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Clinical pharmacology of the tyrosine kinase inhibitors imatinib and sunitinib

Erp, P.H. van

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**Influence of CYP3A4 Inhibition
on the Steady-State Pharmacokinetics
of Imatinib**

3

Chapter 3



Nielka P. van Erp, Hans Gelderblom, Mats O. Karlsson, Jing Li,
Ming Zhao, Jan Ouwerkerk, Johan W. Nortier, Henk-Jan Guchelaar,
Sharyn D Baker, and Alex Sparreboom

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Abstract

Purpose: To evaluate the effects of ritonavir, a potent inhibitor of CYP3A4, on the steady-state pharmacokinetics of imatinib.

Experimental Design: Imatinib pharmacokinetics were evaluated in cancer patients receiving the drug for at least 2 months, after which ritonavir (600 mg) was administered daily for 3 days. Samples were obtained on the day before ritonavir (day 1) and on the third day (day 4). The *in vitro* metabolism of imatinib with or without ritonavir and the effect of imatinib on 1-OH-midazolam formation rate, a probe for CYP3A4 activity, were evaluated with human CYP3A4 and pooled liver microsomes.

Results: In 11 evaluable patients, the geometric mean (95% confidence interval) area under the curve of imatinib on days 1 and 4 were 42.6 (33.0-54.9) $\mu\text{g}\cdot\text{h}/\text{mL}$ and 41.2 (32.1-53.1) $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively ($P = 0.65$). A population analysis performed in NONMEM with a time-dependent covariate confirmed that ritonavir did not influence the clearance or bioavailability of imatinib. *In vitro*, imatinib was metabolized to the active metabolite CGP74588 by CYP3A4 and CYP3A5 and, to a lesser extent, by CYP2D6. Ritonavir (1 $\mu\text{mol}/\text{L}$) completely inhibited CYP3A4-mediated metabolism of imatinib to CGP74588, but inhibited metabolism in microsomes by only 50%. Imatinib significantly inhibited CYP3A4 activity *in vitro*.

Conclusion: At steady-state, imatinib is insensitive to potent CYP3A4 inhibition and relies on alternate elimination pathways. For agents with complex elimination pathways that involve autoinhibition, interaction studies that are done after a single dose may not be applicable when drugs are administered chronically.

Introduction

The first rationally designed inhibitor of a signal transduction pathway, imatinib, is a competitive inhibitor of Bcr-Abl, platelet-derived growth factor receptors (α and β), and c-KIT receptor tyrosine kinases¹⁻⁴. It was first approved for the treatment of Philadelphia chromosome-positive chronic myelogenous leukemia and, shortly thereafter, for c-KIT positive metastatic and unresectable gastrointestinal stromal tumor^{5,6}.

The pharmacokinetic properties of imatinib have been investigated in healthy volunteers and in patients with chronic myelogenous leukemia, gastrointestinal stromal tumor, and other tumors^{7,8}. Imatinib is well absorbed after oral administration with a bioavailability exceeding 90%⁹. It is extensively metabolized, with up to 80% of the administered dose being recovered in feces, predominantly as metabolites¹⁰. Imatinib is metabolized *in vitro* principally by cytochrome P450 (CYP) 3A4 and CYP3A5 with CYP1A2, CYP2C9, CYP2C19, and CYP2D6 playing a minor role⁸. The main circulating metabolite of imatinib is an N-desmethyl derivative, CGP74588, which has *in vitro* activity similar to that of imatinib, and the systemic exposure represents approximately 10% to 15% of that for imatinib¹⁰. The pharmacokinetic profile of a single dose of imatinib is sensitive to CYP3A4 modulation, with a 74% and 30% reduction in imatinib area under the curve (AUC) observed with coadministration of the CYP3A4 inducers rifampin¹¹ or St. John's wort¹², respectively, and a 40% increase in imatinib AUC observed with coadministration of the CYP3A4 inhibitor ketoconazole⁸. Interestingly, imatinib itself is known to decrease the clearance of simvastatin, a CYP3A4 substrate, by 70% in patients with chronic myelogenous leukemia¹³.

