INTERACTION STUDY BETWEEN LEVOFLOXACIN AND OMEPRAZOLE USING URINARY PHARMACOKINETIC DATA

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ABSTRACT

The objective of this study was to observe the drug interaction between levofloxacin and omeprazole using urinary excretion data. Levofloxacin tablet and omeprazole capsule were administered separately as well as in combination in fasting condition with a wash out period of two weeks after each administration. Urine was collected at different time intervals of 0, 0-2, 2-4, 4-8, 8-12, 12-24, 24-36 and 36-48 hr post-dose and analyzed using a validated HPLC with UV detection. Different pharmacokinetic parameters for both drugs were determined using non-compartmental method. The maximum rate of excretion (R_{max}) of levofloxacin was not decreased significantly when co-administered with omeprazole (p>0.05). Similarly no significant difference (p = 0.350) was observed for R_{max} of omeprazole when co-administered with levofloxacin. Again the fraction of levofloxacin excreted (f_e/f) was not changed significantly (p = 0.953) due to the co-administration of omeprazole. Similarly fraction of omeprazole excreted (f_e/f) also remained unaffected (p = 0.672) when co-administered with levofloxacin. No significant change was observed for the area under the rate of excretion versus midpoint of time interval curve from zero to 48 hours ($AURC_{0-48}$) for levofloxacin and omeprazole (p = 0.816 and 0.792 respectively) when administered separately and co-administered with each other. The study clearly revealed that levofloxacin and omeprazole do not undergo any kind of interactions when administered together. So it can be concluded that these two drugs can be prescribed together to achieve optimum therapeutic activity.

Keywords: Drug interaction, Levofloxacin, Omeprazole, Urinary pharmacokinetics.

INTRODUCTION

Omeprazole is a widely prescribed proton pump inhibitor and metabolized primarily by the cytochrome P450 isoenzyme CYP2C19 (Fist and Chow, 1997). It has no significant effect on CYP3A4 which is an important cytochrome enzyme for drug metabolism (Tateishi, 1995). On the other hand, Levofloxacin is the optical *S* (−) isomer of the racemic quinolone ofloxacin and exhibits activity against a variety of gram-positive and gram-negative bacteria (Thornsberry *et al*., 1999; Soussy *et al*., 1999). Most of the drug is excreted unchanged through kidney (80 − 86%) and it undergoes limited metabolism (Fist and Chow, 1997; Nakashima *et al.,* 1992). Most of the quinolones inhibit cytochrome P450 enzyme activity that may result in a prolonged half-life for some drugs that are also metabolized by this system (Fuhr *et al*., 1992). The extent of this inhibition varies among different quinolones. It was very interesting to investigate the drug interaction between levofloxacin and omeprazole as they are prescribed in combination for the treatment of *Helicobacter pylori* infection; the main cause of gastritis, gastro duodenal ulcer and gastric cancer (Caro *et al.,* 2002; Cammarota *et al*., 2000).

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MATERIALS AND METHODS

Subjects

Six healthy, non-smoking, adult volunteers (4 males and 2 females) were enrolled in this study whose mean age ± SD was 24.0 ± 4.0 (range 18 to 27) years. Their mean body weight and height were 69.83 ± 9.95 kg (range 63 to 81) and 1.68 ± 0.1 m (range 1.55 to 1.73 m) respectively, with a mean body mass index (BMI) of 24.65 ± 1.96 kg/m^2. Their mean creatinine clearance was greater than 90 ml/min per 1.73 m^2, which had been referred as normal renal function according to CKD guideline (Drusano *et al.*, 1987). The inclusion criteria for the subjects included normal medical history, physical examination, cardiac, hepatic, renal, gastrointestinal and hematological profile.

Exclusion criteria included previous history of allergy to any fluoroquinolones or proton pump inhibitors and need for any chronic medication, donation of blood or use of an investigational agent within 30 days prior to the first dose of the study. Potential subjects were also excluded if they use any medication within one week 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 and taking alcohol or caffeine or 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 and its amendments (World Medical Association Declaration of Helsinki, 2000). Ethical permission was taken from Bangladesh Medical Research Council (BMRC) to approve the protocol and consent form of the investigation and each volunteer had to sign the consent document before entering the study.

