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ORIGINAL ARTICLE

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Pharmacokinetic interaction between ketoconazole and imatinib mesylate (Glivec) in healthy subjects

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Abstract The study under discussion was a drug–drug interaction study in which the effect of ketoconazole, a potent CYP450 3A4 inhibitor, on the pharmacokinetics of Glivec (imatinib) was investigated. A total of 14 healthy subjects (13 male, 1 female) were enrolled in this study. Each subject received a single oral dose of imatinib 200 mg alone, and a single oral dose of imatinib 200 mg coadministered with a single oral dose of ketoconazole 400 mg according to a two-period cross-over design. The treatment sequence was randomly allocated. Subtherapeutic imatinib doses and a short exposure were tested in order not to overexpose the healthy volunteers. There was a minimum 7-day wash-out period between the two sequences. Blood samples for determination of plasma concentrations were taken up to 96 h after dosing. Imatinib and CGP74588 (main metabolite of imatinib) concentrations were measured using LC/MS/MS method and pharmacokinetic parameters were estimated by a non-compartmental analysis. Following ketoconazole coadministration, the mean imatinib C_{max}, $AUC_{(0–24)}$ and $AUC_{(0–\infty)}$ increased significantly by 26% ($P < 0.005$), 40% ($P < 0.0005$) and 40% ($P < 0.0005$), respectively. There was a statistically significant decrease in apparent clearance (CL/f) of imatinib with a mean reduction of 28.6% ($P < 0.0005$). The mean C_{max} and $AUC_{(0–24)}$ of the metabolite CGP74588 decreased significantly by 22.6% ($P < 0.005$) and 13% ($P < 0.05$) after ketoconazole treatment, although the $AUC_{(0–\infty)}$ of CGP74588 only decreased by 5% ($P = 0.28$). Coadministration of ketoconazole and imatinib caused a 40% increase in exposure to imatinib in healthy volunteers. Given its previously demonstrated safety profile, this increased exposure to imatinib is likely to be clinically significant only at high doses. This interaction should be considered when administering inhibitors of the CYP3A family in combination with imatinib.

Keywords Imatinib · Ketoconazole · Pharmacokinetics · Healthy volunteers · Drug interaction

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Introduction

Imatinib (Glivec, imatinib mesylate, formerly STI571) is a phenylaminopyrimidine derivative and a member of a new class of drugs collectively known as signal transduction inhibitors [25]. Imatinib is a protein-tyrosine kinase inhibitor of ABL [6], BCR-ABL, ARG [21] as well as the structurally related family of tyrosine kinases that includes the receptor for stem cell factor KIT and platelet-derived growth factor receptors α and β [7, 14].

Imatinib has proven clinically effective in the treatment of all phases of chronic myelogenous leukemia (CML) by virtue of its ability to inhibit the constitutively activated BCR-ABL tyrosine kinase thought to underlie the development of CML [15, 19, 26, 27]. In addition to CML, imatinib has also demonstrated efficacy in the treatment of gastrointestinal stromal tumor (GIST), a mesenchymal neoplasm characterized by the expression of dysregulated c-Kit. Imatinib is indicated for treatment of malignant and metastatic GIST [9, 24].

During the phase 1 evaluation of imatinib, chronic-phase CML patients were assigned doses of imatinib ranging from 25 to 1000 mg/day [11, 23]. In these studies imatinib was found to be well tolerated such that a maximal tolerated dose was not identified. The recommended dosage of imatinib is currently 400 mg/day for chronic phase CML and 600 mg/day for advanced

phases [12]. Recent trials of CML have indicated that an 800-mg dose has improved efficacy compared with 400 mg, suggesting that optimal dosing may include higher doses of imatinib in the future [8, 16, 17].

CYP3A4 is the major P450 isoenzyme involved in microsomal biotransformation of imatinib [12]. Ketoconazole is a broad-spectrum antifungal drug that selectively inhibits the cytochrome P450 isoenzyme 3A4 (CYP3A4) in vitro and in vivo, with a dissociation constant (K_I) ranging from 0.0037 to 0.7 μM [5, 10, 29, 30]. Therefore, ketoconazole may have an impact on the plasma concentration and pharmacokinetics of other oral drugs metabolized by CYP3A4, such as imatinib, when administrated concomitantly [12]. This study was conducted to investigate the tolerability and pharmacokinetics of imatinib in combination with ketoconazole as a model for other potential drug interactions that involve CYP3A4 inhibition and imatinib therapy.