Because imatinib is a substrate for CYP3A4, there is great potential for drug interactions with co-administered drugs, food, and herbal and nutritional supplements potentially leading to subtherapeutic exposure or concentrations associated with greater than acceptable toxicity for imatinib^{14,15}. The prescribing information for imatinib mesylate indicates a need for caution when imatinib is administered with inhibitors and inducers of CYP3A4, based on drug interaction studies involving single-dose administration of imatinib¹. However, it is unknown if similar drug interactions occur when imatinib concentrations are at steady-state. The purpose of this study was to evaluate the effect of acute administration of ritonavir, a potent inhibitor of CYP3A4, on the steady-state pharmacokinetics of imatinib.

i Novartis Pharma Stein AG, Gleevec (imatinib mesylate): prescribing information [accessed 2007 July 31]. Available from: <http://www.gleevec.com/info/page/prescribing.info>

Methods

Patients. Eligibility for study entry included a histologically or cytologically confirmed diagnosis of gastrointestinal stromal tumor. Patients had to be on single-agent imatinib treatment for at least 2 months and receive a daily dose of at least 400 mg. Patients were ≥ 18 years old, HIV negative, and had a WHO performance status ≤ 2 . Patients were not allowed to have been in surgery within four weeks before entering the study protocol nor experience gastrointestinal toxicity on imatinib treatment during the last 2 weeks before enrollment. Concurrent use of substances known or likely to interfere with the pharmacokinetics of imatinib was not allowed. All patients had adequate clinical functional reserves as defined by absolute neutrophil count $> 1.5 \times 10^9/L$, platelets $> 100 \times 10^9/L$, creatinine clearance > 65 mL/min, and bilirubin $< 1.75 \times$ the upper limit of institutional normal. The study was approved by the institutional ethics committee (Leiden University Medical Center, Leiden, The Netherlands), and all patients gave written informed consent before entering the study.

Study design. The study was designed to evaluate the effect of ritonavir on imatinib pharmacokinetics at steady-state. All patients were treated daily, for at least 2 months, with commercially available imatinib mesylate film-coated tablets (Novartis International AG) at an oral dose ranging between 400 and 800 mg. The study was done over 5 consecutive days. Ritonavir at a dose of 600 mg (6 capsules of 100 mg; Abbott Laboratories) was coadministered on days 2, 3 and 4 of the study, ~ 30 min before the planned administration of imatinib. The selected dose and schedule of ritonavir are associated with significant inhibition of CYP3A4^{16, 17}. On days 2 to 4 during coadministration of ritonavir, the dose of imatinib was reduced by 50% for safety reasons. On the 5th day of the study, patients returned to receiving the imatinib dose they were taking before entering the study.

Pharmacokinetic sampling and analytical assays. Blood samples were collected on the 1st and 4th day of the study for assessment of imatinib and ritonavir pharmacokinetics. Blood was collected into heparin-containing tubes at the following time points: before treatment and after imatinib administration at 1, 2, 3, 4, 5, 6, 8, 10 and 24 hours. Blood samples were centrifuged at $3,000 \times g$ for 10 min and plasma was divided into two tubes, one each for imatinib and ritonavir pharmacokinetics, and stored at -20°C until the day of analysis. Imatinib and CGP74588 concentrations in plasma were measured using a validated method based on reversed-phase high-performance liquid chromatography with UV detection at a wavelength of 270 nm using a Water 2690 Alliance Separation Module and 2487 UV/Vis Dual Wavelength Detector (Waters Corp.). Each analytical run included a calibration curve of imatinib spiked in plasma over the concentration range of 0.2 to 10 $\mu\text{g/mL}$ and quality control samples analyzed in duplicate at three different concentrations of 0.6, 4.0, and 8.0 $\mu\text{g/}$

mL. Analytes were extracted from plasma by protein precipitation with 10% perchloric acid. Separation was achieved on a column (4×125 mm internal diameter) packed with 5- μm particle size Nucleosil C₁₈. The mobile phase consisted of a mixture of acetonitrile-water (20:80, v/v) containing 0.05% trifluoroacetic acid and was delivered isocratically at a flow rate of 1 mL/min. Imatinib eluted at 5.5 ± 0.2 minutes, and CGP74588 eluted at 4.4 ± 0.1 minutes. The metabolite CGP74588 was quantitated indirectly using the imatinib calibration curve at a wavelength of 270 nm. A small amount of CGP74588 was available to confirm the retention time and to ensure the peak areas of imatinib and CGP74588 were similar (within 90-110% of each other) at a concentration of 10 $\mu\text{mol/L}$. Over 4 days of validation, the within- and between-day precision was always $< 10\%$.

Ritonavir plasma concentrations were measured using a validated method based on high-performance liquid chromatography with UV detection as described previously, with minor modifications¹⁸.

Non-compartmental analysis. Individual plasma concentrations of imatinib, CGP74588, and ritonavir were analyzed by noncompartmental methods using WinNonlin version 5.0 (Pharsight, Inc., CA, USA). Pharmacokinetic variables assessed included peak concentration (C_{max}), AUC during the dosing interval (0-24 h), and apparent oral clearance (CL/F), calculated as dose/AUC. To account for the 50% reduction in imatinib dose between days 1 and 4, C_{max} and AUC for imatinib and CGP74588 on both days 1 and 4 were normalized to an imatinib dose of 400 mg. The relative extent of conversion of imatinib to CGP74588 was calculated as the AUC ratio of CGP74588 to imatinib and expressed as a percentage.