Study drugs

The test drugs levofloxacin hemihydrate INN 500-mg film coated tablet and omeprazole magnesium BP 20-mg capsule were kind gift from Eskayef Bangladesh Ltd, a well reputed pharmaceutical company in Bangladesh.

Study design

This study was conducted in the Department of Clinical Pharmacy and Pharmacology, University of Dhaka in collaboration with Dhaka University Medical Centre to evaluate drug-drug interaction between levofloxacin and omeprazole in human model using urinary excretion data. This was an open label, randomized, single dose study. All the volunteers received three treatments separately (single dose of 500 mg of levofloxacin film coated tablet, single dose of 20 mg of omeprazole capsule and co-administration of the both drugs). A washout period of two weeks was maintained between two treatments. Drug was administered orally early in the morning with 250 ml of water after overnight fasting. After 6 hour of dosing, the subjects were provided with a standard lunch. Urine samples were collected according to the following time intervals of 0, 0-2, 2-4, 4-8, 8-12, 12-24, 24-36 and 36-48 hour post dosing. The urine volume was recorded in each time intervals and the samples were kept at -40^0C in marked tubes until further analysis.

Preparation of urine samples

The urine samples were centrifuged at 10,000 rpm for 5 min and then 20 µl of the supernatant was injected into HPLC injector for the determination of concentration of both levofloxacin and omeprazole in urine.

Chromatographic condition for levofloxacin

The urine samples, obtained after the administration of levofloxacin alone and the combination regimen (both levofloxacin and omeprazole), were assayed by a sensitive and validated HPLC method with UV detection described by Sultana et al. (Sultana *et al*., 2008). The samples were analyzed using a Nucleosil C_{18} column (5 µm, 4.6×250 mm) maintained at ambient temperature. The mobile phase consisted of 0.05 M citric acid: 1 M ammonium acetate: acetonitrile (77:1:22), the flow rate was 1 ml/min and data were recorded at 293 nm. The standard curve was linear over the concentration range of 5 to 500 µg/ml with a mean correlation coefficient of 0.999. The concentrations of samples were calculated against the standard curve.

Chromatographic condition for omeprazole

The urine samples, obtained after the administration of omeprazole alone and the combination regimen (both levofloxacin and omeprazole), were assayed by a sensitive and validated HPLC method with UV detection, for the determination of omeprazole concentration, described earlier with some modifications (Miroshnichenko and Yurchenko, 2002). The separation was done using a XTerra® RP8 (5 µm, 4.6×250 mm) (Waters, Ireland) column. The mobile phase consisted of 34% acetonitrile and 66% of phosphate buffer and eluted at a flow rate of 1 ml/min and the detection was done at 280 nm. The standard curve was linear over the concentration ranges of 1.0 to 200.0 µg/ml with a mean correlation coefficient of 0.9985. The concentrations of samples were determined against the standard curve.

Pharmacokinetic evaluation

Based on the urinary concentrations of levofloxacin and omeprazole and the scheduled time intervals, non-compartmental pharmacokinetic evaluation was performed according to the standard methods (Gieschke *et al*., 1999). The urinary parameters were calculated from the rate of drug excretion versus midpoint of time interval curve using the software Kinetica (Version 2.1, Thermo Fisher Scientific Inc. 81, Wyman Street, Waltham, MA, 02454). The following input data were applied: start and end time of each urine collection interval (Δt), urine concentrations (C), and urine volumes (V), the midpoint of each collection interval and the renal excretion rate for each interval (R) was computed according to the following equation (I):

$$R = CV/\Delta t \qquad \ldots\ldots\ldots (I)$$

Where Δt denotes the sampling interval.

