Autoinhibition of CYP3A4 Leads to Major Role of CYP2C8 in Imatinib Metabolism: Variability in CYP2C8 Activity May Alter Plasma Concentrations and Response

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Major role for CYP2C8 in imatinib metabolism

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Abbreviations: AUC, area under the concentration-time curve; Cmax, peak concentration; CLint, hepatic clearance; CLint, intrinsic clearance; DDC, diethyldithiocarbamate; GEM-G, gemfibrozil 1-O-β glucuronide; HLM, human liver microsomes; ISEF, intersystem extrapolation factor; 8-M-P, 8-methoxy-psoralen; MBI, mechanism-based inhibition; N-DMI, N-desmethylimatinib; P450, cytochrome P450; PBPK, physiologically-based pharmacokinetic.
Abstract

Recent data suggest that the role of CYP3A4 in imatinib metabolism is smaller than presumed. This study aimed to evaluate the quantitative importance of different cytochrome P450 (P450) enzymes in imatinib pharmacokinetics. First, the metabolism of imatinib was investigated using recombinant P450 enzymes and human liver microsomes with P450 isoform-selective inhibitors. Thereafter, an in silico model for imatinib was constructed to perform pharmacokinetic simulations to assess the roles of P450 enzymes in imatinib elimination at clinically used imatinib doses. In vitro, CYP2C8 inhibitors and CYP3A4 inhibitors inhibited the depletion of 0.1 µM imatinib by 45% and 80%, respectively, and the formation of the main metabolite of imatinib, N-desmethylimatinib, by >50%. Likewise, recombinant CYP2C8 and CYP3A4 metabolized imatinib extensively, whereas other isoforms had minor effect on imatinib concentrations. In the beginning of imatinib treatment, the fractions of its hepatic clearance mediated by CYP2C8 and CYP3A4 were predicted to approximate 40% and 60%, respectively. During long-term treatment with imatinib 400 mg once or twice daily, up to 65% or 75% of its hepatic elimination was predicted to occur via CYP2C8, and only about 35% or 25% by CYP3A4, due to dose- and time-dependent autoinactivation of CYP3A4 by imatinib. Thus, although CYP2C8 and CYP3A4 are the main enzymes in imatinib metabolism in vitro, in silico predictions indicate that imatinib inhibits its own CYP3A4-mediated metabolism, assigning a key role for CYP2C8. During multiple dosing, pharmacogenetic polymorphisms and drug interactions affecting CYP2C8 activity may cause marked interindividual variation in the exposure and response to imatinib.
Introduction

The tyrosine kinase inhibitor imatinib (Gleevec, Glivec) has revolutionized the treatment of chronic myelogenous leukemia, advanced gastrointestinal stromal tumours and certain other hematologic and oncologic malignancies (Kovacsovics and Maziarz, 2006; Duffaud and Le Cesne, 2009; Stegmeier et al., 2010). The success of imatinib is mostly due to its excellent efficacy but also because of its tolerability. Both the clinical response and adverse effects of imatinib are associated with its plasma levels, which exhibit a wide interindividual variability, and therapeutic drug monitoring has been recommended for imatinib (Peng et al., 2004b; Picard et al., 2007; Teng et al., 2012). According to clinical studies, achieving and maintaining an imatinib plasma level of >1,000 ng/ml is associated with better response rates, whereas doses of >600 mg daily have been linked to an increased risk of adverse events (Picard et al., 2007; Larson et al., 2008; Takahashi et al., 2010; Teng et al., 2012). Accordingly, it is important to know the potential sources of variability in imatinib pharmacokinetics.

Imatinib has a rapid absorption, complete oral bioavailability (>97%) and a terminal elimination half-life of 18-20 h (Peng et al., 2004a; Peng et al., 2004b), making it suitable for once or twice daily oral administration. Imatinib undergoes extensive hepatic metabolism and its metabolites are excreted principally into feces and, to a lesser extent, into urine (Gschwind et al., 2005). Following a single oral dose, parent imatinib accounts for approximately 70% of the drug concentration in plasma, whereas its pharmacologically active main metabolite N-desmethylimatinib (N-DMI; CGP74588) accounts for a respective 10% (Gschwind et al., 2005; Peng et al., 2005)(Supplementary Fig. S1). Several other, less important metabolites have been identified, including both oxidative and glucuronide conjugated metabolites of imatinib and N-DMI (Gschwind et al., 2005; Rochat et al., 2008).

According to imatinib product information, imatinib metabolism is mainly mediated by cytochrome P450 3A4 (Rochat et al., 2008; Nebot et al., 2010). Recently, however, we demonstrated that imatinib is a potent mechanism-based inhibitor of this enzyme in vitro, and clinically relevant imatinib concentrations were predicted to cause
up to 90% inhibition of hepatic CYP3A4 activity (Filppula et al., 2012). These findings suggest that imatinib can affect its own CYP3A4-mediated metabolism, which may reduce the significance of this enzyme in its elimination in vivo. In fact, strong CYP3A4 inhibitors have had only little effect on imatinib pharmacokinetics in the beginning of therapy, and no effect at all during long-term treatment. In healthy volunteers, the CYP3A4-inhibiting antifungal agent ketoconazole increased the area under the plasma concentration-time curve (AUC) of imatinib after single dose administration by 40% only (Dutreix et al., 2004), and in patients steady-state imatinib pharmacokinetics were unchanged by ritonavir, a CYP3A4-inhibiting antiretroviral drug (van Erp et al., 2007).