Modeling conditions. To more accurately account for variations in drug doses and to apply a more formal method for estimating CL/F values, additional analyses were done with the first-order conditional estimation method in the NONMEM program, version VI (Icon Development Solutions). Exponential variable distributions were used with exploration of off-diagonal elements (covariances). An additive residual error model following log transformation of imatinib concentrations was used. Identification of a structural model was initially done using the imatinib data obtained on day 1. The same model was then applied to all imatinib data (days 1 and 4), and the variables were reestimated. Next, a dichotomous covariate was introduced (RITA), which was given the value zero before the first ritonavir dose and the value one thereafter. The basic model using all data was compared to a model where CL and/or bioavailability (F) were allowed to change with the value of RITA. A change in CL would affect CL/F only, whereas a change in F would affect both CL/F and the apparent volume of distribution (V/F). Based on anticipated changes in imatinib pharmacokinetic variables, the effect of RITA was constrained to potentially decrease CL and/or an increase F. The basic model was then refined by introducing interoccasion variability, where each

dosing interval was treated as a new occasion. This model was further refined by allowing the morning trough samples to have a different (higher) residual variability magnitude compared with other samples. As an alternative to the dichotomous RITA covariate, the predicted ritonavir concentration was evaluated. This was based on individual predictions from a linear one-compartment model with first-order absorption and an absorption lag time (not shown). It was hypothesized that ritonavir potentially could increase F and/or decrease CL, this time via maximum-effect models. Identification of the best structural model and subsequent improvement of the model was based on differences in the objective function value (Δ OFV) from the NONMEM output and on interpretation of diagnostic plots using the Xpose program, version 4 (Uppsala University, Uppsala, Sweden).

In vitro metabolism studies. The *in vitro* metabolism of imatinib and CGP74588 (50 μ mol/L each) was determined using human CYP3A4, CYP3A5, CYP1A2, CYP1A1, CYP2D6, CYP2C9 and CYP2C19 Supersomes (10 – 160 pmol/mL) and pooled human liver microsomes (1.6 mg/mL; Gentest, BD Biosciences). The effect of 30 min coinubation of ritonavir (0.1 – 20 μ mol/L) with imatinib on the formation of CGP74588 from imatinib (50 μ mol/L), as well as the effect of imatinib (1 – 20 μ mol/L) on the formation rate of 1-OH-midazolam from midazolam (10 μ mol/L) was assessed in CYP3A4 Supersomes and pooled human liver microsomes. Reaction mixtures were made in duplicate and consisted of 1.3 mmol/L NADP+, 3.3 mmol/L glucose-6-phosphate, 0.4 units/mL glucose-6-phosphate dehydrogenase, 3.3 mmol/L magnesium chloride, 50 μ mol/L sodium citrate, and 100 mmol/L potassium phosphate buffer in a total volume of 0.2 mL (pH 7.4). Reaction mixtures were incubated for 30 min at 37°C and terminated by adding 100 μ L of acetonitrile and centrifugation at 14,000 rpm at 4°C for 10 min. The supernatant was analyzed for imatinib, CGP74588 and any other unknown metabolites absorbing at 270 nm as described above with minor modifications. Using this modified assay, imatinib and CGP74588 eluted at approximately 12.0 and 9.5 minutes, respectively. Like CGP74588, concentrations of unknown metabolites were quantitated by interpolation on the imatinib calibration curve. Column effluents containing suspected unknown metabolites were subjected to high-performance liquid chromatography analysis with tandem mass spectrometric detection in the scan mode using a Micromass Quattro LC triple-quadrupole mass spectrometer (Waters) to obtain initial structural information. Midazolam and 1-OH-midazolam were analyzed in supernatant using a previously described method based on high-performance liquid chromatography with tandem mass spectrometric detection¹⁹.

Statistical considerations. Data are presented as a geometric mean along with 95% confidence intervals, unless stated otherwise. Statistical analysis was based on a two-tailed paired t-test of logarithmically transformed data, and *P* values of < 0.05 were considered

to be statically significant. Calculations were done using the software package Number Cruncher Statistical Systems, version 2005 (NCSS, J. Hintze).

Results

Patients. Twelve patients with a diagnosis of gastrointestinal stromal tumor were enrolled on the study, and 11 were evaluable for pharmacokinetic analysis (Table 1). One patient did not take the ritonavir dose on days 2, 3 and 4 and was excluded from analysis. No severe or unexpected side effects were observed during the concurrent administration of imatinib and ritonavir for 3 days.

