Effect of Orange Juice on Bioavailability of Levofloxacin

Rebeka Sultana, MPharm[1]
Ashik Ullah, MPharm[1]
Maruf Mohammad Akbor, MPharm[1]
Mohammad Abul Kalam Azad, MPharm[2]
AHM Mahbub Latif, PhD[3]
Abul Hasnat, PhD[1]

[1]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh
[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh
[3]Institute of Statistical Research and Training, University of Dhaka, Dhaka, Bangladesh

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ABSTRACT

Objective: This work was designed to observe the effect of orange juice on the bioavailability of levofloxacin in healthy Bangladeshi volunteers.

Methods: A randomized 2-way crossover design was used with a washout period of 2 weeks. The volunteers ingested either 200 mL of orange juice or water 3 times a day for the first 3 days and 2 times a day on the fourth day. On the morning of Day 3, each subject was given a 250-mg levofloxacin tablet under fasting condition with 200 mL of orange juice or water. Thirteen blood samples were collected from each volunteer over a 24-hour period. Serum levofloxacin concentrations were determined by high performance liquid chromatography using UV detection, and pharmacokinetic parameters were determined by the non-compartmental method.

Results: The mean value of the peak plasma concentration (C_{max}) of levofloxacin decreased significantly (26.36%, P value < 0.001; 90% CI, 125.18%-145%) in the volunteers who had taken the drug with orange juice (C_{max}, 2.57 ± 0.46 µg/mL) than those who had taken the drug with water (C_{max}, 3.49 ± 0.75 µg/mL). The area under the serum concentration-time curve extrapolated from t = 0 to infinity ($AUC_{0-\infty}$) value was also reduced by 17.33%; this change was not within the acceptable range of bioequivalence (90% CI, 111.38%-127.90%). Similarly, the value of area under the serum concentration-time curve extrapolated from t = 0 to t = 24 hours (AUC_{0-24}) was decreased by 14.98%; this change was marginally within the bioequivalence acceptable range (90% CI, 113.31%-122.10%). The values of $AUMC_{0-\infty}$, serum elimination half-life, time to reach peak serum concentration, elimination rate constant, and mean resident time did not change significantly.

Conclusion: As the values of C_{max} and $AUC_{0-\infty}$ were not within the bioequivalence acceptable range, the serum therapeutic concentration of levofloxacin will be severely affected in the presence of orange juice, ultimately affecting its

Table 1. Precision and Accuracy of the Method for the Determination of Levofloxacin in Human Serum (n = 6).

	Concentration (ng/mL)					
	25	**50**	**100**	**250**	**500**	**1000**
Intra-day R.S.D. (%)	4.82	2.00	7.48	9.31	3.01	6.90
Inter-day R.S.D. (%)	7.10	3.49	2.22	4.77	6.82	4.14

bioavailability and therapeutic efficacy. So, levofloxacin should not be taken with orange juice under any circumstances.

INTRODUCTION

Levofloxacin is a synthetic broad-spectrum fluoroquinolone antibacterial agent available both as intravenous and oral formulations.[1] Levofloxacin pharmacokinetics are linear and predictable after single and multiple oral dosing regimens. It is stereochemically stable in plasma and urine and undergoes limited metabolism in human.

The seriousness of food-drug interaction depends on the therapeutic index of each drug. Modern drugs having lower therapeutic indices have a greater possibility of toxic effects due to food-drug interactions, which ultimately may affect treatment efficacy. These effects may lead to treatment failure or severe adverse effects, some of which may be life-threatening.[2,3] Therefore, care should be taken to prevent any type of food-drug interaction. A previous study showed that ciprofloxacin and calcium-fortified orange juice significantly decreased 2 bioequivalence parameters (peak plasma concentration [C_{max}] and area under the serum concentration-time curve extrapolated from t = 0 to infinity [$AUC_{0-\infty}$]) when they are co-administered.[4] Again, a recent study demonstrated lack of bioequivalence when levofloxacin and calcium-fortified orange juice are co-administered to healthy volunteers.[5] The current study was conducted with Bangladeshi people to find any variation in the pharmacokinetic parameters of levofloxacin when it