Ketoconazole is well tolerated and is therefore the model drug under current regulatory guidance for the investigation of the effect of a potential drug–drug interaction on imatinib CYP3A4 metabolism [28].

Materials and methods

Subjects

A total of 14 healthy subjects (13 male, 1 female) were enrolled in this study. Before participation, male or postmenopausal or sterile female subjects ranging in age from 40 to 60 years underwent a medical screening that included medical history, physical examination, and routine laboratory tests. Subjects with a history of smoking or alcohol abuse or who were using any prescription drug or over-the-counter medication within 2 weeks prior to dosing were excluded from the study. Any subjects who discontinued prematurely were to be replaced.

Study design

This study was performed at Swiss Pharma Contract, Clinical Pharmacology Unit, Allschwil, Switzerland, in accordance with the provisions of the Declaration of Helsinki and revisions. The protocol was approved by an independent ethics review committee in the Canton of Baselland, and all subjects gave their written informed consent to participate in the study. This was a single-center, open-label, randomized, crossover design study. In most published drug–drug interaction studies with ketoconazole, the dose selected for ketoconazole is a 400-mg single dose. The clinical dose range for ketoconazole is 200–400 mg/day [3, 13]. The dose for this single-dose study was selected to reflect the lower plasma concentrations and to not overexpose the healthy volunteers. Each subject received an oral dose of 200 mg imatinib in capsule form and an oral dose of 200 mg imatinib in capsule form together with the oral coadministration of 400 mg ketoconazole in two different sequences. Subjects were allocated at random to one of the two treatment sequences. There was a minimum 7-day washout phase between treatments. For each subject, there were a 21-day screening period, two treatment periods (each consisting of a baseline evaluation, drug administration and a 48-h post-dose observation and pharmacokinetic sampling phase), and a study completion evaluation approximately 96 h after the last dosing. In each of the two treatment periods, subjects reported to the study site 12–14 h prior to dosing for baseline evaluations and remained at the center for at least 48 h after dosing. Immediately after breakfast, they received either imatinib 200 mg or imatinib 200 mg together with ketoconazole 400 mg. Lunch was served 4 h after drug administration.

Blood sampling

Blood samples for determination of plasma imatinib concentrations were obtained up to 96 h after dosing. At the time of each imatinib determination in plasma, 5.5 ml of venous blood was drawn from a forearm vein into heparin-containing tubes. The time-points analyzed were pre-dose and 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h after dosing. Parent drug and its *N*-desmethyl metabolite CGP74588 were analyzed by a validated method. All samples were kept frozen at −18°C or below pending analysis. Subjects were eligible for discharge after completion of the 48-h pharmacokinetic sampling; all samples scheduled later than 48 h after drug administration were obtained on an ambulatory basis. After the last dose, the study completion evaluation coincided with the 96-h post-dose evaluations.

Plasma imatinib and CGP74588 analyses

Concentrations of imatinib and its main metabolite, CGP74588, were determined in plasma by liquid chromatography/tandem mass spectrometry (LC/MS/MS) using deuterium-labeled D_8-STI571 as internal standard. The LC/MS/MS analyses were carried out on a Sciex API 3000 mass spectrometer (Applied Biosystems, Foster City, Calif.). The instrument was operated in the atmospheric pressure chemical ionization mode with selected reaction monitoring. LC was performed on a Shimadzu LC system (Shimadzu Scientific Instruments, Columbia, Md.), operated in isocratic mode with a 4.6×50-mm C-18 column. Samples were prepared using protein precipitation with acetonitrile. All concentrations are reported in terms of the free-base form of imatinib. Within-study assay validation was performed by analysis of quality control samples together with the study samples. The limit of quantitation (LOQ) was 4 mg/ml for both imatinib and the metabolite