Table 1 Patient characteristics

Characteristic	Value
Number of patients	11
Sex (female / male)	6 / 5
Age, years ^a	62 (51 – 79)
Baseline renal and liver function parameters ^a	
Creatinine, μ M	86 (70 – 95)
Total bilirubin, μ M	7 (6 – 24)
ALT, units/L	20 (9 – 27)
AST, units/L	32 (22 – 45)
Gamma-glutamyltransferase, units/L	16 (9 – 38)
Alkaline phosphatase, units/L	85 (61 – 112)

^aValues are median with range in parenthesis

Imatinib pharmacokinetics. Ritonavir did not significantly alter the steady-state exposure to imatinib with dose-normalized geometric mean AUC values (95% confidence interval) on days 1 and 4 of 42.6 (33.0 – 54.9) μ g·h/mL and 41.2 (32.1 – 53.1) μ g·h/mL, respectively (*P* = 0.65; Table 2; Fig. 1). Imatinib dose-normalized C_{max} and CL/F values were also unchanged after 3 days of ritonavir administration. However, ritonavir administration resulted in a > 40% increase in plasma exposure to CGP74588, with mean values for CGP74588 to imatinib AUC ratio on days 1 and 4 of 16.8% (14.6 – 19.4%) and 24.0% (19.9 – 29.0%), respectively (*P* < 0.0001; Table 2; Fig. 1).

Population analysis. A one-compartment model with linear elimination and first-order absorption adequately described the imatinib concentration-time data, and a two-compart-

ment model did not significantly improve the fit ($\Delta\text{OFV} = 7.3; P > 0.05$). For the one-compartment model, CL/F (percent interindividual variability) was estimated to 9.18 ± 0.95 L/h (33%), the apparent volume of distribution to 225 ± 31 L (38%), and absorption rate constant to 1.64 ± 0.39 h⁻¹ (38%). The estimate of CL/F obtained when applying the same model to all imatinib data was 9.99 ± 1.05 L/h. Introduction of the dichotomous covariate RITA did not offer an improvement of the model compared with the basic model ($\Delta\text{OFV} = 0.0; P > 0.05$). Introducing interoccasion variability into the basic model resulted in a significant decrease in ΔOFV of 121.1 ($P < 0.001$) and provided an estimate for CL/F of 9.40 ± 1.16 L/h. This model was further refined by allowing the morning trough samples to have a higher residual variability magnitude compared to other samples ($\Delta\text{OFV} = 34.7; P < 0.001$), with an error magnitude of 42.3% for morning trough samples and 10.2% for all other samples. To this refined model, the influence of RITA on CL and/or F was again tested, but the improvement in the model fit was negligible ($\Delta\text{OFV} = 0.3; P > 0.05$). Similarly, a model that incorporated a predicted ritonavir concentration offered no improvement in the description of imatinib data ($\Delta\text{OFV} = 0.0; P > 0.05$).

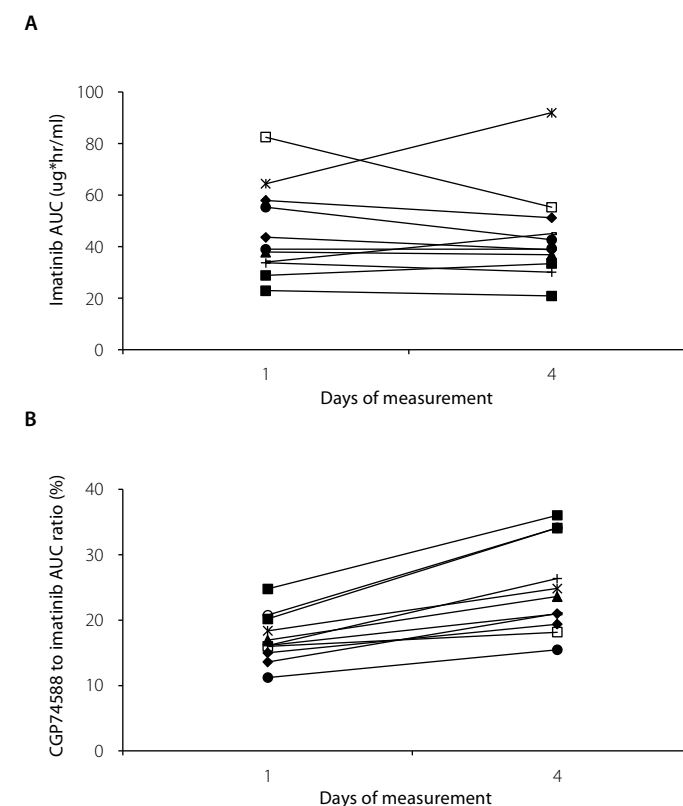
Table 2 Pharmacokinetic parameters obtained with non-compartmental analysis