was co-administered with nonfortified orange juice orally. Orange juice is one of the most frequently used beverages not only in Bangladesh but also in other parts of the world. Both nonfortified orange juice and calcium-fortified orange juice are consumed by people all over the world. Calcium-fortified orange juice contains approximately 148 mg of calcium in a 100-mL preparation; nonfortified orange juice contains approximately 6.8 mg of calcium in a 100-mL preparation (used in this study). The effect of calcium-fortified orange juice on bioavailability of levofloxacin has been reported, but there has been no report of the effect of nonfortified orange juice on bioavailability of levofloxacin. Interestingly, after the coadministration of levofloxacin and nonfortified orange juice, we found different results than those that have been reported when levofloxacin and calcium-fortified orange juice were coadministered.

SUBJECTS AND METHODS
Subjects

Twelve healthy, non-smoking, adult Bangladeshi subjects participated in this study. Their mean age, mean body weight, mean height, and mean body mass index (BMI) were 25.63 ± 1.41 (range, 24 to 28) years, 69.50 ± 4.72 (range, 60 to 75) kg, 1.74 ± 0.04 (1.68 to 1.80) m, and 22.89 ± 1.50 (20.50 to 25.0) kg/m^2, respectively. Subjects were qualified for the study if they had normal pre-study medical history (ie, physical examination, chest x-ray, electrocardiogram, and urine analysis) before entry

Table 2. Mean Pharmacokinetic Parameters After Oral Administration of 250 mg of Levofloxacin Single Dose With Orange Juice.

Pharmacokinetic Parameters (n = 12)	Levofloxacin With Orange Juice						
	Geometric Mean	Median	Mean	SD	CV (%)	Min	Max
C_{max} (μg/mL)	2.53	2.64	2.57	0.46	18.13	1.75	3.35
t_{max} (hr)	1.27	1.50	1.33	0.43	32.31	0.75	2.00
AUC_{0-24} (hr μg/mL)	26.13	26.35	26.23	2.30	8.77	21.73	30.03
$AUC_{0-\infty}$ (hr μg/mL)	41.82	36.41	44.57	19.82	44.47	31.46	98.67
$t_{1/2}$ (hr)	15.89	12.66	19.49	15.83	81.20	8.45	61.14
Kel (hr^{-1})	0.04	0.05	0.05	0.02	44.22	0.01	0.08
$AUMC_{0-24}$ (hr^2 μg/mL)	233.95	228.42	234.95	22.81	9.71	205.30	269.93
$AUMC_{0-\infty}$ (hr^2 μg/mL)	939.97	664.00	1609.26	2349.17	145.98	437.30	8424.12
MRT (hr)	22.48	18.47	27.33	21.85	79.95	12.65	85.38
C_{max}/AUC_{0-24}	0.06	0.08	0.06	0.47	34.02	0.02	0.09

C_{max} = peak plasma concentration; t_{max} = time to reach peak serum concentration; AUC_{0-24} = area under the serum concentration-time curve from t = 0 to t = 24 hr; $AUC_{0-\infty}$ = the area under the serum concentration-time curve extrapolated from t = 0 to infinity; $t_{1/2}$ = serum elimination half-life; Kel = elimination rate constant; $AUMC_{0-24}$ = area under the first moment-versus-time curve from t = 0 to t = 24 hr; $AUMC_{0-\infty}$ = area under the first moment-versus-time curve from t = 0 to infinity; MRT = mean resident time; SD = standard deviation; CV = coefficient of variation.