Recent in vitro data suggest that in addition to CYP3A4, also CYP2C8 contributes to the formation of N-DMI (Nebot et al., 2010); recombinant human CYP2C8 catalyzed the N-demethylation of imatinib at a much higher rate than did CYP3A4 and CYP3A5. However, the significance of CYP2C8 to the human metabolism of imatinib is difficult to estimate because the recombinant CYP2C8 and CYP3A5 enzymes used in this previous study were produced in insect cells, whereas the recombinant CYP3A4 originated from human lymphoblastoid cells, which typically show a several-fold lower activity than enzymes from insect cells. Furthermore, because the contributions of P450 enzymes to the overall metabolism of imatinib, including both its N-demethylation and other metabolic pathways, and to the further metabolism of N-DMI were not investigated, the clinical significance of the previous in vitro results cannot be estimated.

Using recombinant human P450 enzymes, human liver microsomes (HLM) and chemical P450 inhibitors, we carefully examined the contributions of different P450 enzymes, particularly those of CYP2C8 and CYP3A4, to the main metabolic pathway of imatinib and the further metabolism of N-DMI. Furthermore, in order to translate our laboratory findings to clinical relevance, we used our data for pharmacokinetic and drug-drug interaction simulations by constructing a physiologically-based model of imatinib pharmacokinetics, incorporating the time-dependent inhibitory effect of imatinib on its own CYP3A4-mediated metabolism.
Materials and Methods

In vitro study

Chemicals and Microsomes.

Imatinib mesilate (mesylate salt), clopidogrel and montelukast were purchased from Sequoia Research Products Ltd. (Pangbourne, UK), N-DMI and N-DMI-d8 from SynFine Research Inc. (Richmond Hill, ON, Canada), imatinib-d8 mesilate and gemfibrozil 1-O-β-glucuronide (GEM-G) from Toronto Research Chemicals Inc. (North York, ON, CAN), diethyldithiocarbamate (DDC), furafylline, 8-methoxy-psoralen (8-M-P), omeprazole, quinidine, sulfaphenazole, troleandomycin, and β-NADPH from Sigma-Aldrich (St. Louis, MO, USA), and ketoconazole from Janssen Biotech (Olen, Belgium). Pooled HLM and Supersomes™ (human recombinant P450 enzymes and control supersomes) were obtained from BD Biosciences (Woburn, MA, USA). Other chemicals were from Merck (Darmstadt, Germany).

Incubation conditions.

Incubations were carried out in a shaking water bath (37°C) in triplicates (mean value used as the result). The incubations contained HLM or recombinant P450 enzymes in 0.1 M sodium phosphate buffer (pH 7.4). To keep the possible nonspecific binding of imatinib equal in the experiments and to enable precise comparisons of results with different P450 isoforms, an equal protein concentration (0.5 mg/ml; unless otherwise indicated) was used in parallel experiments (resulting in variable P450 contents in recombinant isoform studies). Except for inhibition studies, experiments were started by premixing imatinib for 3 min with microsomes, followed by addition of β-NADPH (final concentration 1 mM) to initiate the reaction. In competitive inhibition studies, imatinib and inhibitor or buffer control were (simultaneously) premixed with HLM for 3 min before β-NADPH addition. In mechanism-based inhibition (MBI) studies, except for that with GEM-G, the inhibitor or buffer control was first premixed with HLM for 3 min before addition of β-NADPH. After a 15-min preincubation, including β-NADPH, imatinib was added to start the reaction. Inhibition by GEM-G (60 µM) was investigated by premixing it with 10 mg/ml HLM in buffer. After addition of β-NADPH, the solution was preincubated for 20 min. An aliquot of 25 µl was then moved to another tube containing β-NADPH in buffer, and imatinib was immediately added to start the reaction in a final incubation volume of 0.5 ml. Thus, the protein and
Inhibitor concentrations had been diluted 20-fold to avoid possible competitive inhibition of other enzymes by the inhibitor.

After incubation, reactions were stopped by moving incubation mixture to ACN containing the internal standards imatinib-d8 and N-DMI-d8, 10% MeOH and 0.1% HCOOH, diluting the incubation mixture 3-fold. Samples were immediately vortexed, put on ice for at least 10 minutes, and vortexed further two times before centrifugation at 20,800 g for 10 minutes. All stock solutions of imatinib and N-DMI were prepared in methanol. All incubations (including controls) contained the same concentration of organic solvent (1% methanol or 0.5%:0.5% methanol:acetonitrile). As appropriate, the incubation time was optimized within the linear range for metabolite formation depending on the turnover conditions of each specific experiment. The unbound fraction of 0.1 µM imatinib in microsomal incubations had previously been determined (Filppula et al., 2012), and the nonspecific binding of N-DMI to microsomes was assumed to equal that of imatinib.

**Measurement of imatinib and its metabolites.**

Imatinib and N-DMI concentrations were quantified by use of an API 3000 liquid chromatography-tandem mass spectrometry system (Sciex Division of MDS Inc, Toronto, ON, Canada). Concentrations of imatinib and N-DMI were measured in positive turbo ion spray mode. Chromatography was performed on an Atlantis Hilic Silica analytic column (2.1×100 mm, 3.0 µm) (Waters, Milford, MA, USA) by use of gradient elution. The mobile phase was (A) 10 mM ammonium formate in 0.1% HCOOH, and (B) 0.1% HCOOH in acetonitrile:MeOH 10:1, v/v, and the gradient comprised 0 min at 95% (B), 15 min to 50% (B), 5 min at 50% (B), 0.1 min to 95% (B), and finally 14.9 min at 95% (B). The flow rate was 0.2 ml/min. The mass spectrometer was operated in multi reaction mode (MRM) and ion transitions monitored were m/z (mass-to-charge ratio) 494.3 to 394.3 for imatinib, 480.3 to 394.3 for N-DMI, 502.2 to 394.3 for imatinib-d8 and 488.2 to 394.3 for N-DMI-d8. The peaks of a hydroxy benzylic metabolite (M5), piperidine N-oxide imatinib (M6) and pyridine N-oxide imatinib (M8) were monitored during the assays based on ion transitions reported (Marull and Rochat, 2006; Rochat et al., 2008), and their quantities were measured as arbitrary units relative to the ratio of the peak height of the metabolite to the peak height of N-DMI-d8. The limit of quantification was 0.005 µM for imatinib, 0.01 µM for N-DMI, and a signal/noise ratio of 10:1 was used as the limit of quantification for
M5, M6, and M8. For imatinib and N-DMI, the between-day coefficient of variation (CV) was <15% at relevant concentrations.