The following pharmacokinetic parameters were subsequently determined from urinary excretion rate versus time curve like the maximal renal excretion rate (R_{max}), the midpoint of the respective collection interval associated with the maximal observed excretion rate (t_{max}) and the area under the renal excretion rate curve from time 0 to the last measured rate ($AURC_{0\text{-}tz}$).

$AURC_{0\text{-}tz}$ was calculated using the linear trapezoidal rule. The renal elimination rate constant (k_{el}) was assessed by ln-linear regression of the terminal segment of the excretion rate versus time curve. The optimal regression fit was determined by the software "Kinetica" using at least the three last excretion rates as the period of the highest possible coefficient of correlation. The negative value of the slope of the fitted linear regression line of the un-weighted data represents k_{el} and Ln 2 divided by k_{el}

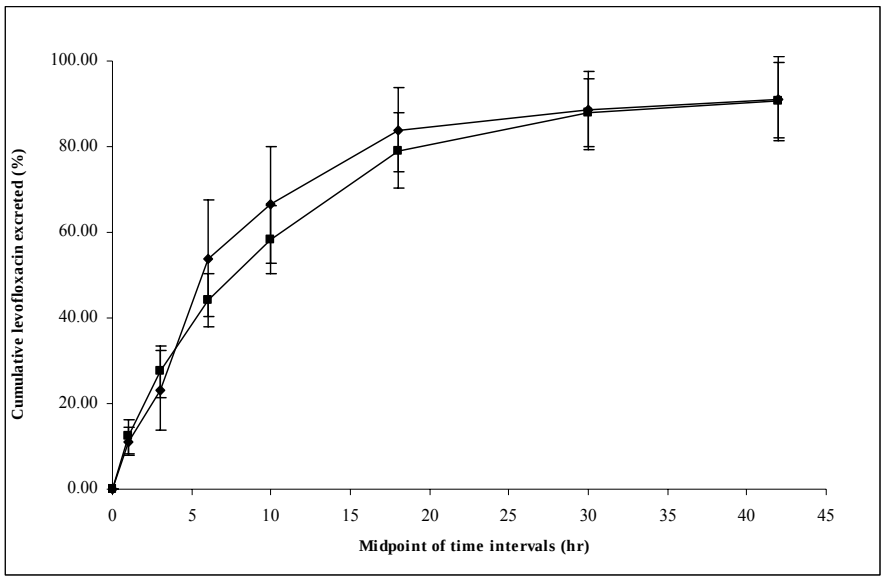

Fig. 1: Comparison of cumulative levofloxacin excreted (%) following administration of levofloxacin alone (–♦–) and levofloxacin and omeprazole in combination (–■–) in all the volunteers.

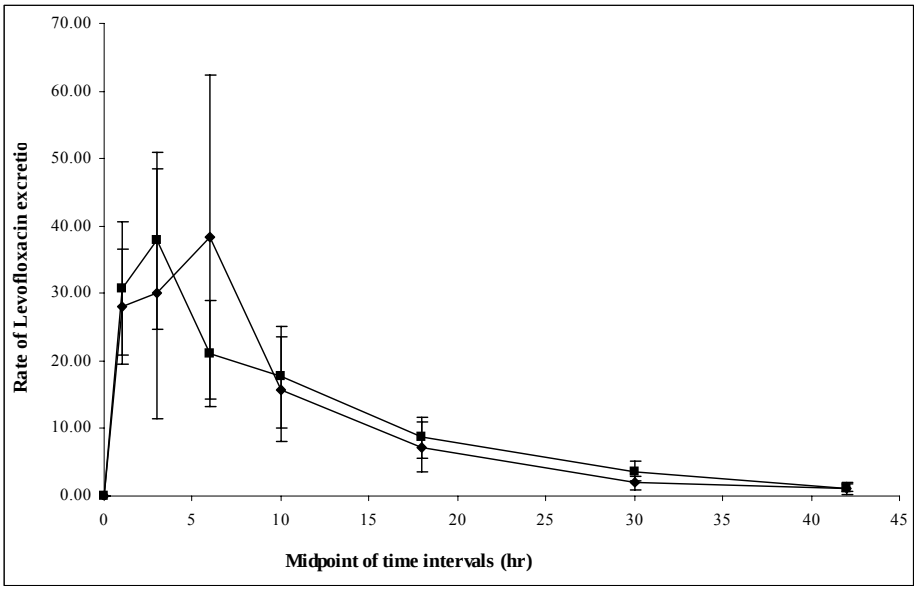

Fig. 2: Comparison of rate of excretion of levofloxacin following administration of levofloxacin alone (–♦–) and levofloxacin and omeprazole in combination (–■–) all the volunteers.

