} (period and subject).

The point estimate and the 90% confidence limits for AUC_{0-t}, AUC_{0-} , and $C_{max,}$ as well as the results of the Schuirmann's two one-sided *t* tests, are shown in Table 3.

DISCUSSION

Assessment of bioequivalence of generic drugs to innovator drugs is required to exclude any clinically important differences in the rate or extent at which the active entity of the drugs becomes available at the site of action. Two drugs are considered to be bioequivalent if they are pharmaceutically equivalent and their bioavailabilities are so similar that they are unlikely to produce clinically relevant differences in regard to safety and efficacy.[9]

The aim of this study was to compare the bioavailability of two formulations of gatifloxacin 400 mg tablet, a generic formulation, Tymer, and an innovator formulation, Tequin. The study revealed that the 90% confidence intervals of AUC_{0-t}, AUC_{0-} , and C_{max} (102.06%-107.34%, 101.69%-107.10%, and 94.00%-106.10%, respectively) are all well within the bioequivalence

Table 3. Parametric 90% Confidence Intervals, and Two One-Sided *t* tests for Main Pharmacokinetic Parameters of Gatifloxacin

	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
90% Confidence Interval			
Point Estimate*	104.67%	104.36%	99.87%
Lower Limit†	102.06%	101.69%	94.00%
Upper Limit†	107.34%	107.10%	106.10%
Schurimann's test			
Critical *t* value	1.717	1.717	1.717
df	22	22	22
Calculated *t* value			
ITLI‡	18.294	17.640	10.081
ITUI‡	12.086	11.976	10.184

*The antilog of mean difference (ln Tymer - ln Tequin).
†The antilog of the confidence limits on the mean difference (ln Tymer - ln Tequin).
‡ITLI and ITUI are calculated test statistics for the lower and upper limits, respectively

acceptable range of 80% to 125%.[10,11] These results were confirmed by the Schuirmann's two one-sided *t* tests, which indicated that the lower and upper limits of the calculated *t* value were greater than the critical *t* value for the three parameters (Table 3). Therefore, the two formulations can be considered bioequivalent in regard to the extent and rate of absorption.

The significant period effect for AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, K_{el}, $t_{1/2}$, and $C_{max}/AUC_{0-\infty}$ (period two more than period one for all parameters except K_{el}) is not easily explained by the data. A significant period effect could conceivably reflect different positioning, timing and degree of physical activity, timing and composition of food/beverages ingested, or the temperature of the water administered in the two periods. To our knowledge, none is applicable in the current study. However, it is possible that the psychological status of the subjects differed between the two periods, which may in turn affect their bowel transit and drug absorption. Theoretically, the previously administered treatment may permanently (or for prolonged period) alter the metabolism of the subjects in some manner, so that they will react differently to any treatment administered from that time onward. Nevertheless, period effects are not expected to influence the comparison of formulations.[12]

The observation that there was a significant formulation effect (Tymer more than Tequin by about 7.5%) despite the fact the two formulations are equivalent is interesting and gives the somewhat discomforting conclusion that the two formulations are both different and equivalent. It indicates a simultaneous rejection of both the null hypothesis of no difference (ANOVA) and the null hypothesis of no equivalence (90% confidence interval and two one sided *t* tests). This occurs when the entire confidence interval does not cross unity (in the current study, 102.058%-107.339% and 101.694%-107.096% for AUC_{0-t} and $AUC_{0-\infty}$, respectively) and could happen in a situation where large sample size (24 subjects in the current study) relative to sample variance provides "too much power"; resulting in a trivial difference (too small to be important clinically) being statistically significant.

ACKNOWLEDGEMENT

The study was supported in part by a

grant from Jamjom Pharma, Jeddah, Saudi Arabia, to the Center for Clinical Research. The authors acknowledge the contribution of Mr. Abdelraheem Abdulgaleel Mohammed, Research Coordinator, in conducting this study.

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