into the study. Participation in the study was limited to those subjects with no evidence of clinically significant abnormal hematological, serum chemistry, and urine analysis values. Exclusion criteria included any history of a significant gastrointestinal condition that could potentially impair the absorption or disposition of the study drug, previous history of allergy to any fluoroquinolone, need for any chronic medication (eg, theophylline, antacid, glibenclamide, phenytoin, iron, or vitamins), donation of blood within 30 days preceding the first dose of the study, or use of an investigational agent within 30 days before starting the experiment. Subjects were also excluded if they used any medication within 1 day before administration of the first dose. The volunteers were asked to abstain from taking any medication (including over-the-counter drugs) throughout the study and from smoking, using alcohol or caffeine, or consuming xanthene-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. The study was conducted according to the Declaration of Helsinki (1964). Each volunteer signed an informed consent form before entering the study. Ethical permission was taken to approve the protocol and consent form of the clinical investigation from Bangladesh Medical Research Council (BMRC).

Study Design and Drug Administration
The study was performed in 12 healthy adult Bangladeshi subjects. The subjects were selected randomly and divided into 2 groups (Group 1 and Group 2). Each group consisted of 6 volunteers, also selected randomly. The volunteers ingested 200 mL of orange juice (composed of carbohydrate 8.3 g, fat 0.1 g, protein 0.8 g, Vitamin C 33.2 mg, and calcium 6.8 mg per 100 mL of juice) or water 3 times a day (8 AM, 2 PM, and 8 PM) for the first 3 days. On the morning of Day 3, each subject was given a 250-mg single levofloxacin film-coated tablet under fasting condition with either 200

Table 3. Mean Pharmacokinetic Parameters After Oral Administration of 250 mg of Levofloxacin Single Dose With Water.

Pharmacokinetic Parameters (n = 12)		Levofloxacin With Water					
	Mean	Geometric Median	Mean	SD	CV (%)	Min	Max
C_{max} (μg/mL)	3.42	3.36	3.49	0.75	22.81	2.40	5.35
t_{max} (hr)	1.20	1.25	1.27	0.46	36.04	0.75	2.00
AUC_{0-24} (hr μg/mL)	30.74	30.85	30.84	2.72	8.81	27.48	35.69
$AUC_{0-\infty}$ (hr μg/mL)	44.00	44.03	44.78	8.89	19.85	33.39	63.25
$t_{1/2}$ (hr)	13.77	12.90	15.01	6.34	42.22	7.01	24.29
Kel (hr^{-1})	0.05	0.05	0.05	0.02	43.76	0.03	0.10
$AUMC_{0-24}$ (hr^2 μg/mL)	262.71	258.86	264.45	31.94	12.08	223.49	314.76
$AUMC_{0-\infty}$ (hr^2 μg/mL)	841.44	763.25	963.91	528.74	54.85	377.60	2044.16
MRT (hr)	19.12	17.82	20.32	7.39	36.36	10.86	32.32
C_{max}/AUC_{0-24}	0.08	0.07	0.08	0.02	28.45	0.06	0.13

C_{max} = peak plasma concentration; t_{max} = time to reach peak serum concentration; AUC_{0-24} = area under the serum concentration-time curve from t = 0 to t = 24 hr; $AUC_{0-\infty}$ = the area under the serum concentration-time curve extrapolated from t = 0 to infinity; $t_{1/2}$ = serum elimination half-life; Kel = elimination rate constant; $AUMC_{0-24}$ = area under the first moment-versus-time curve from t = 0 to t = 24 hr; $AUMC_{0-\infty}$ = area under the first moment-versus-time curve from t = 0 to infinity; MRT = mean resident time; SD = standard deviation; CV = coefficient of variation.

mL of orange juice or water at 8 AM. In addition, the subjects received 200 mL of orange juice or water twice (8 AM and 2 PM) on Day 4. Group 1 received treatment A (administration of drug with water) followed by treatment B (administration of drug with orange juice) with a washout period of 1 week. This sequence of treatment is denoted by AB. Group 2 received treatment B followed by treatment A with a washout period of 2 weeks. This sequence of treatment is denoted as treatment BA. In the first period, Group 1 received treatment A and Group 2 received treatment B. In the second period, Group 1 received treatment B and Group 2 received treatment A. This type of study is known as a 2-way crossover design in statistical literature.[6] A standard lunch was allowed after 4 hours of dosing. The volunteers were ambulatory during the study but were prohibited from strenuous activity. Volunteers were monitored constantly for the 24-hour period by a medical doctor.