CGP74588 [2]. The in-study validation showed the following accuracy and precision for the quality control specimen coanalyzed with unknown samples: at the LOQ, 103 ± 8.1% for imatinib and 114 ± 4.6% for CGP74588. Overall accuracy for imatinib ranged from 97% to 110% and precision ranged from 1.1% to 8.1%. For CGP74588 accuracy ranged from 95.9% to 114% and precision ranged from 2.4% to 5.5%.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined using non-compartmental method(s) with WinNonlin Pro 3.1 (Pharsight Corporation, Mountain View, Calif.). For plasma concentrations of imatinib and CGP74588, the following parameters were determined to investigate the effects of ketoconazole on the pharmacokinetics of imatinib: area under the concentration-time curve from time zero to the last measurable sampling time point ($AUC_{(0-t)}$), AUC from time zero to time infinity ($AUC_{(0-\infty)}$), maximum plasma concentration (C_{max}), time to reach C_{max} (t_{max}), elimination half-life ($t_{1/2}$), apparent volume of distribution at steady state (V_z/f), and apparent clearance (corrected for bioavailability) (CL/f).

Statistical analysis

The following pharmacokinetic parameters were used to assess the interaction between ketoconazole and imatinib: $AUC_{0-2.5\ h}$, $AUC_{0-4\ h}$, $AUC_{0-24\ h}$, AUC_{inf}, C_{max}, V_z/f, CL/f, $t_{1/2}$, and t_{max}. Except for t_{max}, all parameters underwent an analysis of variance (ANOVA). All AUCs and C_{max} were log-transformed prior to ANOVA.

Treatment differences were assessed by means of contrasts in the ANOVA. The null hypothesis (''no interaction'') was tested by comparing the *t*-statistic of these contrasts with the appropriate quartiles of Fisher's *t*-distribution. T_{max} was analyzed non-parametrically. An α-level equal to 0.05 was considered statistically significant. No α-adjustment was made for multiple testing.

Results

Safety and tolerability

A total of 14 healthy subjects (13 male, 1 female) were enrolled in this study. They were aged 35–59 years and weighed 64–103 kg. (One 35-year-old subject, whose age was not within the 40–60 year range of the inclusion criteria, was allowed to participate in the study because it was not foreseen that this would affect the safety and pharmacokinetic data.) All 14 subjects completed the study. No subjects discontinued prematurely.

No serious adverse events or deaths were reported in this study. Only one moderate headache possibly related to study drug treatment was reported the day after imatinib-plus-ketoconazole intake; it resolved after one night and did not require any medical treatment. No clinically significant laboratory value or ECG abnormalities were observed. One subject had a systolic blood pressure of 162 mmHg and a diastolic blood pressure of 98 mmHg at the pre-dose assessment. As the values returned to normal after 1 week, no treatment was needed.

Table 1 Imatinib pharmacokinetic parameters following oral administration of 200 mg of imatinib alone and combined with oral administration of 400 mg of ketoconazole. T_{max}, time to reach maximum plasma concentration; C_{max}, maximum plasma concentration; $t_{1/2}$, elimination half-life; $AUC_{(0-24)}$, area under the concentration–time curve from time zero to 24 h; $AUC_{(0-\infty)}$, AUC from time zero to time infinity; V_z/f, apparent volume of distribution at z state; CL/f, apparent clearance (corrected for bioavailability). Values are means ± SD, except T_{max} median (range)

	Imatinib plus ketoconazole	Imatinib alone	*P* value
T_{max} (h)	4.0 (2.0–6.0)	2.5 (1.5–4.0)	
C_{max} (ng/ml)	1,213 ± 528	942 ± 311	< 0.005
$t_{1/2}$ (h)	19.2 ± 4.5	20.5 ± 4.4	
$AUC_{(0-24)}$ (ng h/ml)	13,498 ± 5,561	9,618 ± 4,191	< 0.005
$AUC_{(0-\infty)}$ (ng h/ml)	19,667 ± 8,932	14,228 ± 7,359	< 0.005
V_z/f (l)	318 ± 113	472 ± 163	
CL/f (l/h)	11.6 ± 4.0	16.3 ± 5.5	< 0.005

Imatinib pharmacokinetics

The pharmacokinetic parameters of imatinib and its main metabolite, CGP74588, for the 14 healthy subjects as determined by non-compartmental model analyses are listed in Tables 1 and 2. The mean and standard deviation for each parameter are given for the two treatment periods in which imatinib was administered. Figures 1 and 2 show the mean plasma concentrations of imatinib and CGP74588, respectively, following oral administration of imatinib either alone or combined with oral administration of ketoconazole.