**Recombinant P450 isoform studies.**

First, the depletion of 0.1 µM imatinib by CYP1A2 (50 pmol/ml), CYP2A6 (30 pmol/ml), CYP2B6 (40 pmol/ml), CYP2C8 (158 pmol/ml), CYP2C9 (120 pmol/ml), CYP2C19 (174 pmol/ml), CYP2D6 (72 pmol/ml), CYP2E1 (296 pmol/ml), CYP3A4 (66 pmol/ml), and CYP3A5 (50 pmol/ml) was investigated in a 30 min incubation. Then, metabolite formation by these enzymes was studied by incubating 1 µM imatinib with each enzyme for up to 60 min. Finally, the enzyme kinetics of N-DMI formation was determined by incubating imatinib (0.10-320 µM) with CYP2C8 (33 pmol/ml) for 4 min, CYP3A4 (13 pmol/ml) for 3 min, and CYP3A5 (10 pmol/ml) for 30 min. The protein concentration was 0.5 mg/ml in the depletion and metabolite formation studies, and 0.1 mg/ml in the enzyme kinetic incubations.

**Effects of CYP-selective inhibitors on imatinib metabolism.**

In order to confirm the results from the recombinant P450 studies, inhibition studies with HLM were carried out. Montelukast (5 µM), sulfaphenazole (10 µM), omeprazole (10 µM), quinidine (10 µM), and ketoconazole (1 µM) were tested as competitive inhibitors of CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, respectively (Baldwin et al., 1995; Newton et al., 1995; Bourrie et al., 1996; Ko et al., 1997; Eagling et al., 1998; Walsky et al., 2005). Furafylline (20 µM), 8-M-P (0.5 µM), clopidogrel (1 µM), GEM-G (60 µM), DDC (100 µM), and troleandomycin (50 µM) were examined as mechanism-based inhibitors of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2E1, and CYP3A4, respectively (Newton et al., 1995; Bourrie et al., 1996; Draper et al., 1997; Koenigs et al., 1997; Eagling et al., 1998; Richter et al., 2004; Ogilvie et al., 2006). 1 µM imatinib was incubated with each inhibitor (or after preincubation), and metabolite formation rate was measured. Then, selected inhibitors (GEM-G, montelukast, ketoconazole, and troleandomycin) were incubated with 0.1 µM imatinib for up to 60 min.

**Metabolism of N-DMI.**

The further metabolism of N-DMI was investigated by incubating 0.1 and 1 µM N-DMI with 0.5 mg/ml HLM and following its depletion for up to 60 min. N-DMI (0.1 µM) was also incubated for 30 min with CYP1A2 (50 pmol/ml), CYP2C8 (158 pmol/ml), CYP2D6 (72 pmol/ml), CYP3A4 (66 pmol/ml), CYP3A5 (50 pmol/ml), and an enzyme mix containing CYP2A6 (59 pmol/ml), CYP2B6 (7.7 pmol/ml),
CYP2C9 (24 pmol/ml), CYP2C19 (35 pmol/ml), and CYP2E1 (25 pmol/ml), wherein the total protein concentration was 0.5 mg/ml. Finally, the depletion of 0.05 µM N-DMI by CYP2C8, CYP3A4, and CYP3A5 was examined in detail.

**Data analysis.**

The kinetics of N-DMI formation were analyzed using SigmaPlot software (version 9.01; Systat Software, Inc., San Jose, CA). Selection of the best-fit enzyme model was based on the Akaike information criterion, on R², and on the examination of Michaelis-Menten plots. For CYP2C8 and CYP3A4, the results were best described by an uncompetitive substrate inhibition model: 

\[ v = \frac{V_{\text{max}} \times S}{(K_m \times S + S^2 / K_i)} \]

where \( v \) is velocity (pmol/min/pmol), \( V_{\text{max}} \) is the maximal velocity (pmol/min/pmol), \( S \) is substrate concentration (µM), \( K_m \) is the Michaelis-Menten constant (µM), and \( K_i \) is the inhibitory constant (µM). CYP3A5-mediated N-demethylation was best described by traditional Michael-Menten kinetics: 

\[ v = \frac{V_{\text{max}} \times S}{K_m \times S} \]

Intrinsic clearance for N-DMI formation (CL_{\text{int,N-DMI form}}) was calculated according to 

\[ \text{CL}_{\text{int,N-DMI form}} = \frac{V_{\text{max}}}{K_m} \]

