Human hepatocyte assessment of imatinib drug–drug interactions – complexities in clinical translation

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Imatinib is a CYP3A4 substrate that has been shown to be a victim of 3A4 drug–drug interactions in clinical trials.
- Antiretroviral therapy may act as a drug–drug interaction perpetrator in HIV patients treated for a malignancy.

WHAT THIS STUDY ADDS

- Antiretroviral agents were able to change imatinib clearance as predicted by hepatocyte studies and *in vitro–in vivo* extrapolations.
- Our results are validated by accurate predictions of the reported ketoconazole and rifampicin interactions in patients.

AIM

Inducers and inhibitors of CYP3A, such as ritonavir and efavirenz, may be used as part of the highly active antiretroviral therapy (HAART) to treat HIV patients. HIV patients with chronic myeloid leukemia or gastrointestinal stromal tumour may need imatinib, a CYP3A4 substrate with known exposure response–relationships. Administration of imatinib to patients on ritonavir or efavirenz may result in altered imatinib exposure leading to increased toxicity or failure of therapy, respectively. We used primary human hepatocyte cultures to evaluate the magnitude of interaction between imatinib and ritonavir/efavirenz.

METHODS

Hepatocytes were pre-treated with vehicle, ritonavir, ketoconazole, efavirenz or rifampicin, and the metabolism of imatinib was characterized over time. Concentrations of imatinib and metabolite were quantitated in combined lysate and medium, using LC-MS.

RESULTS

The predicted changes in imatinib CL_{oral} (95% CI) with ketoconazole, ritonavir, rifampicin and efavirenz were 4.0-fold (0, 9.2) lower, 2.8-fold (0.04, 5.5) lower, 2.9-fold (2.2, 3.5) higher and 2.0-fold (0.42, 3.5) higher, respectively. These predictions were in good agreement with clinical single dose drug–drug interaction studies, but not with reports of imatinib interactions at steady-state. Alterations in metabolism were similar after acute or chronic imatinib exposure.

CONCLUSIONS

In vitro human hepatocytes predicted increased clearance of imatinib with inducers and decreased clearance with inhibitors of CYP enzymes. The impact of HAART on imatinib may depend on whether it is being initiated or has already been dosed chronically in patients. Therapeutic drug monitoring may have a role in optimizing imatinib therapy in this patient population.

Introduction

Highly active antiretroviral therapy (HAART) has significantly reduced the rates of AIDS defining malignancies (Kaposi's sarcoma, non-Hodgkin lymphoma and cervical cancer) [1]. As HIV patients live longer with newer and effective antiretroviral therapy, they increasingly experience non-AIDS defining malignancies. Antineoplastic therapy and HAART therapy will need to be applied concomitantly to achieve the simultaneous therapeutic goals of antiretroviral and anticancer activity [2, 3].

Imatinib mesylate (Gleevec®, Glivec®) is widely used to treat chronic myeloid leukemia (CML) based on its inhibition of Bcr-Abl and to treat gastrointestinal stromal tumours (GIST) based on its inhibition of c-KIT [4–6]. The oral bioavailability of imatinib is reported to vary between 70% and 100% and was recently reported to be dependent on the length of treatment [7, 8]. The metabolism of imatinib is mainly mediated by CYP3A4 and, to a lesser extent, by CYP2C8 during short term treatment. Ketoconazole (archetype inhibitor of CYP3A) and rifampicin (archetype inducer of CYP3A) have been documented to increase and decrease, respectively, the systemic exposure of imatinib in single dose drug interaction studies [9, 10]. Upon chronic treatment, CYP2C8 has been reported to play a major role in the metabolism of imatinib due to mechanism based auto-inhibition of CYP3A4 by imatinib [11–14].

Many HAART drugs inhibit and/or induce cytochrome P450, uridine diphosphate glucuronyl transferases (UGTs), ATP binding cassette (ABC) efflux transporters or solute carrier uptake transporters [15, 16]. Ritonavir, a HIV protease inhibitor, is the most potent inhibitor of CYP3A [17], while efavirenz, a non-nucleoside reverse transcriptase inhibitor (NNRTI), is a mixed inducer and inhibitor of CYP3A [18].

Although prospective, randomized confirmatory studies are lacking, there is ample evidence that imatinib trough concentrations are associated with toxicities on the one hand [19], and with clinical benefit in patients with CML and advanced GIST on the other hand [19–22] suggesting that achieving defined exposures is important.

In this study, we aimed to assess the effect of ketoconazole, ritonavir, rifampicin and efavirenz on the metabolism of imatinib in primary cultures of human hepatocytes. The predicted change in oral clearance of imatinib in the presence of ritonavir/efavirenz was also determined using *in vitro–in vivo* scale up. Since chronic treatment with imatinib results in auto-inhibition and metabolic switch from CYP3A4 to CYP2C8, we also sought to assess the utility of hepatocytes to assess whether ritonavir would alter the imatinib metabolism after chronic exposure in human hepatocytes.

Methods

Materials

Imatinib, N-desmethyl imatinib (CGP74588) and [D_8]-imatinib were obtained from Novartis Pharmaceuticals Co. (East Hanover, NJ, USA). [D_8]-N-desmethyl imatinib and ritonavir were procured from Toronto Research Chemicals (Ontario, Canada). Ketoconazole was obtained from Janssen Research Foundation (Titusville, NJ, USA). Hepatocyte maintenance medium was procured from Lonza Inc (Walkersville, MD, USA). Organic solvents used for the extractions and the analytical assays were obtained from Fisher Scientific (Fairlawn, NJ, USA). All other chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA).

