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Pharmacokinetic Interactions between Ciprofloxacin and Itraconazole in Healthy Male Volunteers

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ABSTRACT: *Objective.* To investigate the pharmacokinetic interaction between ciprofloxacin and itraconazole in healthy male volunteers. *Methods.* Ten healthy male volunteers were assigned into a 2-sequence, 3-period pharmacokinetic interaction study. In phase 1, all subjects were randomly assigned to receive 500 mg of ciprofloxacin alone and 200 mg of itraconazole alone twice daily for 7 days with a 14 day wash-out period in a crossover design. Phase 2 was performed 14 days after finishing phase 1, all subjects received 500 mg of ciprofloxacin in combination with 200 mg of itraconazole twice daily for 7 days. Ciprofloxacin and itraconazole pharmacokinetics were studied and adverse effects noted. *Results.* Ciprofloxacin significantly increased the C_{max} and $AUC_{0-\infty}$ of itraconazole by 53.13% and 82.46%, respectively. The half-life and *CL* of itraconazole were not changed significantly. The combination of itraconazole and ciprofloxacin could therefore result in an increase in adverse drug reactions. Conversely, itraconazole had no significant effect on the pharmacokinetics of ciprofloxacin. *Conclusion.* Ciprofloxacin decreases the metabolism of itraconazole, most likely through inhibition of CYP3A4. The dosage of itraconazole should be reduced and its therapeutic outcome should be monitored closely when these two agents are concomitantly administered. Copyright © 2011 John Wiley & Sons, Ltd.

Key words: pharmacokinetics; ciprofloxacin; itraconazole; drug interaction

Introduction

Co-infection with a fungus and a bacterium may occasionally occur in immunocompromised hosts and immunosuppressed patients. These patients often have to receive medications to treat any complications that might arise. Ciprofloxacin is a fluoroquinolone antibiotic in use worldwide. Ciprofloxacin is effective against a variety of bacterial infections [1]. It is an inhibitor of cytochrome P450 (CYP)3A and 1A family [2] and is also a substrate for the organic anion and/or cation transporters (OAT/OCT) [3]. Itraconazole is a triazole antifungal agent that may be used to treat either superficial or systemic fungal infections. Itraconazole is metabolized by CYP3A4 [4] and is also a potent inhibitor of CYP3A4 [5]. It has been shown to be a P-glycoprotein transporter (P-gp) inhibitor [6]. So, if ciprofloxacin and itraconazole were coadministered, there may be a drug–drug interaction. In addition, a previous study in mice found that the C_{max} and $AUC_{0-\infty}$ of ciprofloxacin when coadministered with ketoconazole and itraconazole were increased significantly by 1.4–2.8 fold greater than after receiving ciprofloxacin alone [7]. However, to date there has been no study

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aimed at determining any potential for pharmacokinetic interaction of these two agents in humans. The objective of this study was to provide such data.

Materials and Methods

Subjects

The study cohort consisted of ten healthy, nonsmoking, nonalcoholic, nonobese, male volunteers. All of the subjects were aged over 18 years. Their mean $\pm$ SD age was 24.7 ± 4.3 years (19–32 years) and the mean $\pm$ SD body mass index was $21.48 \pm 2.54\,kg/m^2$ ($18.29–24.42\,kg/m^2$). At the time of study, all of the subjects were not currently on any medication known to influence ciprofloxacin and itraconazole pharmacokinetics, nor had any been on such medication within 6 weeks before starting the study. Any subject with a history of ciprofloxacin or itraconazole intolerance, renal or hepatic impairment and diarrhea or vomiting during the study period were excluded. The protocol for the study was approved by the Ethics Committee of the Faculty of Science, Prince of Songkla University, and written informed consent was obtained from each subject.