Pseudo-first-order depletion rate constants (k_{dep}) were determined for the depletion of 0.1 µM imatinib, and 0.05 and 0.1 µM N-DMI in HLM and recombinant enzyme incubations using nonlinear regression analysis (SigmaPlot). Imatinib k_{dep} values were calculated on the basis of the time points at 0-8 min for CYP2C8, 0-4 min for CYP3A4, 0-30 min for the other P450 isoforms and HLM. N-DMI k_{dep} values were estimated based on time points at 0-30 min for all P450 isoforms and 0-60 min for HLM. Percent inhibition of imatinib depletion was calculated by comparing k_{dep} values to those of control incubations. Assuming that imatinib and N-DMI concentrations were <<K_m for their metabolic pathways, their intrinsic clearance in depletion experiments was expressed as 

\[ \text{CL}_{\text{int, dep}} = \frac{k_{\text{dep}}}{[M]} \]

where [M] is the microsomal protein concentration or P450 concentration in recombinant enzyme incubations (Venkatakrishnan et al., 2003). In addition, an intrinsic clearance value for the formation of all primary metabolites of imatinib other than N-DMI was defined as 

\[ \text{CL}_{\text{int,u,ima dep}} - \text{CL}_{\text{int,N-DMI form}} \]

for each P450 isoform, where \( \text{CL}_{\text{int,u,ima dep}} \) and \( \text{CL}_{\text{int,N-DMI form}} \) denote the unbound intrinsic clearance values for imatinib depletion and N-DMI formation, respectively. To estimate the relative contributions of different P450 enzymes to imatinib and N-DMI metabolism, the CL int values, adjusted for nonspecific binding in microsomes, were multiplied with intersystem extrapolation factors (ISEFs)(Proctor et al., 2004) of each P450 isoform and with average P450 isoform abundance (pmol/mg microsomal protein)(Table 1). The obtained values were then scaled to in vivo using 40 mg of microsomal protein per gram liver (Houston and Galetin, 2008) and
25.7 g of liver weight per kg body weight (Davies and Morris, 1993). Hepatic blood clearance (CL\textsubscript{H}) values were calculated using the well-stirred model: \( \text{CL}\textsubscript{H} = \frac{Q\textsubscript{h} \times f\textsubscript{u,b} \times \text{CL}\textsubscript{int, in vivo}}{Q\textsubscript{h} + (f\textsubscript{u,b} \times \text{CL}\textsubscript{int, in vivo})} \) where \( Q\textsubscript{h} \) is the hepatic blood flow (20.7 ml/min/kg)(Houston and Galetin, 2008), \( f\textsubscript{u,b} \) is the unbound fraction of imatinib or N-DMI in blood, and \( \text{CL}\textsubscript{int, in vivo} \) is the scaled \( \text{CL}\textsubscript{int} \).

**In silico study**

**Construction and validation of a Simcyp model for imatinib.**

Based on the present *in vitro* investigations and literature data, compound files for imatinib and N-DMI (see Supplementary Tables S1 and S2 for data) were constructed within the Simcyp Population-Based Simulator (V11.00; Simcyp Limited, UK). Using physiologically-based pharmacokinetic (PBPK) modeling, Simcyp simulates change in drug concentration over time. The software generates virtual populations reflecting variability in genetic, physiological, and demographic variables using Monte Carlo methods so that interindividual variability in drug elimination and the importance of different metabolizing P450 isoforms in a population can be assessed (Howgate et al., 2006; Rostami-Hodjegan and Tucker, 2007; Jamei et al., 2009; Rowland Yeo et al., 2010).

The models for imatinib and N-DMI comprised first order oral absorption from the intestine (for imatinib), intestinal metabolism, a minimal physiologically-based distribution model including a single adjusting compartment, and elimination by hepatic CYP-mediated and additional (non CYP-mediated) metabolism and by renal excretion. The hepatic elimination input parameters were obtained from the present *in vitro* studies. ISEF factors in Tables 1 and 2 and other parameters provided by the software were used to scale \( \text{CL}_{\text{int}} \) to \( \text{CL}\textsubscript{H} \), and \( \text{CL}\textsubscript{H} \) values were then predicted using the well-stirred model. Renal clearance was estimated from literature values of systemic clearance and amount excreted unchanged in the urine (Bornhauser et al., 2005; Gschwind et al., 2005). Then, hepatic and renal clearances were combined with additional clearance to derive the total clearance. Furthermore, the compound files for imatinib and N-DMI included values for direct inhibition of different P450 enzymes, and the final model for imatinib also included kinetic constants for MBI of CYP3A4. When the literature and experimental values had been entered into the compound files, the model was refined by adjusting the additional clearance and the volume of the single adjusting compartment so that published single dose pharmacokinetic data could be described accurately (see Supplementary Methods and Supplementary Table S3 for details). Thereafter, the model was validated by comparing...
simulations to published multiple-dose pharmacokinetic studies, and to interaction studies with ketoconazole, ritonavir, and rifampicin (Supplementary Methods and Supplementary Tables S4-S5).

**Simulation of the clinical consequences of in vitro findings.**

To demonstrate the significance of MBI of CYP3A4 to the metabolism of imatinib, multiple-dose pharmacokinetics of imatinib 400 mg once daily were simulated, both with and without MBI. In addition, the time- and dose-dependent effects of imatinib on intestinal and hepatic CYP3A4 activity, and on the roles of CYP2C8 and CYP3A4 in imatinib biotransformation were simulated using the final model and imatinib doses ranging from 100 mg once daily to 400 mg twice daily (Supplementary Methods and Supplementary Table S4). Furthermore, the effects of CYP2C8 polymorphisms and of a strong CYP2C8 inhibitor (gemfibrozil 600 mg twice daily), strong CYP3A4 inhibitor (itraconazole 100 mg twice daily) or their combination on imatinib pharmacokinetics after a single dose and/or multiple doses of imatinib 400 mg once daily were predicted as detailed in the Supplementary Methods and Supplementary Tables S6-S8.
Results

In vitro study

Imatinib metabolism by recombinant enzymes.