Primary human hepatocytes cultures

Human liver samples ($n = 8$) were obtained from Life Technologies (CA, USA) or the Hepatocytes Transplantation Laboratory, University of Pittsburgh or Xenotech, LLC (KS, USA) and processed for hepatocytes as described before [23]. These serum free cultures of human hepatocytes are suitable for drug–drug interaction studies as reported before [24]. All the drug incubations were carried out 48 h after seeding. The CYP induction potential of the hepatocytes used in all the studies was also evaluated at the end of the study by measuring the activity of CYP3A (formation of testosterone 6β hydroxylation) in separate sets of untreated and rifampicin (10 μM) treated hepatocytes.

Time course of imatinib metabolism

The time course of imatinib metabolism was assessed in the absence and presence of ritonavir or ketoconazole or efavirenz or rifampicin. Hepatocytes were incubated daily with ritonavir (10 μM), ketoconazole positive control (10 μM), efavirenz (10 μM), rifampicin positive control (10 μM) or vehicle control (0.1% dimethyl sulfoxide (DMSO)) for 4 days. On day 5, imatinib (2.5 μM) was incubated along with the above agents for an additional 24 or 48 h. Hepatocyte samples were collected at 0, 4, 8, 24 and 48 h for inhibition studies. The duration of incubation for induction studies was reduced to 24 h and samples were obtained at 0, 2, 4, 8 and 24 h. Drug concentrations were selected based on the steady-state maximum plasma concentration at clinically recommended doses [25]. At the end of the study, hepatocytes were scraped from the plates along with culture medium supernatant on ice and the cells were lyzed by sonication. Samples were stored at –80°C until analysis. Additionally, the cytotoxicity assay (MTT assay) was performed to ensure that the selected drug concentrations did not affect the viability of human hepatocytes (data not shown).

Effect of chronic imatinib exposure on metabolism

In an effort to differentiate metabolism of imatinib after chronic exposure relative to acute exposure, 24 h incubations were performed in the absence and presence of ritonavir. To model acute exposure, hepatocytes were incubated daily with ritonavir (10 μM) or vehicle control (0.1% DMSO) for 4 days as described above. On day 5, imatinib was incubated along with the above agents for an additional 24 h. To model chronic exposure, hepatocytes were incubated daily with imatinib and ritonavir (10 μM) or imatinib for 4 days. On day 5, imatinib was incubated along with the above agents for an additional 24 h. These incubations were performed at 2.5 and 10 μM imatinib. Hepatocyte samples were collected on day 5 at 0 and 24 h as a point estimate of alterations of metabolism.

LC-MS quantitation of imatinib and desmethyl imatinib

Imatinib and active desmethyl-metabolite were quantitated with an Agilent 1100 Autosampler and Binary pump (Agilent, Delaware, USA) hyphenated to a Thermo Electron MSQ detector (Thermo Fisher Scientific, MA, USA). Analytes were chromatographically separated on a Phenomenex Luna C18(2) column (5 μm, 2 × 150 mm) kept at ambient temperature. The gradient mobile phase system was comprised of solvent A (methanol 0.1% formic acid) and solvent B (water 0.1% formic acid). The initial mobile phase was 30% A and 70% B at a flow rate of 0.2 ml min^{-1} held for 4 min. Subsequently, solvent A was increased to 60% over 6 min, where it was held until 13 min. Next, A was increased to 80% over 0.5 min with a flow rate of 0.3 ml min^{-1} and held until 16 min. Finally, A was lowered back to initial conditions over 0.5 min, where it was held until 24 min, followed by injection of the next sample. Mass spectrometer settings were probe voltage 3 kV, cone voltage 40 V and probe temperature 500°C. The SIM monitored were m/z 493.9, 501.9, 479.9 and 487.9 for imatinib, [D$_8$]-imatinib, desmethyl imatinib, and [D$_8$]-desmethyl imatinib, respectively. Aliquots of 200 μl of hepatocyte medium were extracted with 1000 μl acetonitrile and 10 μl of internal standard mix (2 μg ml^{-1} of [D$_8$]-imatinib (internal standard for imatinib) and [D$_8$]-desmethyl imatinib (internal standard for desmethyl imatinib) in methanol : water (50 : 50; v/v)). After vortexing and centrifugation, the supernatant was transferred to a glass tube and then evaporated to dryness under nitrogen. Subsequently, the dried residue was reconstituted with 100 μl of methanol : H$_2$O 30 : 70, (v/v) mobile phase and then 2 μl of the sample was injected into the LC-MS system. The ion chromatograms were integrated and quantified using Thermo Electron Excalibur 1.4 (Thermo Fisher Scientific, MA, USA). The assay was linear between 10 and 1000 ng ml^{-1} with acceptable QC accuracy (97.5–106%

and 98.8–106%) and precision (<7.9 CV% and <7.6 CV%) for imatinib and desmethyl imatinib, respectively, as determined from independent QC samples ($n = 6$). Samples with concentrations above the upper limit of the calibration range were diluted to within the calibration range with control media.

Data analysis

Each study was performed in at least three different cultures of human hepatocytes in duplicate. The half-life ($t_{1/2}$) and area under the concentration–time curve from time 0 to 48 h (AUC(0,48 h) for imatinib in primary cultures of human hepatocytes were calculated non-compartmentally with Phoenix WinNonlin (Pharsight Corp, Cary, North Carolina, USA).

In the acute and chronic exposure experiment, each study was performed once in duplicate.