Drugs and chemicals

Itraconazole (Sporal®) was obtained from Janssen-Cilag, Bangkok, Thailand. Ciprofloxacin (Ciprobay®) from Bayer, Bangkok, Thailand. Pure powder of itraconazole (R51211), ciprofloxacin and an internal standard (R51012) were obtained from Fitzgerald Industries International, Inc., MA, USA. Pure powder of quinine sulfate was generously donated by the Nutritional Biochemicals Corporation, USA. Anhydrous disodium hydrogen orthophosphate and glacial acetic acid were obtained from Merck Darmstadt, Germany. All of the solvents were high-performance liquid chromatography (HPLC) grade.

Study design

The study was conducted in two phases. In phase 1 (day 1–day 28), all subjects were randomly assigned to a two-way crossover study of a ciprofloxacin regimen and an itraconazole regimen with a 14 day washout period. The ciprofloxacin regimen consisted of a 500 mg tablet of ciprofloxacin (Ciprobay®) given every 12 h immediately after a standard meal for 7 days. The itraconazole regimen consisted of two 100 mg capsules of itraconazole (Sporal®) given at 12 h intervals in a similar way to the ciprofloxacin regimen. In phase 2 (day 42–day 49), all subjects were assigned to receive 500 mg of ciprofloxacin and 200 mg of itraconazole every 12 h immediately after a standard meal for 7 days. All subjects were monitored for adverse reaction according to CTCAE v4.02 criteria [8].

Sample collection

Ciprofloxacin and itraconazole pharmacokinetic studies were carried out on days 7, 28 and 49. Blood samples (approximately 5 ml) were obtained by direct venipuncture at the following times: before (time 0) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24 and 48 h for ciprofloxacin and up to 72 and 96 h for itraconazole after drug administration. Blood samples were added into heparinized tubes and centrifuged at $1000 \times g$ for 15 min. Plasma samples were stored at $-70^{\circ}C$ until analysis.

Ciprofloxacin assay

The concentrations of ciprofloxacin were determined by reverse-phase HPLC. The assay was modified from the method of Drusano [9]. Five hundred microliters of the plasma sample and 50 µl of internal standard (10 µg/l of quinine sulfate dissolved in 20% methanol) were transferred into an Eppendorf microcentrifuge tube and an equal volume of acetonitrile was added for deproteinizaton. The mixture was vortexed for 30 s and centrifuged at $1000 \times g$ for 15 min. One hundred microliters of supernatant was transferred to 400 µl of mobile phase and then the mixture was vortexed for 30 s. Twenty microliters of the supernatant was injected into the HPLC system, using a pump and automated injection system (Waters 2695, Waters Associates, Milford, MA, USA), onto a µ-Bondapak® C_{18} column (Waters Associates, Milford, MA, USA). The mobile phase was composed of phosphate buffer (0.08 mM KH_2PO_4; 0.06 mM Na_2HPO_4; pH 3): methanol: tetrahydrofuran (78.0:21.2:0.8%

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v/v/v) at a flow rate of 1.2 ml/min. The column effluent was monitored by fluorescence detection (Waters 2470, Waters Associates, USA) at excitation/emission values of 277/440 nm, respectively. The system was controlled and monitored by a single computer operated with Empower software (Waters Associates, Milford, MA, USA). The lower limit of detection of ciprofloxacin was 31.25 ng/ml.

The intra-assay reproducibility values characterized by coefficients of variation (CV) were 0.82%, 1.76% and 4.46% for samples containing 0.25, 2 and 6 μg/ml, respectively. The inter-assay reproducibility precision values, calculated by CV, were 0.33%, 1.00% and 0.67% for samples containing 0.25, 2 and 6 μg/ml, respectively.