Blood Sampling

The timing of blood collection was planned according to the previously reported value of time to reach peak serum concentration (t_{max}) and serum elimination half-life ($t_{1/2}$).[7,8] An intravenous cannula was placed into the volunteers' forearm vein before drug administration and left in place for 24 hours until blood samples were collected. Venous blood samples were collected before and at 0.25, 0.50, 0.75, 1.00, 1.50, 2, 3, 5, 7, 9, 12, and 24 hours after drug administration. The blood samples were collected in coded, evacuated tubes, kept 30 minutes for clotting, and centrifuged at room temperature (3000 rpm for 15 minutes). The serum was collected in coded eppendorf tubes and serum protein was separated by precipitation with methanol followed by centrifugation at 10,000 rpm for 5 minutes. The serum was separated and stored at -80°C until further analysis.

Table 4. Large Sample-Based 90% Confidence Intervals (CI) for Different Pharmacokinetic Parameters From Log-Transformed Data for Assessment of Bioequivalence.

Parameters	Mean Ratio (%) (Juice/Water)	90% Confidence Interval (CI) for Different Pharmacokinetic Parameters	
		Upper Limit (%)	Lower Limit (%)
C_{max} (μg/mL)	134.92	145.00	125.00
AUC_{0-24} (hr μg/mL)	117.62	122.10	113.31
$AUC_{0-\infty}$ (hr μg/mL)	119.36	127.90	111.38
C_{max}/AUC_{0-24}	128.23	150.25	109.44

C_{max} = peak plasma concentration; AUC_{0-24} = area under the serum concentration-time curve from t = 0 to t = 24 hr; $AUC_{0-\infty}$ = the area under the serum concentration-time curve extrapolated from t = 0 to infinity.

Levofloxacin Concentration Determination by High Performance Liquid Chromatography (HPLC)

Levofloxacin concentration was determined at room temperature using 5-µm (particle-size), 4.6 × 250-mm Kromasil ODS C18 column. The compounds of interest were detected using a UV detector set at 293 nm wavelength. The mobile phase consisted of 0.05 M citric acid (1 M ammonium acetate and acetonitrile [77:1:22 v/v)) and was delivered at a flow rate of 1.0 mL/min. Samples were injected in the HPLC system by an autosampler. The retention time was 4.8445 ± 0.0016 minutes. The standard curves were linear over the concentration range of 25 to 1000 ng/mL with a mean correlation coefficient of 0.9958. The lower limit of quantification (LLOQ) of levofloxacin in the serum was found to be 25 ng/mL. All the blood samples were analyzed within 1 week of collection. The precision and accuracy of the method for determining the presence of levofloxacin were investigated at concentrations of 25, 50,100, 250, 500, 1000 ng/mL. The results are shown in Table 1. The intra-day and inter-day coefficient of variation for 5 samples were satisfactory with R.S.D.s less than 9.31%.