denoting the terminal elimination half-life ($t_{1/2}$). $AURC_{0-\infty}$ was determined by the equation (II):

$$AURC_{0-\infty} = AURC_{0-tz} + R_{tZ} / \lambda_Z \quad \ldots\ldots\ldots \text{(II)}$$

Moreover, the observed total amount of levofloxacin and omeprazole recovered in the urine from time 0 up to 48h (Ae_{0-48}) was determined by multiplying the concentration with the urine volume of the respective sample in each collection interval and summing up all intervals after dosing subsequently. The fraction of orally administered levofloxacin and omeprazole excreted in urine within 48 h (*fe/f*) was calculated by dividing Ae_{0-48} by the levofloxacin and omeprazole dose administered (Frank *et al*., 2005).

Statistical Analysis

The mean and standard deviation (SD) values of all the pharmacokinetic parameters were calculated and given in tabulated format. The ratio of parameters obtained following administration of levofloxacin alone and the combination and omeprazole alone and the combination were calculated to get idea about the interaction. Paired *t*-

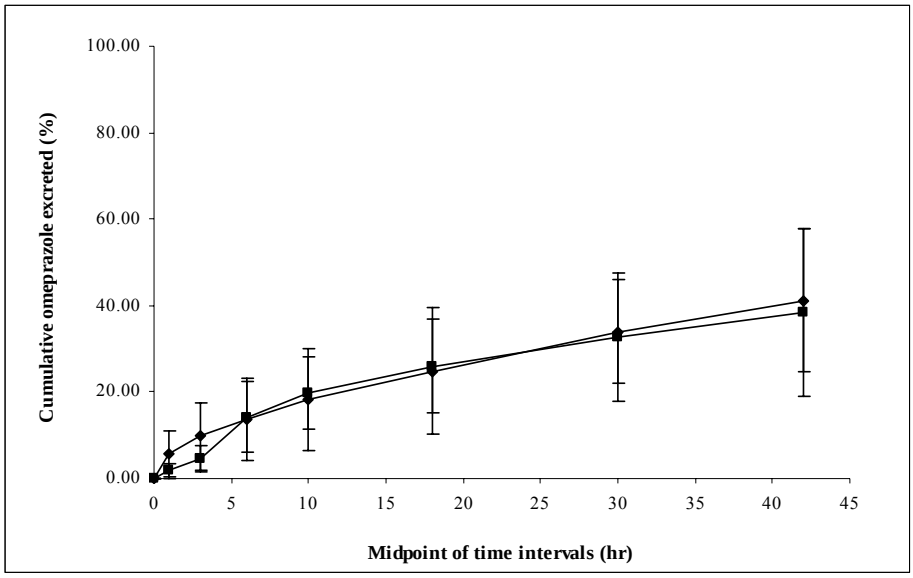

Fig. 3: Comparison of cumulative amount of omeprazole excreted (%) versus midpoint of time interval (hr) following administration of omeprazole alone (–♦–) and levofloxacin and omeprazole in combination (–■–) in all the volunteers.