Table 2 Imatinib metabolite (CGP74588) pharmacokinetic parameters following oral administration of 200 mg of imatinib alone and combined with oral administration of 400 mg of ketoconazole. T_{max}, time to reach maximum plasma concentration; C_{max}, maximum plasma concentration; $t_{1/2}$, elimination half-life; $AUC_{(0-24)}$, area under the concentration–time curve from time zero to 24 h; $AUC_{(0-\infty)}$, AUC from time zero to time infinity. Values are means ± SD, except T_{max} median (range)

	Imatinib plus ketoconazole	Imatinib alone
T_{max} (h)	4.0 (1.5–8.0)	2.5 (1.5–4.0)
C_{max} (ng/ml)	84.4 ± 34.2	108 ± 40.0
$t_{1/2}$ (h)	45.6 ± 13.2	48.9 ± 13.0
$AUC_{(0-24)}$ (ng h/ml)	1120 ± 472	1302 ± 651
$AUC_{(0-\infty)}$ (ng h/ml)	3280 ± 1,269	3638 ± 2,246

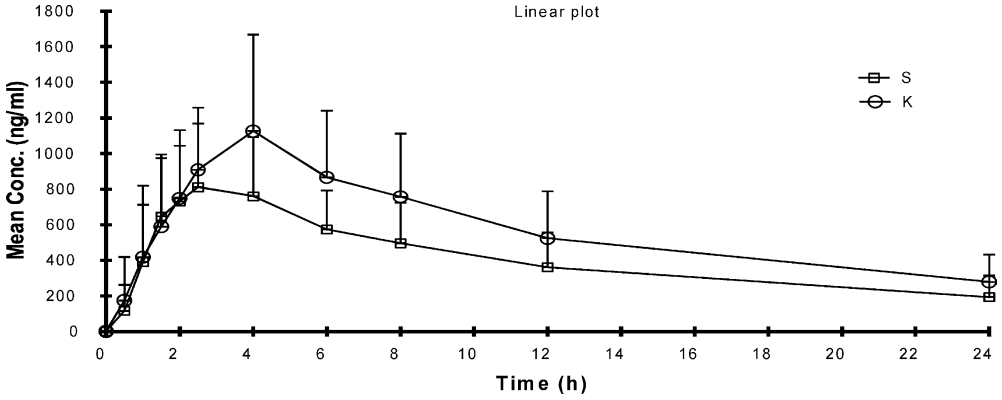

Fig. 1 Mean plasma concentrations of imatinib following oral administration of imatinib alone (*squares*, *S*) and combined with ketoconazole (*circles*, *K*)

Following ketoconazole coadministration, the mean imatinib C_{max} increased significantly by 26%, the $AUC_{(0–24)}$ increased by 40%, and $AUC_{(0–\infty)}$ also increased by 40% (Table 1). There was a statistically significant decrease in CL/f, with a mean reduction of 28.6%.

Discussion

Following imatinib and ketoconazole coadministration, the mean C_{max} of imatinib was found to be increased by 26% and the $AUC_{(0–24)}$ and $AUC_{(0–\infty)}$ by 40%; however, the C_{max} and AUC of CGP74588 decreased by only 13% and 5%, respectively. This difference between the effects of ketoconazole on the imatinib and CGP74588 values may have been due to the decreased rate of formation of other metabolites mediated by CYP3A4; these metabolites were not determined quantitatively in the present study.