Itraconazole assay

The concentrations of itraconazole were determined by reverse-phase HPLC. The assay was modified from the method of Badcock [10]. Five hundred microliters of the plasma sample and 50 μl of internal standard (5 μg/l of R51012) were transferred into an Eppendorf microcentrifuge tube and an equal volume of acetonitrile was added for deproteinizaton. The mixture was vortexed for 30 s and centrifuged at $1000 \times g$ for 15 min. The supernatant was transferred into a new vial and 200 μl was injected, using a pump and the automated injection system (Waters 2695, Waters Associates, Milford, MA, USA), into a Symmetry® C_{18} column (Waters Associates). The mobile phase was composed of acetonitrile: water (60:40%v/v), containing diethylamine 300 μl/l, and the pH was adjusted to 7.8 with phosphoric acid at a flow rate of 1.2 ml/min. The column effluent was monitored by UV detection (Waters 2470, Waters Associates, Milford, MA, USA) at 263 nm. The system was controlled and monitored by a single computer operated with Empower software (Waters Associates, Milford, MA, USA). The lower limit of detection of itraconazole was 25 ng/ml.

The intra-assay reproducibility values characterized by CV were 3.54%, 4.14% and 1.66% for samples containing 0.05, 0.8 and 3.2 μg/ml, respectively. The inter-assay reproducibility precision values, calculated by CV, were 0.80%, 1.84% and 0.37% for samples containing 0.05, 0.8 and 3.2 μg/ml, respectively.

Pharmacokinetic and statistical analysis

The maximum plasma concentration (C_{max}), the minimum plasma concentration (C_{min}) and time to maximum plasma concentration (T_{max}) were read directly from the obtained data of the

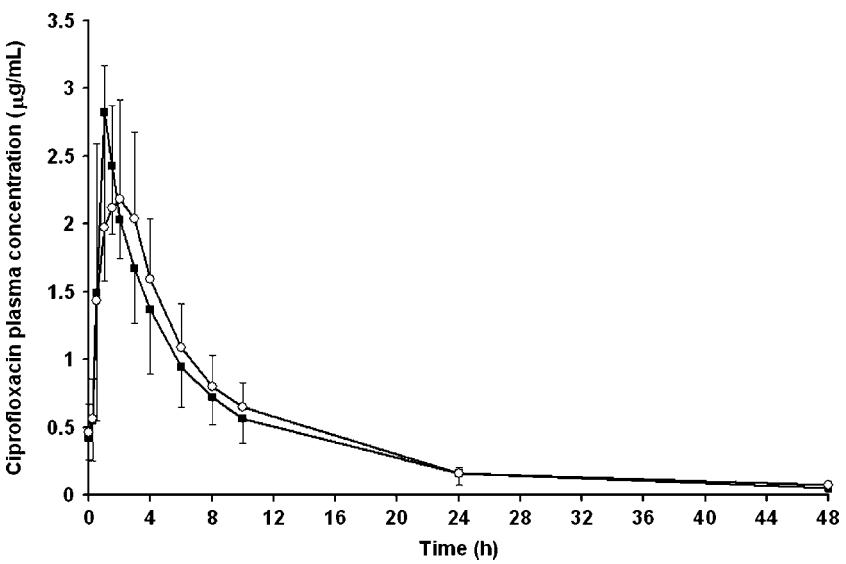

Figure 1. Mean plasma concentration–time curves of ciprofloxacin after multiple oral doses of 500 mg ciprofloxacin alone twice daily for 7 days (Phase 1: filled squares) and multiple oral doses of 500 mg ciprofloxacin in combination with 200 mg itraconazole twice daily for 7 days (Phase 2: open circles)

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individual plasma concentration–time profiles. The elimination half-life ($T_{1/2}$), the elimination rate constant (k_e), the area under the concentration–time curve between zero and 96 h ($AUC_{0–96}$), the area under the concentration–time curve between zero and infinity ($AUC_{0-\infty}$), the total clearance (CL_{tot}) and the volume of distribution (V) were determined by non-compartmental analysis. The model was fitted to the data using WinNonlin Version 1.1 (Scientific Consulting Inc, NC, USA). The results were expressed as mean ± standard deviation (SD) and statistical comparisons were made using paired *t*-test because the data showed normal distribution. Values of $p < 0.05$ were considered significant.