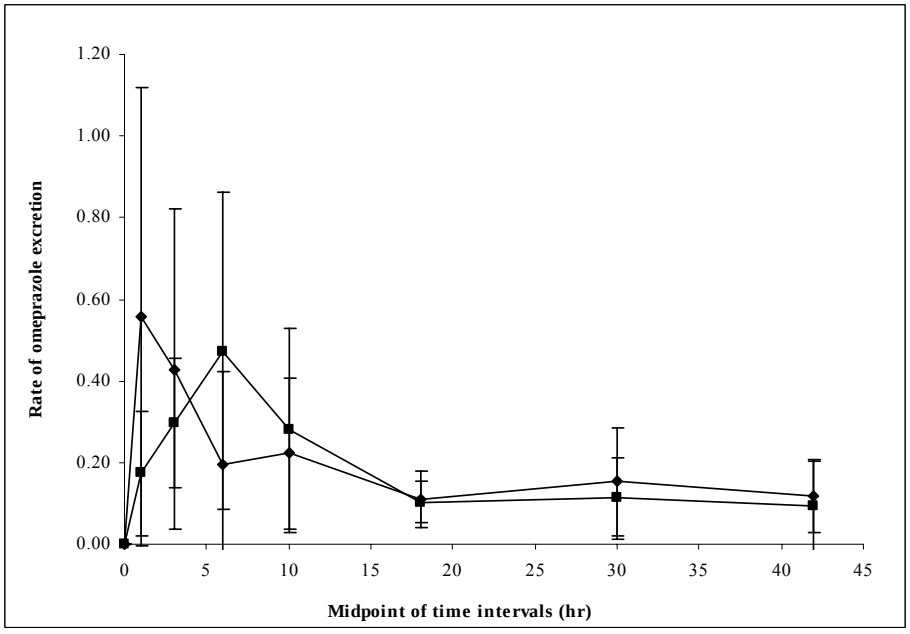

Fig. 4: Comparison of rate of excretion of omeprazole following administration of omeprazole alone (–♦–) and levofloxacin and omeprazole in combination (–■–) in all the volunteers.

test was performed for all the urinary parameters at 5% level of significance. This test indicates whether the differences between the pharmacokinetic parameters obtained during different treatments are significant or not. All the statistical analysis were done using the software SPSS (Version 12.0; Chicago, IL).

RESULTS AND DISCUSSIONS

The mean urinary excretion data, following oral administration of single dose of levofloxacin and levofloxacin in combination with omeprazole, is presented in comparative graphical presentations in figures 1 and 2 for all the volunteers.

The mean values of R_{max} for levofloxacin were found to be 42.72 ± 4.61 mg/hr when levofloxacin was administered alone and 40.63 ± 11.21 mg/hr when levofloxacin was administered with omeprazole. The mean values of t_{max} were found to be 3.67 ± 1.97 hr and 2.00 ± 1.10 hr when levofloxacin was administered alone and in combination with omeprazole respectively. The

Table 1: Mean urinary parameters of levofloxacin following administration of levofloxacin alone and levofloxacin and omeprazole in combination in all the volunteers

Parameters	Levofloxacin alone (Mean ± SD)	Levofloxacin and Omeprazole (combination) (Mean ± SD)	*p*-values*
R_{max} (mg/hr)	42.72 ± 4.61	40.63 ± 11.21	0.605
t_{max} (hr)	3.67 ± 1.97	2.00 ± 1.10	0.215
Ae $_{0-48}$ (mg)	455.67 ± 48.89	454.14 ± 44.30	0.955
f_e/f (%)	91.13 ± 9.78	90.83 ± 8.86	0.953
AURC $_{0-48}$ (mg)	447.87 ± 62.38	456.81 ± 49.93	0.816
AURC $_{0-\infty}$ (mg)	460.16 ± 67.59	472.94 ± 56.74	0.754
k_{el} (hr^{-1})	0.1207 ± 0.0571	0.0855 ± 0.0123	0.145
$t_{1/2}$ (hr)	6.53 ± 2.10	8.232 ± 1.074	0.091

* *p*-value at 5% level of significance.