Parameter ^{a,b}	Day 1 (imatinib alone)	Day 4 (imatinib with ritonavir)	Day 4 / Day 1 ratio	P
Imatinib				
C _{max} , µg/mL	2.88 (2.27 – 3.65)	2.50 (1.93 – 3.24)	0.869 (0.744 – 1.02)	.072
AUC, µg-h/mL	42.6 (33.0 – 54.9)	41.2 (32.1 – 53.1)	0.969 (0.835 – 1.125)	.65
CL/F, L/h	9.40 (7.29 – 12.1)	9.69 (7.53 – 12.5)	1.032 (0.889 – 1.198)	.65
CGP74588				
C _{max} , µg/mL	0.467 (0.356 – 0.612)	0.521 (0.411 – 0.661)	1.050 (0.893 – 1.235)	.52
AUC, µg-h/mL	7.16 (5.74 – 8.93)	9.92 (7.85 – 12.5)	1.385 (1.159 – 1.656)	.0023
CGP74588/imatinib AUC ratio, %	16.8 (14.6 – 19.4)	24.0 (19.9 – 29.0)	1.429 (1.317 – 1.551)	<.0001

^aValues are geometric mean with 95% confidence interval in parenthesis; P-values were obtained from a paired t-test. ^bC_{max} and AUC values are normalized to an imatinib dose of 400 mg.

Abbreviations: C_{max}, peak concentration; AUC, area under the concentration-time curve; CL/F, apparent oral clearance.