Table 1. Pharmacokinetic parameters (mean ± SD) of ciprofloxacin in subjects after receiving multiple oral doses of 500 mg ciprofloxacin alone (Phase 1) and in combination with 200 mg itraconazole (Phase 2) twice daily for 7 days

Parameter (units)	Phase 1	Phase 2	% changed
C_{max} (μg/ml)	3.19 ± 0.83	3.00 ± 0.70	−6.33
C_{min} (μg/ml)	0.052 ± 0.034	0.066 ± 0.031	+26.92
T_{max} (h)	1.25 ± 0.68	1.90 ± 0.91	+52.00
$AUC_{0–48}$ (μg.h/ml)	20.14 ± 4.26	21.62 ± 4.06	+7.35
$AUC_{0-\infty}$ (μg.h/ml)	20.95 ± 4.22	22.64 ± 3.99	+8.07
CL_{tot} (l/h)	0.020 ± 0.010	0.023 ± 0.004	+15.00
$T_{1/2}$ (h)	9.42 ± 2.49	9.93 ± 2.11	+5.41
k_e (h^{-1})	0.08 ± 0.02	0.07 ± 0.02	−12.50
V (l)	0.33 ± 0.12	0.33 ± 0.10	

Results

In both phases of this study, there were no significant differences in demographic or laboratory characteristics.

The mean plasma ciprofloxacin concentration-time data of phase 1 and 2 in normal subjects are depicted in Figure 1. The mean ciprofloxacin pharmacokinetic parameters of phases 1 and 2 are shown in Table 1. There was no statistically significant change in the mean C_{max}, $AUC_{0–48}$, $T_{1/2}$ and CL of ciprofloxacin. The mean plasma itraconazole concentration–time data of phases 1 and 2 in normal subjects are depicted in Figure 2. The individual C_{max}, $AUC_{0–96}$ and $T_{1/2}$ of itraconazole are shown in Figure 3. The mean itraconazole pharmacokinetic parameters of phase 1 and 2 are shown in Table 2. There were statistically significant increases in the mean C_{max}, C_{min}, $AUC_{0–96}$ and $AUC_{0-\infty}$ of itraconazole after being co-administered with ciprofloxacin. The V of itraconazole was significantly decreased when compared with itraconazole alone. The $T_{1/2}$ and CL of itraconazole were slightly changed.

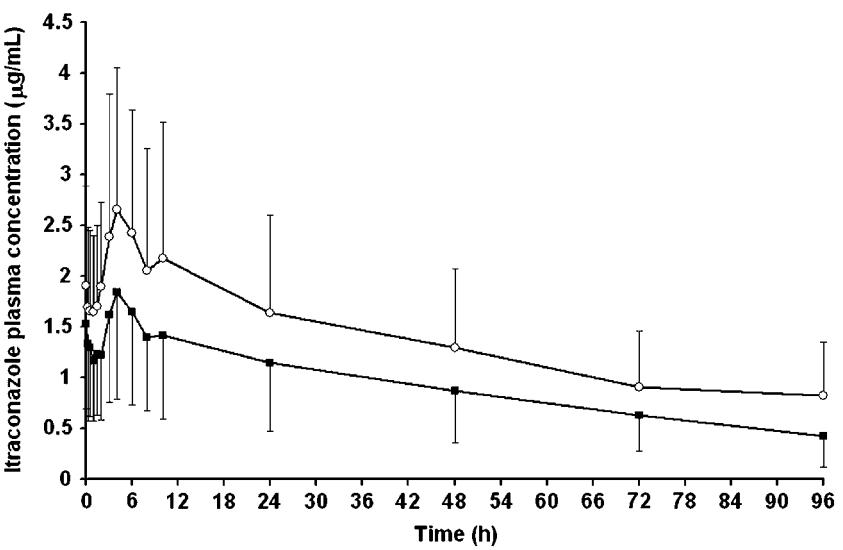

Figure 2. Mean plasma concentration–time curves of itraconazole after multiple oral doses of 200 mg itraconazole alone twice daily for 7 days (Phase 1: filled squares) and multiple oral doses of 200 mg itraconazole in combination with 500 mg ciprofloxacin twice daily for 7 days (Phase 2: open circles)