Calculation of apparent intrinsic clearance

The apparent intrinsic clearance (CL$_{int, app}$) of imatinib was calculated from the half-life of imatinib in the absence and presence of ritonavir, ketoconazole, efavirenz or rifampicin in primary cultures of human hepatocytes using equation 1 [26],

$$CL_{int,\ app}\left(ml\ min^{-1}kg^{-1}\right) = \frac{0.693}{t_{1/2}} \times \frac{ml\ incubation}{number\ of\ cells}$$
$$\times \frac{130 \times 10^6 cells}{g\ liver}$$
$$\times \frac{20\ g\ liver}{kg\ per\ body\ weight} \quad (1)$$

where incubation volume is 1 ml and the number of cells is 1.5×10^6.

Prediction of hepatic clearance

The hepatic clearance (CL$_h$) of imatinib was predicted from CL$_{int, app}$ using the well-stirred liver model as follows [27–29],

$$CL_h = Q_h(1 - F_h) \quad (2)$$

Hepatic blood flow (Q_h) = 21 ml min^{-1} kg^{-1} and hepatic availability (F_h), which was calculated using equations 3 and 4,

$$F_h = \frac{Q_h}{\left(Q_h + f_{u,inc} \times CL_{int,\ app}\right)} \quad (3)$$

$f_{u,inc}$ = fraction unbound in incubation medium

$$= \frac{1}{1 + K_p(V_c/V_m)} \quad (4)$$

where K_p = partition ratio = 4.38 [30], V_c = adherent cell volume = 0.0051 ml [31] and V_m = volume of culture medium = 1 ml. Equation 3 was chosen [27–29] as in

our experience it was found to predict best for low extraction ratio drugs.

Prediction of oral clearance

Imatinib has been reported to be well-absorbed from the gastro-intestinal tract [8]. Consequently, the intestinal clearance is assumed to be negligible for imatinib, particularly in the presence of ritonavir or ketoconazole. Therefore, the fraction absorbed (f_a) and fraction of dose escaping gut metabolism (F_g) are assumed to be 1 for the prediction of oral clearance using equation 5 [32].

$$CL_{oral} = \frac{CL_h}{f_a \times F_g \times F_h} \quad (5)$$

The bias of CL_{oral} prediction was estimated from the geometric mean ratio of the predicted and observed values, and calculated as average fold-error (AFE) from equation 6 whereas the precision of the prediction was assessed from root mean squared prediction error (RMSE) using equations 7 and 8, respectively [33].

$$AFE = 10^{\left| \frac{1}{N} \sum \log \frac{Predicted}{Observed} \right|} \quad (6)$$

$$MSE = \frac{1}{N} \sum (Predicted - Observed)^2 \quad (7)$$

$$RMSE = \sqrt{MSE} \quad (8)$$

Statistical analysis

All the results are expressed as mean ± 95% CI. The statistical differences among the different treatment groups were analyzed by non-parametric Friedman analysis of variance (ANOVA) followed by Dunn's multiple comparison *post-hoc* test. $P < 0.05$ was considered as statistically significant. The data from the chronic exposure experiment were handled descriptively.

Results

The time course of metabolism of imatinib in the absence and in the presence of ritonavir or efavirenz was assessed in primary cultures of human hepatocytes and compared with that of the corresponding positive controls, ketoconazole and rifampicin. The demographics of human liver donors are shown in Table 1. The CYP3A4/5 activities in control hepatocytes ranged from 51 to 265 pmol min^{-1} per million cells (based on formation of 6-β-hydroxy testosterone from testosterone) at the end of the study period. All the batches of human hepatocytes used in this study retained CYP induction potential as confirmed by

Table 1

Demographic profiles of human liver donors

Liver ID	Age (years)	Gender	Race	Diagnosis
Hu 1595	31	Female	Caucasian	Liver mass – Focal nodular hyperplasia
Hu 1600	40	Male	Caucasian	Metastatic rectal adenocarcinoma
H 1165	13	Male	Caucasian	Anoxia
H 1174	21	Female	Caucasian	Cardiovascular arrest
HH 2018	68	Male	Caucasian	Cholangiocarcinoma
HH 2019	68	Male	Caucasian	Metastatic colon cancer
Hu 1488	31	Male	Caucasian	Colorectal cancer
HH 2020*	62	Female	Caucasian	Metastatic colon cancer

*Used in chronic exposure experiment.

the greater than 2.5-fold increase in CYP3A activity upon treatment with rifampicin.

The average concentration–time profile for imatinib and the formation of desmethyl imatinib in primary cultures of human hepatocytes treated with vehicle, ritonavir and positive control (ketoconazole) are shown in Figure 1A and B. The individual and mean values of AUC(0,48 h) for the time course of metabolism of imatinib and the formation of desmethyl imatinib in the absence and presence of ritonavir or ketoconazole in human hepatocytes are shown in Table 2. Treatment with ritonavir resulted in a 1.6-fold increase in the exposure (AUC(0,48 h)) of imatinib with a 4.4-fold reduction in the exposure to desmethyl imatinib as compared with vehicle treatment. Ketoconazole treatment increased the exposure of imatinib 1.8-fold and reduced the exposure of desmethyl imatinib 3.2-fold. Treatment with ritonavir significantly increased the half-life of imatinib 2.8-fold. Additionally, metabolite to parent imatinib (M : P) AUC (0,48 h) ratio was decreased 7.4-fold, respectively, by ritonavir. Ketoconazole treatment resulted in a 4.0-fold increase in half-life and 6.3-fold reduction in M : P AUC (0,48 h) ratio as compared with vehicle treatment.