Recombinant CYP2C8 and CYP3A4 metabolized 0.1 and 1 µM imatinib rapidly and <13% of the parent drug remained after 30 min incubation (Fig. 1A and 2A). In CYP3A5 incubations ~80% of imatinib was left at 30 min, whereas the other P450 isoforms tested had a minor effect on imatinib concentrations (>90% of parent drug was left). At 1 µM imatinib, N-demethylation was mediated by CYP2C8, CYP3A4, and CYP3A5, but very small amounts of N-DMI were formed also by CYP2D6, CYP2C19 and CYP2C9 (Fig. 1B). However, at 0.1 µM imatinib, only CYP2C8, CYP3A4 and CYP3A5 formed detectable amounts of N-DMI (Fig. 1B and 2B). At 1 µM imatinib, CYP2C8 also formed detectable amounts of M6, and CYP2D6 catalyzed formation of M5, whereas CYP3A4 formed both M5 and M6 (data not shown). The enzyme kinetics of N-DMI formation by CYP2C8, CYP3A4, and CYP3A5 is presented in Fig. 2C.

Comparison of CL<sub int</sub> values obtained from the depletion (total metabolism) of 0.1 µM imatinib and N-demethylation kinetics, indicated that formation of N-DMI accounts for the majority (88%) of imatinib metabolism by CYP2C8, whereas the metabolite accounts for 27% and 75% of imatinib metabolism by CYP3A4 and CYP3A5 (Table 1), respectively. With use of the static ISEF approach, CYP2C8 and CYP3A4 were estimated to be the most important enzymes in imatinib metabolism in vivo (contributions of 40% and 57%, respectively, with <3% contribution by other P450 isoforms; Table 1).

Effects of CYP-selective inhibitors on imatinib metabolism.

The CYP2C8 inhibitors montelukast and GEM-G, and the CYP3A4 inhibitors ketoconazole and troleandomycin inhibited the formation of N-DMI by ≥50% (Fig. 1C) and were further investigated in a depletion study. Herein, montelukast and GEM-G inhibited the depletion of 0.1 µM imatinib by ~45%, whereas the CYP3A4 inhibitors inhibited it by almost 80% (data not shown).

Metabolism of N-DMI.

The further metabolism of N-DMI was mainly catalyzed by CYP3A4, with a less significant contribution by CYP2C8, and minor contribution by other P450 enzymes (Fig. 1D and 2D). Incubation with a
mixture containing CYP2A6, CYP2B6, CYP2C9, CYP2C19, and CYP2E1 did not lead to significant depletion of N-DMI. Using the ISEF approach, the contribution of CYP3A4 to the hepatic metabolism of N-DMI in vivo was estimated to average 40%, followed by CYP2C8 (11%), whereas the contributions by CYP1A2, CYP2D6 and CYP3A5 were <5% each (Table 2).

**In silico study**

**Comparison of a PBPK model of imatinib to its clinical pharmacokinetics.**

Based on physical-chemical and pharmacokinetic data available from the literature, a PBPK model was created to estimate the contributions of different P450 enzymes to imatinib and N-DMI metabolism in humans over time. The clearances of imatinib and N-DMI were predicted on the basis of the present in vitro data together with literature data on renal and additional clearance, and the final model included MBI of CYP3A4 by imatinib. The simulated concentration-time curves as well as the mean pharmacokinetic parameters of imatinib and N-DMI matched well with published data on imatinib single dose pharmacokinetics in healthy volunteers (Fig. 3A-B, Supplementary Table S3).

Compared to when only direct inhibition of CYP3A4 by imatinib was considered, the final model simulated the peak concentrations, AUC₀-2₄, and trough concentrations of imatinib on day 30 that were on average 32%, 58%, and 113% higher, respectively, and those of N-DMI were on average 5%, 16%, and 24% higher, respectively (Fig. 4A-B). The final model also matched well with observed trough and peak concentrations of imatinib during treatment with 400 mg once daily, averaging 910-1,530 ng/ml and 2,200-3,405 ng/ml, respectively, and with those of N-DMI averaging 170-301 ng/ml and 290-556 ng/ml, respectively (Peng et al., 2004b; van Erp et al., 2007; Gibbons et al., 2008; Demetri et al., 2009; Kim et al., 2011; Teng et al., 2012) (Fig. 4A-B, Supplementary Table S4). Similarly, the simulations predicted well the effects of ketoconazole, rifampicin and ritonavir on the pharmacokinetics of imatinib (Supplementary Table S5). The validation simulation of gemfibrozil pharmacokinetics is presented in Supplementary Fig. S2.

**Role of CYP2C8 in imatinib metabolism is important during prolonged treatment.**

With the final model, the inactivation of intestinal and hepatic CYP3A4 by imatinib proceeded time- and dose-dependently (Fig. 4A-E). For example, at steady-state, imatinib 100 mg once daily, 400 mg once daily, and 400 mg twice daily, was predicted to reduce the amount of active CYP3A4 to 55%-
59%, 22%-29%, and 13%-15% of its normal amount in the liver, respectively. At the commencement of imatinib treatment, the fractions metabolized by hepatic CYP3A4 and CYP2C8 were predicted to average 59% and 39% of the total hepatic clearance of imatinib, equaling those estimated with the static ISEF model. After multiple doses of imatinib 400 mg once daily, the majority of the hepatic metabolism of imatinib was predicted to occur by CYP2C8 (61%-65%) and a smaller proportion by CYP3A4 (31%-37%)(Fig. 4E). The corresponding values for treatment with 400 mg twice daily were 71%-74% and 22%-25% for CYP2C8 and CYP3A4, respectively.