Figure 1

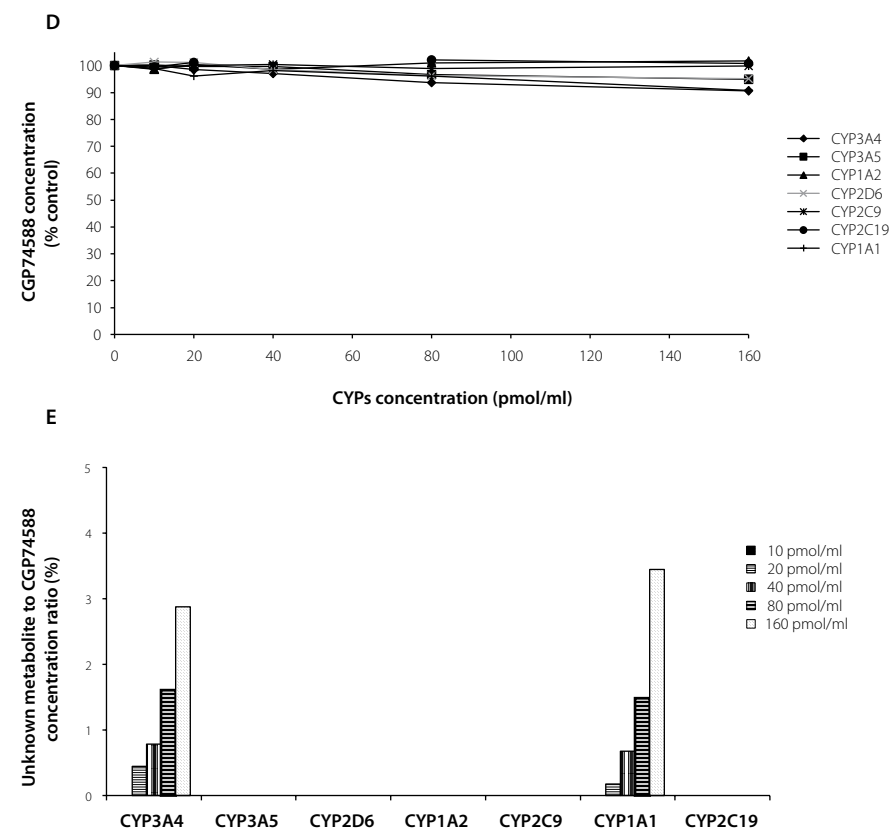
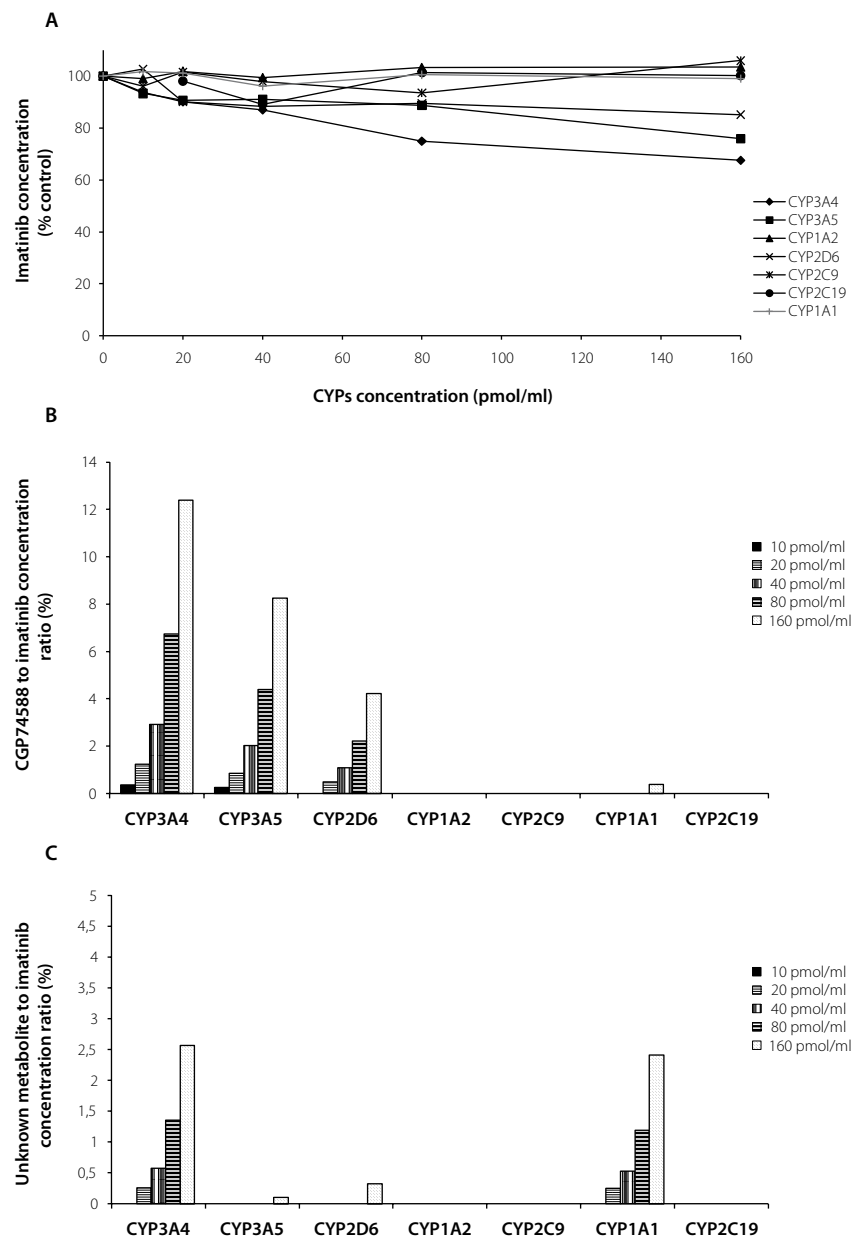


Imatinib dose normalized area under the concentration-time curve (AUC) (A) and CGP74588 to imatinib AUC ratio (B) before (day 1) and after (day 4) ritonavir co-administration in 11 patients.

Ritonavir pharmacokinetics. On day 4, the mean C_{max} and AUC values of ritonavir were 14.7 ± 6.3 µg/mL and 85.3 ± 23 µg-h/mL, respectively, which are similar to those previously published for ritonavir in drug interaction studies to inhibit CYP3A4 when it was administered to enhance the oral absorption of antiretroviral agents^{20,21}.

In vitro metabolism studies. Imatinib was metabolized to CGP74588 by CYP3A4 and CYP3A5, and to a lesser extent by CYP2D6, with CYP1A1 having minor involvement (Fig. 2A and B). Imatinib was also converted to a metabolite with a retention time of 5.5 min by CYP3A4 and CYP1A1, with a minor fraction formed by CYP3A5 and CYP2D6 (Fig. 2C). The UV