No significant difference was observed between treatment with imatinib alone and treatment with imatinib in combination with ketoconazole in the half-life of imatinib. This result is unlikely to have been due to first-pass metabolism since the absolute bioavaila bility of imatinib is 100%, suggesting that imatinib is completely absorbed when given alone and therefore inhibition of first-pass metabolism cannot increase absorption [22]. A more plausible explanation is that the increase in exposure (AUC) to imatinib is due to inhibition of drug elimination, perhaps metabolism. This is supported by the decrease in apparent clearance (CL/f) and the reduction in the apparent volume of distribution in combination with ketoconazole.

In this study, the increased imatinib exposure caused by a single dose of ketoconazole was moderate and was well tolerated by healthy volunteers. If ketoconazole is used in a multiple-dose regimen and its steady-state concentrations are higher than the concentrations in the present study, it is possible that the drug–drug interaction may be greater. The clinical significance of this study may arise in situations where 800 mg/day or higher doses of imatinib are administered. Given that several trials have demonstrated the efficacy of 800 mg compared with lower doses in CML, increased awareness of the drug interaction described here is beneficial. This should be evaluated in further pharmacokinetic studies.

In a recent study in patients with CML, imatinib, also an inhibitor of the cytochrome P450 3A4 isoenzyme, has been shown to reduce the CYP3A4-mediated clearance of a coadministered drug [20]. This study indicated that caution is required when administering imatinib with other CYP3A4 substrates with a narrow therapeutic window. In another study in healthy volunteers, concomitant treatment with rifampicin, a CYP3A4 inducer,

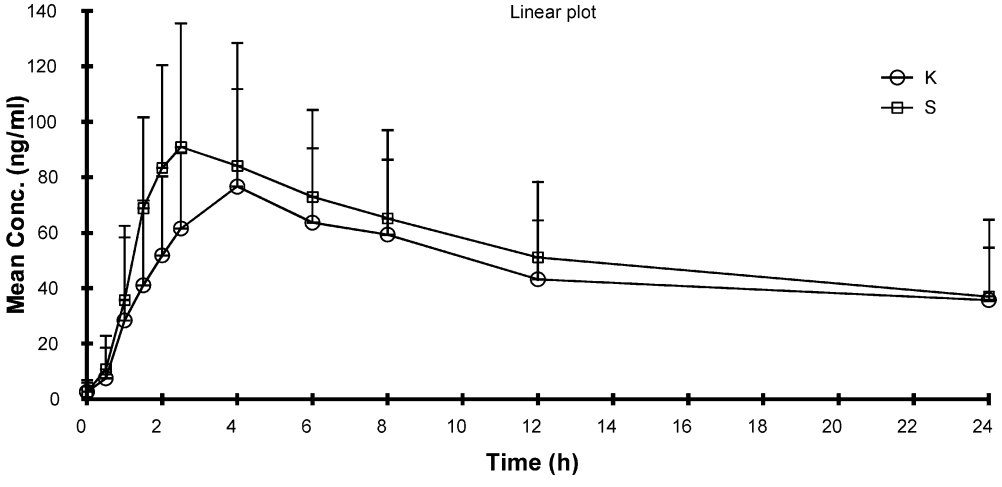

Fig. 2 Mean plasma concentrations of CGP74588 following oral administration of imatinib alone (*squares*, *S*) and combined with ketoconazole (*circles*, *K*)

was found to decrease exposure to imatinib, which could potentially result in subtherapeutic plasma concentrations of imatinib [4]. These studies illustrate other drug interactions that potentially affect imatinib exposure.

Brief treatment with imatinib plus ketoconazole was well tolerated with only one moderate headache possibly related to study drug treatment reported during the study. No significant abnormalities from baseline in laboratory values, vitals signs, or ECG findings were reported.

In conclusion, the results of this drug–drug interaction study are in agreement with those of in vitro human microsome studies indicating that imatinib is metabolized to CGP74588 by CYP3A4 (data not shown). There was a significant increase in exposure to imatinib in healthy volunteers when coadministered with ketoconazole. Ketoconazole (400 mg) coadministered with subtherapeutic doses and short exposure of imatinib (200 mg) was well tolerated in healthy subjects, and no significant clinical effect was reported. However, this study indicates that potent inhibitors of CYP3A4 can markedly increase the plasma concentration of imatinib; therefore, caution should be exercised when administering imatinib with inhibitors of the CYP3A family [1].

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