An otherwise typical 60-year old male, but with a two-fold higher than the average CYP2C8 activity in the liver, was predicted to have a markedly increased steady-state imatinib metabolism (Fig. 5A-B). His plasma concentrations of imatinib were for the most part of the 24 h-dosing interval below the suggested effectiveness threshold of about 1,000 ng/ml (Fig. 5A). Conversely, a low activity CYP2C8 genotype (50% reduction in CYP2C8 activity) decreased imatinib metabolism, leading to increased imatinib concentrations and reduced N-DMI concentrations.

According to drug-drug interaction predictions, a strong CYP3A4 inhibitor (itraconazole) increased the $\text{AUC}_{0-\infty}$ of imatinib and N-DMI by 38% and 6%, respectively, after a single dose of imatinib 400 mg (Fig. 5C-D, Supplementary Table S8), and the dosing interval AUC by 18% and 6%, respectively, following multiple imatinib doses (Fig. 5E-F). A strong CYP2C8 inhibitor (gemfibrozil) was predicted to increase the $\text{AUC}_{0-\infty}$ of a single dose of imatinib 1.8-fold, and the dosing interval AUC after multiple imatinib doses 2.3-fold, whereas it reduced the AUC of N-DMI by 59%-84%. The inhibition of both CYP2C8 and CYP3A4 was predicted to markedly increase imatinib AUC 2.9-fold, and almost completely inhibit N-DMI formation (by >79%).
Discussion

According to the present \textit{in vitro} findings, imatinib metabolism is almost exclusively mediated by CYP2C8 and CYP3A4 at clinically relevant imatinib concentrations. Formation of the active metabolite of imatinib, N-DMI, accounted for the majority of imatinib metabolism by CYP2C8, whereas more than two thirds of imatinib metabolism mediated by CYP3A4 occurred via other metabolic pathways. Moreover, the further biotransformation of N-DMI was mainly catalyzed by CYP3A4. Pharmacokinetic simulations based on these results and on recent data indicating that imatinib is an irreversible mechanism-based inhibitor of CYP3A4 (Filppula et al., 2012), predicted that the role of CYP3A4 in imatinib metabolism is important mainly in the beginning of therapy, but diminishes thereafter, because of time-dependent autoinhibition of CYP3A4-mediated metabolism of imatinib. Consequently, CYP2C8 is likely to be the most important enzyme in imatinib metabolism at steady-state, during long-term treatment.

Imatinib biotransformation occurs via multiple metabolic pathways in addition to the N-demethylation pathway (Gschwind et al., 2005; Marull and Rochat, 2006; Rochat et al., 2008). Therefore, we used a depletion approach to estimate the contributions of different P450 enzymes to the total elimination of imatinib at low, therapeutic concentrations. The depletion rates and inhibition experiments showed that CYP2C8 and CYP3A4 almost exclusively mediate the hepatic microsomal metabolism of imatinib with other isoforms contributing to a minor extent (~5%). For example, CYP3A5 metabolized imatinib to N-DMI, but the total elimination CL\textsubscript{int} of CYP3A5 was small (<10% of that of CYP2C8). Comparison of CL\textsubscript{int} values obtained from the depletion of 0.1 µM imatinib and N-demethylation kinetics indicated that N-demethylation accounts for the majority of imatinib metabolism by CYP2C8. On the contrary, CYP3A4 seemed to produce more than twice as much other metabolites as its produced N-DMI, in line with previous data (Rochat et al., 2008). In our incubations, CYP3A4 formed M5 and M6, but due to lack of authentic standards, the quantitative evaluation of these metabolites was not possible. CYP2D6 also formed a small amount of M5, but the depletion experiment with 0.1 µM imatinib indicated a negligible significance for CYP2D6 in its metabolism. Also CYP2C9, CYP2C19, CYP1A1, CYP1B1, and CYP4F may play minor roles in imatinib biotransformation (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2001/21335_Gleevec.cfm) (Rochat et al., 2008), but in our study the contributions of CYP2C9 and CYP2C19 were negligible at therapeutic concentrations, and CYP1A1, CYP1B1, and CYP4F were not investigated because of their
insignificant hepatic expression. Moreover, our findings indicate that the further metabolism of N-DMI is mediated mainly by CYP3A4, with CYP2C8, CYP3A5, CYP2D6, and CYP1A2 participating to a smaller extent.

Using the static ISEF approach, the contributions of CYP2C8 and CYP3A4 to the hepatic metabolism of imatinib in vivo were estimated to be 40% and 57%, respectively. However, because imatinib inactivates CYP3A4 time-dependently (Filppula et al., 2012), a static model cannot adequately describe the contributions of different P450 enzymes to imatinib metabolism. Therefore, we constructed a dynamic PBPK model for imatinib and N-DMI pharmacokinetics, incorporating previous data on time-dependent inactivation of CYP3A4 by imatinib. Interaction simulations with ketoconazole, rifampicin, and ritonavir, based on this model, generally showed a good agreement with their reference studies (Bolton et al., 2004; Dutreix et al., 2004; van Erp et al., 2007). Thus, it seems that our in vitro data for CYP3A4 generated reliable predictions of its role in imatinib pharmacokinetics.

Compared to a model without MBI of CYP3A4 by imatinib, steady-state simulations based on the final model corresponded much better with clinical data showing that trough and peak concentrations average 910-1,530 ng/ml and 2,200-3,405 ng/ml, respectively, during imatinib 400 mg once daily (Peng et al., 2004b; van Erp et al., 2007; Gibbons et al., 2008; Demetri et al., 2009; Kim et al., 2011; Teng et al., 2012).