The average concentration–time profile for imatinib and the formation of desmethyl imatinib in primary cultures of human hepatocytes treated with vehicle control (DMSO), efavirenz and rifampicin (inducer positive control) are shown in Figure 1C and D. The individual and mean values of AUC(0,24 h) for the time course of metabolism of imatinib and the formation of desmethyl imatinib in the presence and absence of efavirenz or rifampicin are presented in Table 3. Efavirenz treatment decreased the exposure (AUC(0,24 h)) of imatinib 2.3-fold and increased the exposure of desmethyl imatinib by 3.1-fold as compared with treatment with vehicle. Treatment with rifampicin resulted in a 3.0-fold decrease in the imatinib exposure and a 2.1-fold increase in the exposure of desmethyl imatinib. Efavirenz treatment resulted in an
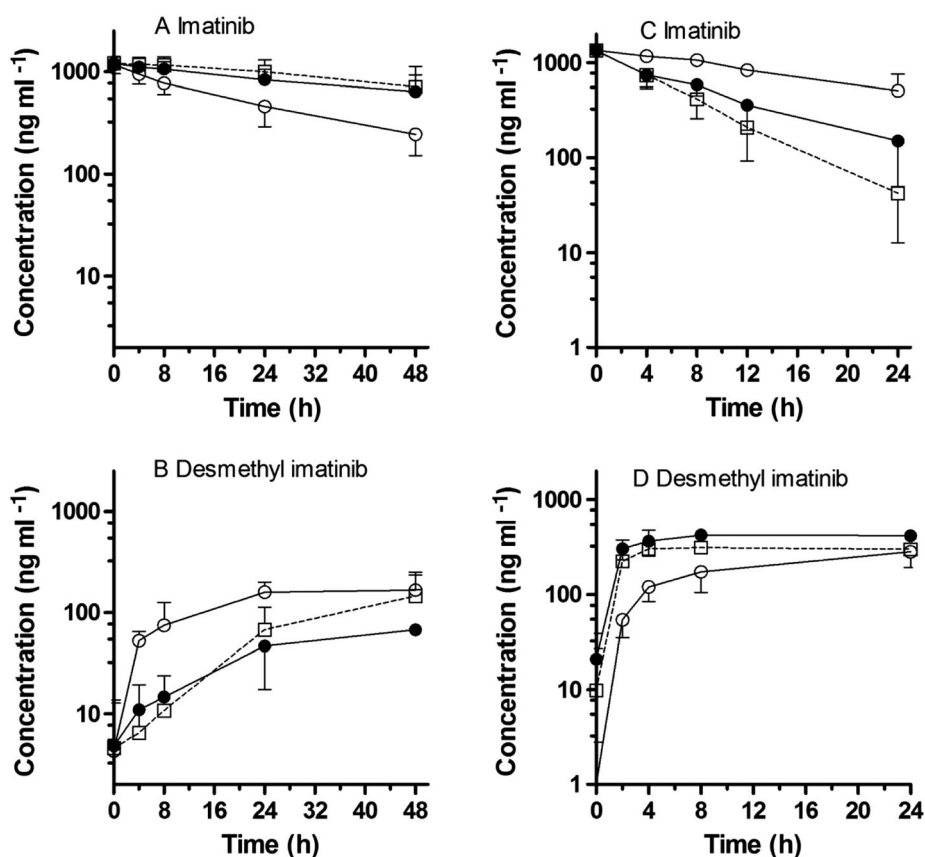
Figure 1

Time course of imatinib depletion and formation of desmethyl imatinib in primary cultures of human hepatocytes treated with either DMSO (○), ritonavir 10 μM (●) and ketoconazole 10 μM (□) [A, B] or DMSO (○), efavirenz 10 μM (●) and rifampicin 10 μM (□) [C, D]. Data are representative of three individual experiments each performed in duplicate. Error bars represent standard deviation

almost 2-fold decrease in half-life of imatinib as compared with vehicle control. The M:P AUC(0,24 h) ratio was increased 7.8-fold, by efavirenz. Rifampicin treatment resulted in a 2.9-fold reduction in the half-life, and a 6.1-fold increase in M:P AUC(0,24 h) ratio, compared with vehicle control.

The individual and mean apparent intrinsic clearance ($CL_{int, app}$) values of imatinib in primary cultures of human hepatocytes treated with either vehicle (DMSO), ritonavir or ketoconazole are shown in Table 2 and these values upon treatment with vehicle, efavirenz or rifampicin are presented in Table 3. The $CL_{int, app}$ of imatinib was decreased 2.8-fold and 4.0-fold (range 1.3–8.0 fold) respectively, by ritonavir and ketoconazole. In case of efavirenz or rifampicin treatment, the $CL_{int, app}$ of imatinib was increased 2.0-fold and 2.9-fold, respectively. The imatinib human CL_{hep} values were predicted from $CL_{int, app}$ using the well-stirred liver model and are presented in Figure 2. Under the given experimental conditions and assumptions for the predictions, the values of both CL_{hep} and CL_{oral} (both expressed as ml min^{-1} kg^{-1}) are similar and, therefore, they are presented in the same figure (Figure 2). The parameters used for the prediction of hepatic clearance and the comparisons of observed

and predicted CL_{oral} values for imatinib with prediction errors are shown in Table 4.