Pharmacokinetic Analysis

The following pharmacokinetic parameters were directly calculated by the standard non-compartmental analysis: (a) maximum serum concentration (C_{max}) and time to reach peak serum concentration (t_{max}); (b) the elimination half-life ($t_{1/2}$), calculated as $t_{1/2}$ = (ln 2)/Kel, where Kel is the apparent elimination rate constant (Kel was calculated by using the software WinNonlin[9]); (c) area under the serum concentration-time curve from t = 0 to t = 24 hours (AUC_{0-24}), area under the first moment curve (AUMC), and mean residence time (MRT), which was calculated from the measured concentration, from time 0 to the time of last quantifiable level, by the linear trapezoidal rule; (d) area under the serum concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$), calculated according to the following formula: $AUC_{0-\infty}$ = AUC_{0-t} + Ct/Kel, where Ct is the last quantifiable serum level; and (e) the rate of absorption, calculated from the ratio of $C_{max}/AUC_{0-\infty}$. Pharmacokinetic parameters were calculated by personal computer using Microsoft Excel (Version 2000) and WinNonlin (Version 2.1).

Statistical Analysis

Let y_{ijk} be the observed value of a pharmacokinetic parameter corresponding to the subject k in period j of group i. The following regression model (6) is assumed for y_{ijk} :

i = 1, 2.

j = 1, 2.

k =1, 2......12.

Table 5. *P* Values for Sources of Variations Obtained by ANOVA.

Sources of Variations	C_{max}	t_{max}	AUC_{0-24}	$AUC_{0-\infty}$	$t_{1/2}$	$AUMC_{0-24}$
Treatment	0.0002	0.6417	0.000049	0.6343	0.3389 0.	0166
Period	0.2171	0.1249	0.06802	0.1944	0.1500	0.83237
Sequence	0.8368	0.7330	0.3269	0.0591	0.8450	0.1253
Subjects	0.0130	0.1640a	0.04000	0.5030	0.0290	0.3890

C_{max} = peak plasma concentration; t_{max} = time to reach peak serum concentration; AUC_{0-24} = area under the serum concentration-time curve from t = 0 to t = 24 hr; $AUC_{0-\infty}$ = the area under the serum concentration-time curve extrapolated from t = 0 to infinity; $t_{1/2}$ = serum elimination half-life; $AUMC_{0-24}$ = area under the first moment-versus-time curve from t = 0 to t = 24 hr.

$$y_{ijk} = \mu + S_{ik} + \pi_j + \tau_{d[i,j]} \lambda_{d[i,j-1]} + \varepsilon_{ijk} \quad 1$$

where μ is the general mean, S_{ik} is the random effect of subject k in group i, π_j is the effect of period j, $\tau_{d[i,j]}$ is the effect of treatment administered in period j of group i, $\lambda_{d[i,j-1]}$ is the carry-over (sequence) effect of the treatment administered in period j-1 of group i with λ[i,0] = 0, and ε_{ijk} is the random error term. It is assumed that random terms S_{ik} and ε_{ijk} follow normal distribution with same mean 0 and variance σ^2 and σ^2_s, respectively. Carry-over effect can be tested by comparing corresponding mean sum of squares with the between subject mean sum of squares and period of a treatment effects are tested by comparing corresponding mean squares with the within subject mean squares.[6]

In our analysis, log-transformed value of the pharmacokinetic parameters AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, Kel, $t_{1/2}$, and $C_{max}/AUC_{0-\infty}$ are used in the model (1). The model (1) can be fitted by usual statistical software. We used statistical software R for fitting the model and drawing inferences about the parameters.[10]

RESULTS

No subject was dropped out of the study, and data obtained from all subjects were included in the analysis. Adverse events were mild. The comparison of mean serum concentration-time profile after administration of drug with water and nonfortified orange juice is shown in Figure 1. The important pharmacokinetic parameters after Treatment A and Treatment B are shown in Table 2 and Table 3.

Table 4 shows the 90% confidence intervals of the ratios (juice/water) between administration of levofloxacin with orange juice and water regarding AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, and $C_{max}/AUC_{0-\infty}$. When comparing the treatments after administration of drug with nonfortified orange juice and water, it was observed that the mean value of C_{max} decreased by 26.36% in the volunteers who had taken the drug with orange juice (2.57 ± 0.46 μg/mL) compared with those who had taken the drug with water (3.49 ± 0.75 μg/mL). This change was beyond the bioequivalence acceptable range (90% CI, 125.18%-145%; *P* = 0.0002). The mean value of AUC_{0-24} was decreased by 14.98% after administration of levofloxacin with orange juice (90% CI, 113.31%-122.10%), which is marginally within the bioequivalence range. The mean $AUC_{0-\infty}$ value was also reduced by 17.33% after administration of levofloxacin with orange juice (90% CI, 111.38%-27.90%). This change of $AUC_{0-\infty}$ was not within the acceptable range of bioequivalence.