MBI of P450 enzymes proceeds in a time- and concentration-dependent manner, because it requires that the inhibitor is metabolized by the enzyme to an intermediate, which immediately binds covalently to the enzyme (Lin and Lu, 1998). Thus, unlike reversible inhibitors, mechanism-based inhibitors inactivate their victim enzymes permanently, and enzyme activity can be regained only by synthesis of new enzyme. Clinically, these characteristics can lead to a slow onset of inhibition and a long-lasting duration of inhibition. MBI poses a particular problem when in vitro studies are carried out to estimate the contributions of P450 enzymes to the metabolism of a drug, which is a mechanism-based inhibitor of a P450 form participating in its own metabolism. Failure to consider MBI leads to overestimation of the role of this enzyme. To overcome this problem with imatinib, we used very short incubations and low imatinib concentrations in order to minimize MBI and obtain reliable initial rates of in vitro metabolism, and applied a dynamic PBPK model for extrapolation of our in vitro data to in vivo.
According to our simulations, autoinhibition of the CYP3A4-mediated metabolism of imatinib is likely to attribute a key role for CYP2C8 in imatinib elimination. The fraction of hepatic clearance mediated by CYP2C8 was simulated to increase from 40% in the beginning of treatment to over 60% after multiple doses of imatinib 400 mg once daily. Conversely, the fraction eliminated by CYP3A4 decreased from 60% in the beginning to <40% during multiple dosing. The significance of CYP2C8 in steady-state imatinib pharmacokinetics became apparent in interaction simulations, where a strong CYP2C8 inhibitor like gemfibrozil (Backman et al., 2002; Honkalammi et al., 2012) was predicted to increase imatinib AUC, following a single imatinib dose, by 80%, and after multiple imatinib doses by 130%. By contrast, a strong CYP3A4 inhibitor like itraconazole (Olkkola et al., 1994) was predicted to increase imatinib AUC by 40% after a single dose of imatinib, and by 20% only, after multiple imatinib doses. This result is comparable to clinical findings from interaction studies with ketoconazole and ritonavir (Dutreix et al., 2004; van Erp et al., 2007). According to our simulations, gemfibrozil could reduce the plasma exposure to N-DMI by up to 85%, whereas itraconazole could increase it by ~5%. Of note, the combination of gemfibrozil and itraconazole was predicted to increase imatinib AUC by almost 200% during multiple dosing, highlighting the potential dangers of multiple interacting medications.

Simcyp has been associated with overpredictions of the magnitude of time-dependent drug-drug interactions. For CYP3A4, the former default degradation rate constant of 0.0077 1/h has been recognized as an important source for overpredictions, whereas the value of 0.019 1/h, used in version 11, has generated relatively reliable predictions (Rowland Yeo et al., 2011). Other possible factors impairing prediction accuracy of drug-drug interactions are failure to consider transporter mechanisms, gut metabolism or simultaneous mechanisms such as concurrent inhibition and induction of enzymes (Peters et al., 2012). In the present work, as evident from steady state simulations and interaction simulations with the CYP3A4 inhibitors, Simcyp did not appear to overpredict the impact of autoinhibition of CYP3A4 on imatinib pharmacokinetics. However, the effect of imatinib on the pharmacokinetics of other CYP3A4 substrates was not investigated in this work and needs to be addressed in future studies. Furthermore, given the above uncertainties, clinical studies are needed to evaluate the prediction accuracy concerning the role of CYP2C8 in imatinib pharmacokinetics and interactions.

There is a wide interindividual variability in imatinib plasma concentrations, associated with nonresponse and disease progression, even in the absence of interacting comediations (Peng et al.,
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2004b; Picard et al., 2007; Teng et al., 2012). Differences in CYP3A4 activity have been suggested to partly explain this variability (Peng et al., 2005; Apperley, 2007). Our findings contrast this suggestion and raise the possibility that pharmacogenetic polymorphisms of CYP2C8 can affect imatinib plasma concentrations, especially during long-term medication. For example, the CYP2C8*3 allele has been associated with a considerably increased clearance of several drugs, such as rosiglitazone and pioglitazone (Kirchheiner et al., 2006; Tornio et al., 2008; Aquilante et al., 2012). In our simulations, a high activity CYP2C8 genotype was predicted to result in an increased imatinib metabolism, leading to mean plasma levels of imatinib below the threshold concentration of 1,000 ng/ml, suggesting that genetic polymorphisms of CYP2C8 could possibly affect the pharmacokinetics of imatinib to a clinically relevant degree. It should be noted, however, that the effect of the CYP2C8*3 polymorphism seems to be substrate-dependent and therefore clinical studies with imatinib are needed. Moreover, imatinib is a substrate and inhibitor of several transporters in vitro, but their clinical significance in imatinib pharmacokinetics has not yet been established, apart from the role of organic cation transporter 1 (OCT1) in uptake of imatinib to leukocytes and leukemic cells (Eechoute et al., 2011; Wang et al., 2012). Our interaction simulations (Fig 5C-F) did not account for possible co-effects of P450 inhibitors on different transporter functions, which could influence imatinib pharmacokinetics. Thus, an inhibition or induction of relevant transporters affecting imatinib pharmacokinetics could modify the effect of CYP2C8 and CYP3A4 inhibitors on imatinib plasma and tissue concentrations.