Acute exposure to imatinib over 24 h resulted in imatinib depletion and desmethyl imatinib generation (Figure 3), as expected. Addition of ritonavir reduced imatinib depletion and abolished desmethyl imatinib generation. After chronic (4 day) exposure, hepatocytes had accumulated imatinib and desmethyl imatinib to some degree. Imatinib depletion and desmethyl imatinib generation patterns were similar after acute and chronic imatinib exposure, respectively.

Discussion

In vitro–in vivo extrapolation (IVIVE) is useful to predict human pharmacokinetic and potential drug interactions [34]. The average predicted human CL_{oral} of imatinib from vehicle treated human hepatocytes cultures was approximately 2.6-fold lower than that of the clinically observed average CL_{oral} of imatinib, which was comparable with reported predictions [28, 32].

Imatinib exhibits wide inter-patient variability in exposure, which is associated with toxicity and clinical

Table 2

Parameters of imatinib metabolism and desmethyl imatinib metabolite formation in primary cultures of human hepatocytes treated with vehicle (DMSO), ritonavir or ketoconazole and incubated with imatinib (2.5 μM).

Treatment liver ID	Imatinib AUC(0,48 h) ($\mu g\ ml^{-1}\ h$)	Metabolite AUC(0,48 h) ($\mu g\ ml^{-1}\ h$)	AUC(0,48 h) M : P ratio	Imatinib % metabolized at 48 h	Imatinib $t_{1/2}$ (h)	Imatinib $CL_{int,\ app}$ ($ml\ min^{-1}\ kg^{-1}$)	Imatinib $CL_{oral,pred}$ (mL/min/kg)
DMSO							
Hu 1595	16.7	8.4	0.51	86	17	1.17	1.15
Hu 1600	25.3	5.8	0.23	73	26	0.78	0.76
H 1165	33.9	4.4	0.13	77	24	0.84	0.82
H 1174	28.0	5.9	0.21	82	19	1.03	1.00
Mean (95% CI)	26.0 (14.6, 37.4)	6.1 (3.5, 8.8)	0.27 (0, 0.53)	80 (71, 89)	22 (15, 28)	0.96 (0.67, 1.24)	0.93 (0.65-1.21)
Ritonavir							
Hu 1595	26.2	4.3	0.16	77	23	0.88	0.86
Hu 1600	43.4	0.94	0.022	40	64	0.32	0.31
H 1165	46.6	1.7	0.036	51	49	0.41	0.40
H 1174	51.1	0.90	0.018	29	104	0.19	0.19
Mean (95% CI)	41.8 (24.5, 59.1)	2.0 (0, 4.5)	0.059 (0, 0.17)	49 (17, 82)	60 (6, 114)	0.45 (0, 0.93)	0.44 (0-0.96
Ratio to DMSO	1.62 (1.31, 1.93)	0.30 (0.02, 0.58)	0.19 (0.003, 0.38)*	0.62 (0.26, 0.98)	2.8 (0, 5.6)*	0.46 (0.09, 0.83)*	0.46 (0.09-0.83)*
Ketoconazole							
Hu 1595	26.7	5.3	0.20	77	23	0.89	0.87
Hu 1600	58.2	1.7	0.028	10	210	0.10	0.093
H 1165	48.5	5.1	0.11	61	32	0.62	0.61
H 1174	55.1	0.88	0.016	25	100	0.20	0.20
Mean (95% CI)	47.1 (24.5, 69.7)	3.2 (0, 6.9)	0.088 (−0, 0.22)	43 (0, 9.3)	91 (0, 229)	0.45 (0, 1.0)	0.44 (0-1.0)
Ratio to DMSO	1.82 (1.21, 2.44)*	0.56 (0, 1.3)	0.36 (0, 0.92)	0.53 (0, 1.1)	4.0 (0, 11)	0.46 (0, 1.0)	0.46 (0-1.0)**

M : P ratio, the ratio of desmethyl imatinib to imatinib. *$P < 0.05$ vs. DMSO, non-parametric Friedman ANOVA followed by Dunn's multiple comparison test. **The individual ratios to DMSO were 0.76, 0.12, 0.74 and 0.20 for a mean of 0.46. This corresponds to a fold decrease in clearance (inverse of the ratio) of 1.3, 8.2, 1.3 and 5.0 for a mean of 4.0.

benefit in patients with CML and advanced GIST [20–22, 35–37]. It is therefore essential to identify the sources of variability and optimize the likelihood that patients achieve exposures associated with response, e.g. through therapeutic drug monitoring [19, 38]. Imatinib is mainly metabolized by CYP3A4 and CYP2C8 and is expected to have major interactions with enzyme inducers and inhibitors. Many HAART drugs modulate metabolizing enzymes and transporters, resulting in complex interactions. Previously, ketoconazole and rifampicin were reported to change imatinib clearance significantly in patients [9, 10]. The present study evaluated the interaction of ketoconazole, rifampicin, and the HAART drugs, ritonavir and efavirenz, on imatinib using primary cultures of human hepatocytes.