Figure 2

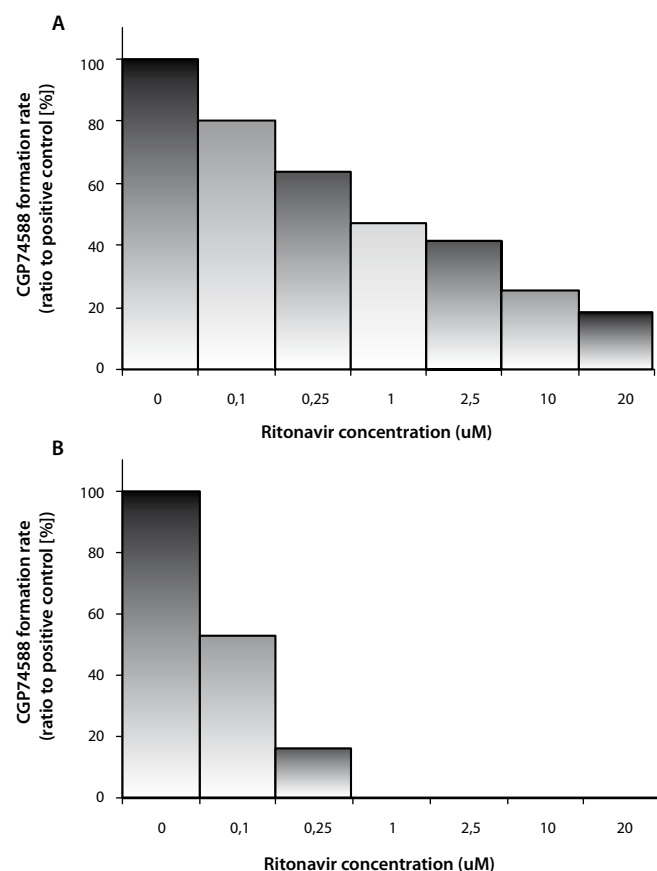


In vitro metabolism of imatinib (A-C) and CGP74588 (D-E) by different cytochrome P450 (CYP) isoforms. Experimental details are provided in the Methods section.

spectrum of this unknown compound indicated the presence of at least two metabolites. Analysis by mass spectrometry suggested that one of the metabolites formed from imatinib is AFN911, whereas the other could be CGP72383 or CGP71422 or a combination of both, as described previously¹⁰. No biotransformation of imatinib was observed by CYP1A2, CYP2C9 and CYP2C19 (Fig. 2A-C). CGP74588 was less sensitive to CYP-mediated metabolism, but an unknown metabolite at a retention time of 4.3 min was observed in the presence of CYP3A4 and CYP1A1 (Fig. 2D). The UV spectrum of this unknown metabolite also indicated the presence of two or more compounds, and mass-spectral analysis suggested that these metabolites are structurally similar to the metabolites formed from imatinib without the N-methyl group.

Ritonavir concentrations of 1 $\mu\text{mol/L}$ and higher completely inhibited the metabolism of imatinib to CGP74588 by CYP3A4 (Fig. 3A). However, in pooled human liver microsomes only 50% inhibition of imatinib metabolism by ritonavir was noted at 1 $\mu\text{mol/L}$, and ~80% inhibition at the highest ritonavir concentration tested (Fig. 3B). This suggests the involvement of other CYPs in imatinib metabolism when CYP3A4 function is inhibited. Finally, imatinib concentrations of 1 $\mu\text{mol/L}$ and higher inhibited the metabolism of midazolam to 1-OH-midazolam both in a CYP3A4-expressed system as well as in human liver microsomes (Table 3).

Figure 3



Influence of ritonavir on the *in vitro* metabolism of imatinib by human liver microsomes (A) and cytochrome P-450 (CYP) 3A4 (B). Experimental details are provided in the Methods section.

Table 3 Effect of imatinib on 1-OH-midazolam formation rate in human liver microsomes and by CYP3A4^a

Imatinib Concentration (μM)	1-OH-midazolam formation rate (% control) ^b
CYP3A4	
1 μM	92.3
5 μM	86.4
20 μM	57.1
Liver microsomes	
1 μM	64.7
5 μM	60.9
20 μM	56.8

^aMidazolam concentration used was 10 μM . Experimental details are provided in the Methods section.

^bThe control is 1-OH-midazolam formation rate in the absence of imatinib.

Discussion

This study shows that acute inhibition of CYP3A4 by the potent enzyme inhibitor ritonavir does not result in a substantial pharmacokinetic interaction with imatinib at steady state. These data not only emphasize the need to consider appropriate trial designs to evaluate the plausibility of pharmacokinetic interactions in the development of anticancer drugs that require daily chronic dosing but also have direct clinical relevance for chemotherapeutic treatment with imatinib.

It was previously established that the most prominent pathway of imatinib elimination consists of CYP3A4-mediated metabolism leading to the formation of CGP74588 and several other metabolites⁸. This suggested that imatinib was potentially subject to a host of enzyme-mediated drug interactions with commonly prescribed medications¹⁴. Indeed, the prototypical CYP3A4 inhibitor ketoconazole has been shown to inhibit the CL/F of imatinib by 40% in healthy volunteers after single-dose imatinib administration⁸. This led to the concern that some degree of interaction is to be expected with simultaneous administration of other potent CYP3A4 inhibitors with imatinib and that concurrent administration should be avoided or that dose adjustments for imatinib should be considered¹.