Table 2: Mean urinary parameters of omeprazole following administration of omeprazole alone and levofloxacin and omeprazole in combination in all the volunteers

Parameters	Omeprazole alone (Mean ± SD)	Levofloxacin and Omeprazole (combination) (Mean ± SD)	*p*-values*
R_{max} (mg/hr)	0.72 ± 0.55	0.61 ± 0.33	0.350
t_{max} (hr)	6.83 ± 11.39	6.33 ± 3.14	0.929
Ae $_{0-48}$ (mg)	8.23 ± 3.32	7.66 ± 3.88	0.672
f_e/f (%)	41.15 ± 16.59	38.29 ± 19.41	0.672
AURC $_{0-48}$ (mg)	7.57 ± 2.94	7.29 ± 3.56	0.792
AURC $_{0-\infty}$ (mg)	12.44 ± 5.05	10.80 ± 8.64	0.671
k_{el} (hr^{-1})	0.0407 ± 0.0194	0.06031 ± 0.0566	0.453
$t_{1/2}$ (hr)	21.303 ± 12.815	17.60 ± 9.62	0.551

**p*-value at 5% level of significance.

mean values of Ae_{0-48} for levofloxacin were also found to be 455.67 ± 48.89 mg and 454.14 ± 44.30 mg for levofloxacin alone and when administered with omeprazole respectively. The mean values of f_e/f for levofloxacin decreased slightly from 91.13 ± 9.78 % to 90.83 ± 8.86 % when levofloxacin was administered alone and in combination. Other pharmacokinetic parameters such as $AURC_{0-48}$ (mg), $AURC_{0-\infty}$ (mg), K_{el} (hr^{-1}) and $t_{1/2}$ (hr) for levofloxacin were also determined. Table 1 features that the changes of $R_{max,}$ $t_{max,}$ $Ae_{0-48,}$ fe/f, $AURC_{0-48,}$ $AURC_{0-inf,}$ $k_{el,}$ $t_{1/2}$ were not significant (p>0.05).

Urinary excretion pattern of omeprazole are well illustrated in the figure 3 and 4. Different pharmacokinetic parameters from urinary data of omeprazole were calculated from the rate of excretion of omeprazole versus midpoint of time interval curve using the software Kinetica and tabulated in the table 2 for all the volunteers. From this the mean values of R_{max} were found to be 0.72 ± 0.55 mg/hr for omeprazole alone and 0.61 ± 0.33 mg/hr for omeprazole and levofloxacin combination. The mean values of t_{max} were found to be 6.83 ± 11.39 hr for omeprazole alone and 6.33 ± 3.14 hr for omeprazole and levofloxacin combination. The mean values of Ae_{0-48} of omeprazole were reduced from 8.23 ± 3.32 mg to 7.66 ± 3.88 mg for individual and combination therapy. The mean values of f_e/f were found to be 41.15 ± 16.59% for omeprazole alone and 38.29 ± 19.41% for omeprazole and levofloxacin combination. Other pharmacokinetic parameters such as $AURC_{0-48}$ (mg), $AURC_{0-\infty}$ (mg), K_{el} (hr^{-1}) and $t_{1/2}$ (hr) were also determined. From table 2, it is clear that the changes of $R_{max,}$ $t_{max,}$ $Ae_{0-48,}$ fe/f, $AURC_{0-48,}$ $AURC_{0-inf,}$ $k_{el,}$ $t_{1/2}$ were not significant (p>0.05).

Antibiotic resistance and poor compliance are the main causes of the failure of *H. pylori* eradication. This study evaluated the compliance of levofloxacin and omeprazole as combined therapy for *H. pylori* eradication. In the present study, oral bioavailability and urinary exposure of levofloxacin were not altered by co-administration of omeprazole. This is an important observation, because fluoroquinolones exhibit concentration dependent bactericidal activity, and reductions in bioavailability could potentially affect drug efficacy, particularly for pathogens that are less susceptible to the drug. Moreover due to lack of interaction, omeprazole also can be effectively prescribed in combination of levofloxacin to *H. pylori* eradication treatment without any alteration in omeprazole dosage regimen, which will support

achievement of optimum therapeutic activity. So it can be concluded that these two drugs can be administered together to achieve optimum therapeutic activity.

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