Our data suggest that ketoconazole would decrease single dose imatinib clearance 1.3 to 8.0-fold, which may be explained by inhibition of both CYP3A and CYP2C8 [39, 40]. Ketoconazole showed variable extent of inhibition with two of the experiments showing 1.3-fold inhibition, and two showing 5.2- and 8.2-fold inhibition. This likely reflects the biological variability for the susceptibility to azole drug–drug interactions, by virtue of CYP3A5 expressers (CYP3A5*1 carriers) being less susceptible to e.g. fluconazole mediated inhibition [41]. The predicted

ketoconazole effect is in agreement with clinical observations of approximately 1.4-fold decreased imatinib clearance [9]. Ritonavir moderately inhibited imatinib metabolism and strongly reduced desmethyl imatinib exposure, in line with the inhibitory effects of ritonavir on CYP3A (strong) and 2C8 (moderate) [11–13, 42–44]. Of note, ritonavir inhibits CYP3A and is also reported to induce CYP3A. Results from our laboratory and other studies with ritonavir in vivo suggest that ritonavir mediated CYP3A inhibitory effects prevail over induction effects [12, 45]. The change in imatinib exposure may not be mirrored by an inverse similar sized effect on metabolite exposure because not only the formation, but also the clearance, of the metabolite is CYP catalyzed and would be modulated by ritonavir. Our data suggest ritonavir decreases imatinib clearance 2.8-fold. Though imatinib is a P-gp substrate, we do not think the inhibitory effect of ritonavir on P-gp would increase imatinib bioavailability because imatinib is nearly completely bioavailable [8].

Rifampicin, which induces CYP3A and CYP2C8, decreased imatinib exposure 3.0-fold and more than doubled desmethyl imatinib exposure [46]. This agrees with clinical observations of a 3.9-fold increased imatinib clearance [10]. Efavirenz is a mixed type inducer/inhibitor

Table 3

Parameters of imatinib metabolism and desmethyl imatinib metabolite formation in primary cultures of human hepatocytes treated with vehicle (DMSO), efavirenz or rifampicin and incubated with imatinib (2.5 μM)

| Treatment liver ID	Imatinib AUC(0,24 h) (μg ml^{-1} h)	Metabolite AUC(0,24 h) (μg ml^{-1} h)	AUC(0,24 h) M : P ratio	Imatinib % metabolized at 24 h	Imatinib $t_{1/2}$ (h)	Imatinib CL$_{int, app}$ (ml min^{-1} kg^{-1})	Imatinib CL$_{oral,pred}$ (ml min^{-1} kg^{-1})
DMSO							
HH 2018	17.6	5.3	0.30	64	17	1.22	1.19
HH 2019	23.5	2.7	0.11	43	16	1.24	1.21
Hu 1488	16.9	2.7	0.16	79	11	1.84	1.80
Mean (95% CI)	19.3 (13.6, 25.1)	3.6 (1.2, 6.0)	0.19 (0.0, 0.35)	62 (33, 91)	15 (10, 20)	1.43 (0.87, 1.99)	1.40 (0.85, 1.95)
Efavirenz							
HH 2018	10.7	9.3	0.87	82	11	1.86	1.82
HH 2019	6.05	10.4	1.7	97	5.3	3.77	3.69
Hu 1488	11.9	10.2	0.86	88	8.6	2.34	2.29
Mean (95% CI)	9.55 (4.6, 14.5)	10.0 (9.1, 10.9)	1.1 (0.38, 1.9)	89 (77, 101)	8.2 (3.8, 12.6)	2.66 (1.08, 4.24)	2.60 (1.05, 4.14)
Ratio to DMSO	0.52 (0.15, 0.90)	3.1 (1.2, 5.0)*	7.9 (0, 18)	1.6 (0.57, 2.5)	0.59 (0.21, 0.97)	1.95 (0.42, 3.47)	1.95 (0.42, 3.48)
Rifampicin							
HH 2018	5.2	6.8	1.30	98	5.0	4.04	3.95
HH 2019	8.9	6.7	0.75	94	6.0	3.32	3.25
Hu 1488	5.5	6.6	1.19	99	4.2	4.79	4.69
Mean (95% CI)	6.5 (3.3, 9.8)	6.7 (6.5, 6.9)	1.1 (0.62, 1.5)	97 (93, 101)	5.1 (3.6, 6.5)	4.05 (2.88, 5.22)	3.96 (2.82, 5.11)
Ratio to DMSO	0.33 (0.27, 0.40)	2.1 (1.0, 3.2)	6.2 (3.6, 8.8)	1.7 (0.89, 2.4)	0.35 (0.28, 0.42)	2.86 (2.24, 3.48)	2.87 (2.25, 3.49)

M : P ratio, the ratio of desmethyl imatinib to imatinib. *$P < 0.05$ vs. DMSO, non-parametric Friedman ANOVA followed by Dunn's multiple comparison test.

of CYP3A and has potent inhibitory effects on CYP2C8 and CYP2B6 [47, 48]. In human hepatocytes, efavirenz decreased imatinib exposure 2.3-fold and more than tripled desmethyl imatinib, indicating that efavirenz mediated CYP3A induction prevails over its CYP3A inhibition [18]. This resulted in a predicted 2.0-fold increase in imatinib clearance.

The interactions predicted and interactions observed in healthy volunteer studies after single imatinib doses were in agreement for ketoconazole and rifampicin [9, 10], which supports the predictions for ritonavir and efavirenz. Interestingly, there are clinical data available on the combination of these agents with imatinib in patients. Ritonavir was added to chronic imatinib therapy in 11 cancer patients and did not change imatinib AUC (subjects serving as their own control) [14]. Studies in expressed enzymes and human liver microsomes (HLM) suggested that ritonavir completely inhibited CYP3A4 catalyzed imatinib metabolism, but only partly (50–80%) inhibited imatinib metabolism in HLM, implicating an important role for other CYP enzyme(s) in the metabolic fate of imatinib [14]. Although this diminishes the role for CYP3A4 in imatinib metabolism, the complete absence of a clinical interaction is still surprising and unexpected. Administration of imatinib to HIV patients on chronic ritonavir or efavirenz resulted in interaction trends, but sample numbers were too small to be conclusive [49].