In consideration of prior knowledge⁸, the current observation that acute inhibition of CYP3A4-mediated metabolism by ritonavir does not lead to substantially altered imatinib steady-state exposure was somewhat unexpected. In particular, ritonavir is generally considered to have similar CYP3A4-inhibitory potency as compared with ketoconazole²², and hence, it is unlikely

that the degree of interaction between imatinib at steady-state and inhibitors of CYP3A4 other than ritonavir would be more substantial than that observed in the current study.

To reconcile the apparent inconsistencies with reported studies on the drug interaction potential of CYP3A4 inhibitors given in combination with imatinib, several additional *in vitro* experiments were done. We found that ritonavir completely inhibited the metabolism of imatinib in CYP3A4 expression system but had only a limited effect on imatinib biotransformation in human liver microsomes. This suggests that the lack of pharmacokinetic interference with ritonavir might be the result of inhibition of only one of multiple enzymes involved in the hepatic metabolism of imatinib, which results in shunting of parent drug to alternative elimination pathways. The present study also showed that imatinib itself is a potent inhibitor of CYP3A4 *in vitro*, and it is plausible that, at steady state, continuous administration of imatinib causes auto-inhibition of the primary metabolic pathway (CYP3A4) and that the presence of another modulator of this route does not result in additional changes in systemic exposure to imatinib.

It should be pointed out that the effect of ritonavir on CYP3A4 activity may be time dependent. Although acute exposure to ritonavir inhibits CYP3A4, extended daily administration of ritonavir may induce CYP3A4. For example, exposure to ritonavir for 7 days or more increased the clearance of the CYP3A4 substrate drugs methadone, alprazolam, mefloquine, dapsone, and cortisol^{20, 23-25}. Several recent trials have evaluated the effects of acute and extended exposure to ritonavir on CYP3A activity in the same individuals. In contrast to previous studies, 200 mg ritonavir given twice daily for three doses (acute exposure) and for 10 days (extended exposure) increased the AUC of triazolam, a CYP3A4 probe drug, by 50-fold and 20-fold, respectively¹⁶. Likewise, acute and extended exposure to 200 mg ritonavir twice daily increased exposure to midazolam, a CYP3A4 substrate probe, by up to 50-fold¹⁷. If induction does occur, it is likely that ritonavir exposure for more than 10 days is required for this phenomenon. Therefore, the present observations may not be extrapolated to the situation where imatinib is coadministered with ritonavir for an extended period of time.

Interestingly, ritonavir may also affect CYP2C9 and CYP2C19 activity²⁰, but this may not be of concern clinically, because CYP2C9 and CYP2C19 seem to play only a minor role in imatinib metabolism (Fig. 2). Compared with imatinib, the *in vitro* experiments indicated that the catalytic activity and the relative affinity of CGP74588 for CYP3A4 were substantially weaker. However, subsequent elimination of CGP74588 seem to be highly dependent on the activity of CYP3A4, and therefore, this metabolite is likely to be more sensitive to an acute interaction with ritonavir. This hypothesis is consistent with the current observation that the systemic exposure to CGP74588 was increased by ritonavir (Table 2).

It is noteworthy that imatinib is also both a substrate and an inhibitor for the ATP-binding cassette transporters ABCB1 (P-glycoprotein) and ABCG2 (breast cancer resistance protein)²⁶⁻³⁰. These transporters influence the oral bioavailability of various substrate drugs by

decreasing the amount of drug absorbed after oral intake due to their localization on the apical surface of intestinal epithelial cells^{31, 32}. Furthermore, these efflux transporters may alter systemic drug elimination, as they are expressed in proximal renal tubular cells and on the biliary surface of hepatocytes³². Indeed, inhibitors of ABCB1 and ABCG2 function, such as elacridar and pantoprazol, have been shown to significantly reduce the systemic clearance of imatinib in mice³³. Because ritonavir is a known inhibitor of both ABCB1 and ABCG2^{34, 35}, the current data suggest that modulation of the activity of these transporters in humans would not result in substantially altered exposure to imatinib under steady-state conditions.

In conclusion, this study suggests that acute inhibition of CYP3A4 by ritonavir does not result in increased steady-state plasma concentrations of imatinib. The current findings suggest that the warning in the prescribing information for imatinib related to the concomitant use of substrates or inhibitors of CYP3A4 should be reconsidered. Furthermore, the design of drug interaction studies with novel agents that require continuous administration should consider additional evaluation at steady-state.

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