The mean $AUMC_{0-24}$ values were found to be 234.95 ± 22.81 hr^2 μg/mL and 264.45 ± 31.94 hr^2 μg/mL after administration of levofloxacin with
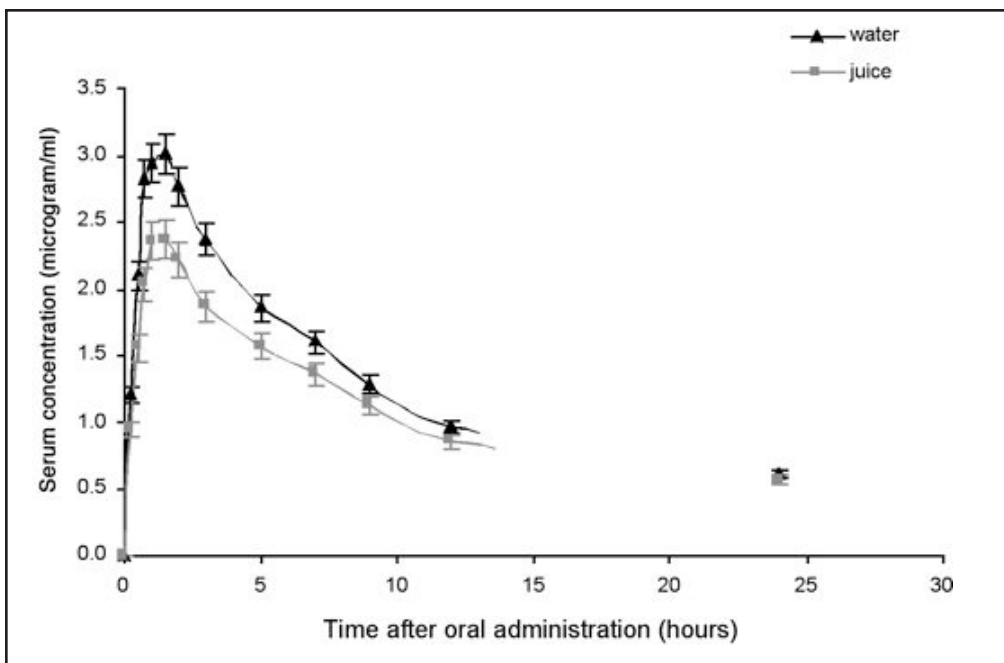
Figure 1. Mean serum levofloxacin concentration-versus-time curve of 12 subjects following oral administration of 250 mg levofloxacin single dose with water and with orange juice.

orange juice and with water, respectively. Here, a significant decrement (11.15%) of $AUMC_{0-24}$ was observed after administration of drug with orange juice. Other pharmacokinetic parameters such as $t_{1/2}$, t_{max}, Kel, $AUMC_{0-\infty}$, and MRT were not changed significantly. Table 5 shows the ANOVA of the model-1. It shows a significant difference of AUC_{0-24} and C_{max} between the 2 treatments (A and B) after controlling for the effects of period, sequence, and subject. Period effects were found to be insignificant for all the parameters. The insignificant sequence effect indicates no carry-over effect of the 2 treatments. Subject variations are also found to be significant for few parameters (AUC_{0-24}, C_{max}, and $t_{1/2}$) between 2 treatments.