There have been recent reports on a metabolic shift of imatinib over time, possibly contributing to the differences in the results of single dose and chronic dosing of imatinib. Imatinib metabolism is predicted to be mainly mediated by CYP3A4 (60%) and to a lesser extent by CYP2C8 (40%) during short term treatment. However, during long term treatment, the contribution of CYP2C8 (65–75%) is larger than that of CYP3A4 (25-35%) due to mechanism based auto-inhibition of CYP3A4 by imatinib [12, 13]. Simulations have shown that this change in the primary enzyme involved causes the 40% increase of single dose imatinib AUC in presence of itraconazole that decreases to about 20% increase in imatinib AUC after multiple imatinib doses [13]. Although the authors considered this result to be in line with the absence of a ritonavir interaction on chronic imatinib as reported by van Erp et al. [14], our data point to the stronger inhibition of imatinib metabolism by ritonavir relative to ketoconazole and would suggest that an interaction might still have been seen. Many of the studies of the imatinib metabolic switch have been performed in HLM, which severely limits the experimental duration. In addition, solvent concentrations of up to 1% were used in incubations, which are known to affect metabolism [50, 51]. It has been reported that it may take several days of in vivo treatment to reach the maximal inhibitory effect of a mechanism-based inhibitor [52–54]. Therefore, we performed an additional experiment with 4 days of

A

B
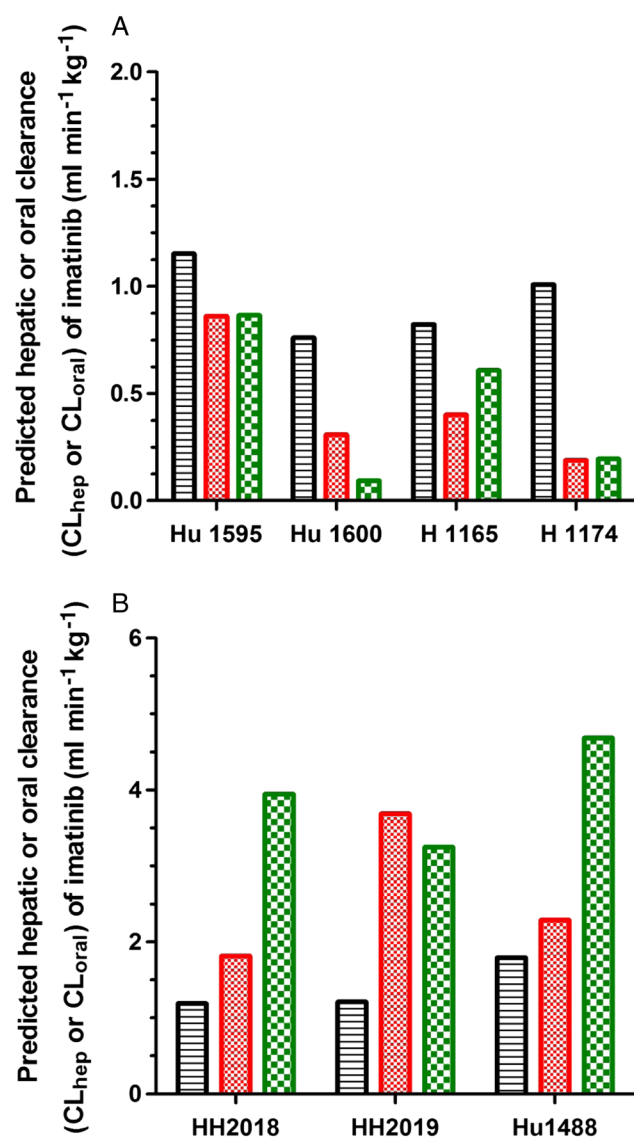
Figure 2

Predicted human hepatic or oral clearance (CL_{hep} or CL_{oral}) of imatinib from primary cultures of human hepatocytes treated with either DMSO (▤), ritonavir 10 μM (▨) and ketoconazole (▨) 10 μM (A) or DMSO (▤), efavirenz 10 μM (▨) and rifampicin 10 μM (▨) (B)

Table 4

The parameters used for the prediction of hepatic clearance, the comparisons of observed and predicted CL_{oral} values for imatinib with prediction errors, and predictions of change in CL_{oral} and literature values

| Parameters	Values	Reference	Comment
K_p	4.38	[30]	
$f_{u, inc}$	0.98		
Imatinib			
Mean observed CL_{oral} (l h^{-1})	12.3		
Mean predicted CL_{oral} (l h^{-1})	4.8		
Bias of prediction (AFE)	0.434		
Precision of prediction (RMSE)	7.7		
Ketoconazole			
Predicted change in CL_{oral}*	1.3–8.0-fold reduction		
Reported change in CL_{oral}*	1.4-fold reduction	[9]	Single imatinib dose
Ritonavir			
Predicted change in CL_{oral}*	2.8-fold reduction		
Reported change in CL_{oral}*	No effect	[14]	Ritonavir added to SS-imatinib
Rifampicin			
Predicted change in CL_{oral}*	2.9-fold increase		
Reported change in CL_{oral}*	3.9-fold increase	[10]	Single imatinib dose
Efavirenz			
Predicted change in CL_{oral}*	2.0-fold increase		
Reported change in CL_{oral}*	1.5-fold increase	[49]	Single imatinib dose added to SS-efavirenz
Reported change in CL_{oral}*	1.2-fold increase	[49]	SS imatinib added to SS-efavirenz