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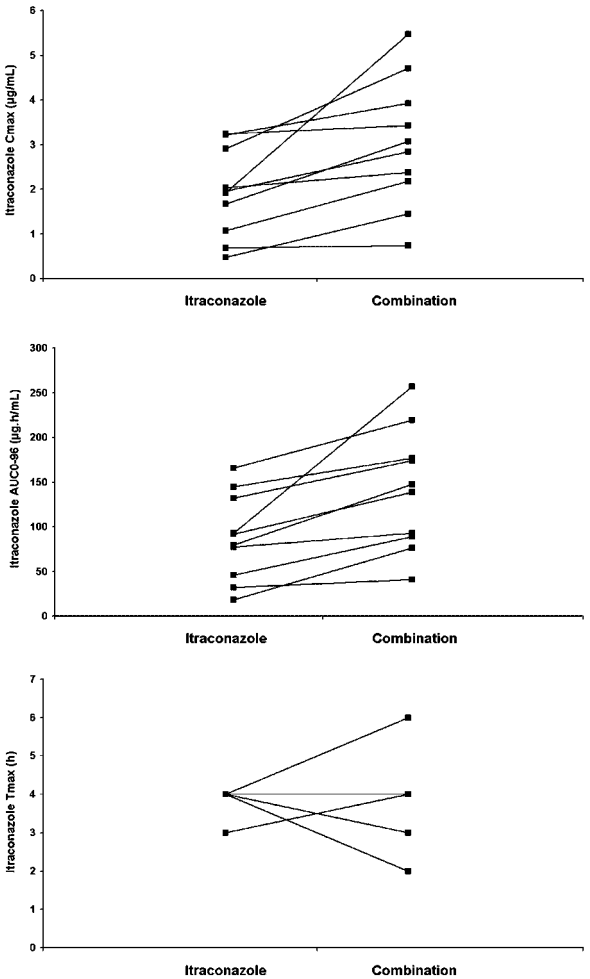

Figure 3. The individual C_{max} and AUC_{0-96} values of itraconazole after multiple oral doses of 200 mg itraconazole alone twice daily for 7 days (itraconazole regimen) and multiple oral doses of 200 mg itraconazole in combination with 500 mg ciprofloxacin twice daily for 7 days (combination regimen)

The results of the present study showed that the plasma concentration–time data of ciprofloxacin and itraconazole fitted closely to the noncompartment model. The adverse drug reactions in both phases are reported in Table 3.

Discussion

The results of the present study revealed that ciprofloxacin decreased the metabolism of itraconazole when they were administered concurrently for 7 days. This was due most likely to the inhibition of CYP3A4. On the contrary, itraconazole did not alter the pharmacokinetics of ciprofloxacin.

Ciprofloxacin has been reported to be a substrate for the OAT/OCT transporters [3] and CYP enzyme system. It has been well documented that itraconazole has also been reported to be P-gp and CYP inhibitors [6]. When multiple oral doses of ciprofloxacin were co-administered with itraconazole for 7 days (phase 2), the means of the C_{max}, AUC_{0-48}, $AUC_{0-\infty}$ and $T_{1/2}$ of ciprofloxacin were not significantly changed when compared with phase 1. Ciprofloxacin has the potential to cause a drug–drug interaction at the level of the CYP enzyme with itraconazole but our results were different from the study of Abou-Auda *et al*. in mice [7] which might be due to a difference in the metabolic pathways. It has been documented that both itraconazole and ketoconazole significantly increased the C_{max}

Table 2. Pharmacokinetic parameters (mean ± SD) of itraconazole in subjects after receiving multiple oral doses of 200 mg itraconazole alone (Phase 1) and in combination with 500 mg ciprofloxacin (Phase 2) twice daily for 7 days