DISCUSSION

The current study demonstrated 2 important clinical findings regarding the pharmacokinetics of a single oral dose of levofloxacin when it was administered

with orange juice: 1. the value of peak plasma concentration (C_{max}) decreased significantly and not within the bioequivalence ranges; and 2. the area under the plasma level time curve (AUC_{0-24}) was also decreased, but the decrement of $AUC_{0-\infty}$ was not within the acceptable range of bioequivalence. Previous studies indicated that fluoroquinolone antibiotics undergo well-described chelation interactions when co-administered with multivalent ions.[11,12] Recent studies demonstrated that a similar interaction occurs with ciprofloxacin when it is administered with calcium-fortified orange juice.[13] In the study of Wallace and colleagues,[5] C_{max} of levofloxacin was decreased significantly and significant prolongation of t_{max} was observed when the drug was administered with orange juice. However, in our study, the change of C_{max} and $AUC_{0-\infty}$ of levofloxacin were not within the bioequivalence range when the drug was administered with orange juice; no sig-

nificant difference was observed for t_{max}.

It may be speculated that this change of C_{max} and $AUC_{0-\infty}$ was obtained due to the interaction between the orange juice and levofloxacin at the intestinal transport system, and it may involve identified mechanisms such as P-glycoprotein or organic anion-transporting polypeptides (OATP) in the gastrointestinal tract in combination with some mild chelation interaction. The early studies involving orange juice have identified that heptamethoxyflavone (HMF), tangeretin, and nobiletin are not only substrates for both P-glycoprotein and OATP, but also are inhibitors that decrease the bioavailability significantly of other substrates, such as fexofenadine.[14,15] A study by Yamaguchi et al[16] demonstrated that both grepafloxacin and levofloxacin undergo intestinal secretion via P-glycoprotein, and it was evidenced by decreases in their bioavailability when co-administered with the P-glycoprotein inhibitor cyclosporine.[16-18] Additional studies have demonstrated that levofloxacin and other fluoroquinolones are substrates for both P-glycoprotein and OATP.[19,20] The limited sampling (plasma only) that was conducted during this study cannot completely rule out other causes of the interactions except potential interaction with P–glycoprotein and OATP.

Regardless of the actual mechanism of the interaction, the significant decrease of C_{max} and $AUC_{0-\infty}$ of levofloxacin is a matter of concern. It has been suggested that levofloxacin is a concentration-dependent killer and needs to achieve a ratio of C_{max} to minimum inhibitory concentration (MIC) of approximately 12 to have optimal clinical and bacteriological outcomes.[21] Based on the results of our study, optimal outcomes could be affected against susceptible pathogens, especially with those having borderline MICs such as streptococci and staphylococci, when a patient takes a dose of 250 mg levofloxacin with orange juice, due to the reduction of C_{max}. As a result of potential suboptimal drug exposure, not only will the patient be put at more risk of clinical failure, but the infecting pathogen may also become resistant to levofloxacin and other fluoroquinolones, thereby restricting treatment options for the patient in the future.[22] The $AUC_{0-\infty}$ value was reduced by 17.33% and the CI value was not within the bioequivalence range (119.36%-127.9%). This ultimately will affect the therapeutic efficacy of the drug. Although previous studies reported no effect of calcium-fortified orange juice on bioequivalence of levofloxacin, our data were different when nonfortified orange juice was co administered with levofloxacin. This difference may be due to the presence of a different concentration of calcium present in calcium-fortified orange juice and nonfortified orange juice. The chance of pharmacogenomic variation of metabolizing enzymes should be negligible as we are observing the effect of orange juice on bioavailability of levofloxacin.

Again, failure in antimicrobial therapy can lead to increased cost of continued medication, adverse effects of protracted courses of antibiotics, development of resistant pathogens, and possible hospitalization requiring intravenous antibiotics. When not considered, this problem has an unappreciated magnitude; regardless of mechanism, prescribers and patients should be aware of these interactions, and levofloxacin should not be taken with orange juice in any circumstances.

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