K_p = Partition ratio; $f_{u, inc}$ = fraction unbound in hepatocytes; CL_{oral} = Oral clearance; AFE = average fold error; RMSE = root mean square error; SS = steady-state; Observed CL_{oral} values (13.3, 8.1, 10, 14.3, 19.2, 9.2, 12 l h^{-1}) were obtained from the literature ($n = 7$) [25, 57–62]; Predicted CL_{oral} values (4.84, 3.19, 3.45, 4.24, 5.0, 5.09, 7.55 l h^{-1}) were obtained from six different vehicle treated human hepatocytes and converting CL_{oral} from ml min^{-1} kg^{-1} to l h^{-1} assuming a 70 kg person.
*relative to imatinib alone

imatinib pre-incubation allowing for the mechanism based inhibition to take place. Ritonavir abolished desmethyl imatinib generation in hepatocytes, which is in contrast to a previous report on HLM experiments where ritonavir could not completely abolish imatinib demethylation [14]. Imatinib depletion and desmethyl imatinib generation patterns were similar after acute and chronic imatinib exposure, respectively, which is not consistent with the hypothesis of time-dependent susceptibility of imatinib to ritonavir mediated metabolic inhibition in hepatocytes. Susceptibility of imatinib metabolism to ritonavir was also not different after acute or chronic exposure. The complete absence of an effect of ritonavir on imatinib exposure in subjects in the van

Erp *et al.* study [14] may be explained by a complete mechanism based inhibition of CYP3A by imatinib after 2 months of imatinib treatment.

Our results underline both the complexity of imatinib metabolism and its drug–drug interactions and the potential limitations of HLM and hepatocyte studies in predicting drug–drug interactions, in particular with regards to limited duration experiments of certain drugs that undergo a shift in metabolic route over time, as exemplified by imatinib and its auto-inhibition of CYP3A4,

A

B
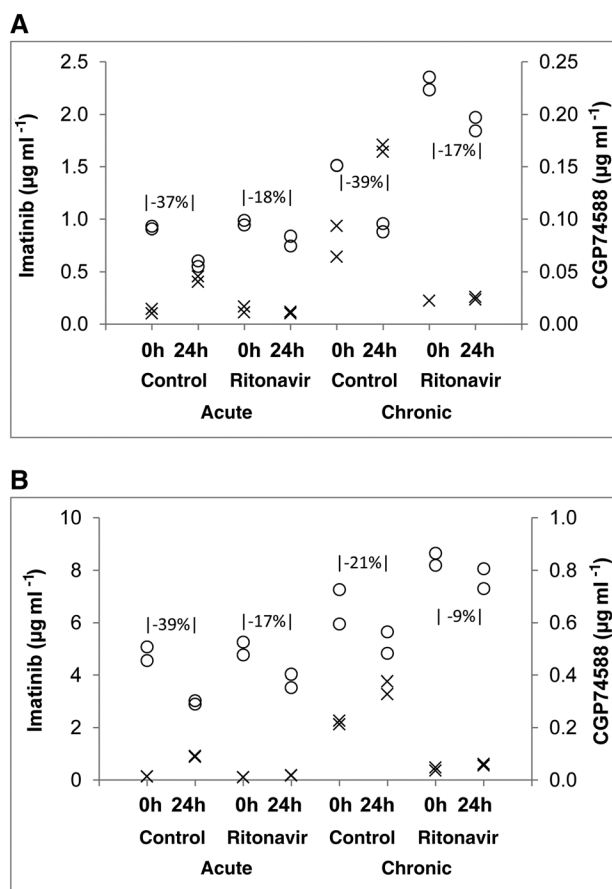
Figure 3

Imatinib depletion and formation of desmethyl imatinib (CGP74588) in primary cultures of human hepatocytes treated with either DMSO or ritonavir simulating acute and chronic imatinib exposure at 2.5 μM [A] or 10 μM [B]. Decrease in imatinib between 0 and 24 h is indicated in the graphs. The open circle (○) indicates imatinib depletion and the cross (x) represents the formation of desmethyl imatinib

and changing oral bioavailability [7, 13]. In addition, this also implies that the metabolic drug–drug interaction studies of imatinib which were limited to single dose administrations of imatinib, such as that of St John's Wort and imatinib [55, 56], may not be reflective of the interaction at steady-state use of imatinib.

Based on our results and the existing literature, suggested imatinib dose adjustments in the context of antiretroviral therapy may be dependent on whether imatinib or HAART is the pre-existing therapy. Patients who are on imatinib therapy for over 3 months, and require addition of ritonavir containing antiretroviral therapy may not require any imatinib dose adjustments, as imatinib clearance is no longer additionally susceptible to ritonavir. However, for patients who are on ritonavir containing antiretroviral therapy and are initiating imatinib therapy, a lower dose of imatinib (200 mg) may be warranted to minimize potential side effects. After chronic treatment, the metabolic fate of imatinib will have achieved steady-state, and the imatinib dose

of 400 mg or higher may be tolerated [14, 49]. Clinical confirmation of our hypotheses is desired. The effect of efavirenz on imatinib exposure in patients on long term imatinib treatment will need to be studied in a well-controlled trial comparing within-subject changes, before more specific recommendations may be formulated.

Given the extensive literature on imatinib exposure–response relationships with a target plasma imatinib concentration of 1000 ng ml^{-1}, the wide inter-patient variability in imatinib clearance, and the limited availability of clinical data on the range of possible imatinib drug interactions, therapeutic drug monitoring may be especially critical in the setting of antiretroviral co-medication [38].

Competing Interests

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