Parameter (units)	Phase 1	Phase 2	% changed
C_{max} (µg/ml)	1.92 ± 0.99	2.94 ± 1.50[a]	+53.13
C_{min} (µg/ml)	0.43 ± 0.32	0.80 ± 0.52[b]	+86.05
T_{max} (h)	3.40 ± 1.26	4.30 ± 2.67	+26.47
AUC_{0-96} (µg.h/ml)	87.96 ± 48.46	131.24 ± 73.75[a]	+49.21
$AUC_{0-\infty}$ (µg.h/ml)	126.02 ± 76.39	229.94 ± 135.34[b]	+82.46
CL_{tot} (l/h)	0.003 ± 0.002	0.002 ± 0.001	−33.33
$T_{1/2}$ (h)	53.13 ± 27.00	72.34 ± 41.14	+36.16
k_e (h^{-1})	0.020 ± 0.001	0.010 ± 0.006	−50.00
V (l)	0.17 ± 0.12	0.11 ± 0.08[a]	−35.29

[a] $p < 0.05$, significantly different compared with phase 1.
[b] $p < 0.01$, significantly different compared with phase 1.

Table 3. Percentage of adverse reactions in subjects after receiving multiple oral doses of 500 mg of ciprofloxacin or 200 mg of itraconazole alone (Phase 1) and 500 mg of ciprofloxacin in combination with 200 mg of itraconazole (Phase 2) twice daily for 7 days

Adverse reaction	Phase 1 (%)	Phase 2 (%)[a]
Nausea	None	60
Dry mouth	None	40
Diarrhea	None	10
Headache	None	10

[a]The number of subjects who had adverse reaction divided by total number of subjects, expressed as a percent.

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and $AUC_{0-\infty}$ of ciprofloxacin. In our study, there was no significant effect of itraconazole on the pharmacokinetics of ciprofloxacin. The differences of ciprofloxacin pharmacokinetics in human and mice may be explained by species differences in CYP3A genes [11]. In addition, the different results may be due to the high K_m (Michaelis-Menten kinetic parameter) of ciprofloxacin for midazolam hydroxylation in canine liver microsomes (38.7 μM) [12]. It can be defined that ciprofloxacin has a low affinity for CYP3A. Moreover, the plasma itraconazole concentrations in this study were lower than the K_i value for itraconazole (2300 nM) when using midazolam as a probe [13].

Coadministration of ciprofloxacin with CYP3A4 substrates has been shown to increase the plasma concentrations of various coadministered drug [14–15]. Because ciprofloxacin is a potent inhibitor of CYP1A2 and CYP3A4 in human and rat microsomes [2], whereas itraconazole is a substrate of CYP3A4 [4]. In our study, itraconazole concentrations were higher when it was administered with ciprofloxacin than when it was administered alone. The mean C_{max}, C_{min}, AUC_{0-96} and $AUC_{0-\infty}$ were significantly increased by 53%, 86%, 49% and 82%, respectively. The mean $T_{1/2}$ of itraconazole seemed to be prolonged when compared with phase 1. The results suggested that the metabolism of itraconazole was decreased. The mean T_{max} for itraconazole was not significantly different when compared with phase 1. Therefore, the result of this study indicated that ciprofloxacin has no effect on the rate of itraconazole absorption. The adverse reactions of nausea (60%), dry mouth (40%), diarrhea (10%) and headache (10%) were noted after coadministration of both agents. These adverse reactions might be due to increases of the C_{max} and AUC of itraconazole after coadministration or are synergistic effects of both drugs. However, any adverse reaction after administration of ciprofloxacin or itraconazole alone was not observed. The adverse drug reactions described in this study were similar to those found in the previous study [16].

In conclusion, ciprofloxacin markedly decreased the metabolism of itraconazole and led to an increase in its plasma concentration and the AUC. These effects may be caused by inhibition of the CYP3A4 isozyme by ciprofloxacin. However, there was no significant effect of itraconazole on the pharmacokinetics of ciprofloxacin. Therefore, the dosage of itraconazole should be reduced and its therapeutic outcome be closely monitored when these two agents are concomitantly administered, especially in patients who will be on a long-term therapy to avoid the risk of adverse drug reaction.

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Conflict of Interest

None of the authors of this paper has a conflict of interest.

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