In this work, we used an integrated approach of carefully optimized in vitro experiments, comprising both depletion and enzyme kinetic experiments, and pharmacokinetic simulations to evaluate the clinical relevance of CYP2C8 and CYP3A4 in imatinib elimination. Although clinical studies are necessary to confirm our in vitro-in silico findings, it is likely that imatinib time-dependently inhibits its own CYP3A4-mediated metabolism, leading to a key role for CYP2C8 in its elimination. Thus, during multiple dosing, both pharmacogenetic polymorphisms and drug interactions affecting CYP2C8 activity may cause marked interindividual variability in the exposure and response to imatinib. Overall, the present findings highlight the need to carefully evaluate the implications of MBI using integrated in vitro and modeling/simulation approaches.
Authorship Contributions

Participated in research design: Filppula, Neuvonen M., Laitila, Neuvonen P.J., and Backman

Conducted experiments: Filppula, Neuvonen M., Laitila, and Backman

Performed data analysis: Filppula, Neuvonen P.J., and Backman

Wrote or contributed to the writing of the manuscript: Filppula, Neuvonen M., Laitila, Neuvonen P.J., and Backman
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**36**:473-497.

variability in pharmacokinetics using virtual human populations and integrating general
knowledge of physical chemistry, biology, anatomy, physiology and genetics: A tale of
'bottom-up' vs 'top-down' recognition of covariates. *Drug Metab Pharmacokinet* **24**:53-75.

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Tsahuridu M, Meineke I, Brockmoller J, and Fuhr U (2006) Pharmacokinetics and


Footnotes

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Figure legends

Fig. 1.

Recombinant P450 isoform and P450 inhibitor screening experiments. A, remaining amount of 0.1 µM and 1 µM imatinib after a 30-min incubation with ten different P450 enzymes (0.5 mg/ml protein). B, initial formation rates of N-DMI in the respective incubations. C, the effects of different P450 isoform inhibitors (concentrations given in Methods) on N-DMI formation rate during incubation of 0.1 µM imatinib with pooled HLM (0.5 mg/ml protein) for 5 min. The inhibitors tested were furafylline (FURA), 8-methoxy-psoralen (8-M-P), clopidogrel (CLOP), gemfibrozil 1-O-β glucuronide (GEM-G), montelukast (MONT), sulfaphenazole (SULF), omeprazole (OME), quinidine (QUIN), diethyldithiocarbamate (DDC), ketoconazole (KETO), and troleandomycin (TAO). A 15- or 20-min preincubation of the inhibitor with HLM preceded the incubation in experiments with FURA, 8-M-P, CLOP, GEM-G, DDC, and TAO. Because recombinant CYP1A2, CYP2A6, CYP2B6, and CYP2E1 did not form N-DMI, the observed weak effects of FURA, 8-M-P, CLOP, and QUIN on N-DMI formation in C are likely to be due to nonspecific inhibition of CYP2C8 or CYP3A4 by these compounds. D, the remaining amount of 0.1 µM N-DMI after incubation for 30 min with CYP1A2, CYP2C8, CYP2D6, CYP3A4, CYP3A5, and a P450 mix containing CYP2A6, CYP2B6, CYP2C9, CYP2C19, and CYP2E1 (0.5 mg/ml protein). The equal protein concentrations used in recombinant P450 experiments resulted in variable P450 concentrations in the incubations (see Materials and Methods for exact P450 concentrations). The bars represent mean±SD values of triplicate incubations.

Fig. 2.

Experiments with recombinant P450 enzymes. A, depletion of 0.1 µM imatinib and B, the subsequent formation of N-DMI by CYP2C8, CYP3A4, and CYP3A5. C, the enzyme kinetics of N-DMI formation by CYP2C8, CYP3A4, and CYP3A5. D, depletion of 0.05 µM N-DMI by CYP2C8, CYP3A4, and CYP3A5. The protein concentration was 0.5 mg/ml in all incubations, except in the enzyme kinetic study, where it was 0.1 mg/ml, resulting in variable P450 concentrations in the incubations (see Materials and Methods for exact P450 concentrations). The data points represent mean±SD values of triplicate incubations.

Figure 3
Pharmacokinetic simulations of the single-dose pharmacokinetics of imatinib. A and B, mean simulated and observed imatinib and N-DMI plasma concentrations in 33 healthy volunteers after a single oral dose of imatinib 400 mg. Lines are simulated mean concentration time-profiles of ten trials (dotted lines represent 95% CI:s of the mean), whereas the circles refer to clinical data from Nikolova et al. (2004).

Fig. 4.
Simulated effects of multiple dosing on imatinib pharmacokinetics and enzyme activities. A and B, simulated mean steady-state concentrations of imatinib and N-DMI during administration of imatinib 400 mg daily, when MBI of CYP3A4 by imatinib is included in the model (final model; full line) or when it is excluded from the model (dotted line). C and D, the simulated mean effects of different doses of imatinib on the intestinal and hepatic amounts of active CYP3A4 (as % of initial active amount when treatment is initiated at time 0, and MBI of CYP3A4 is included in the simulation model). E, the simulated importance of CYP2C8 and CYP3A4 in imatinib pharmacokinetics during treatment with imatinib 400 mg once daily (o.d.) or twice daily (b.i.d.). The curves for CYP1A2, CYP2B6, CYP2D6, and CYP3A5 were omitted for clarity, because their roles were negligible (<5% of total hepatic clearance). The simulations were carried out in 100 North European Caucasian subjects.

Fig. 5.
Simulated effects of CYP2C8 polymorphisms and CYP2C8 and CYP3A4 inhibitors on imatinib pharmacokinetics. A and B, simulated steady-state concentrations of imatinib and N-DMI in a typical 60-year old man with different CYP2C8 activities; normal (CYP2C8 reference genotype), 50% decrease (low activity CYP2C8 genotype) or 100% increase (high activity CYP2C8 genotype) in hepatic CYP2C8 activity, after imatinib 400 mg once daily. The line at 1,000 ng/ml in A represents the suggested therapeutic trough concentration of imatinib. C-F, simulation of the effects of a CYP2C8 inhibitor (gemfibrozil) and a CYP3A4 inhibitor (itraconazole) on the pharmacokinetics of imatinib. The simulated effects of a CYP2C8 inhibitor (gemfibrozil 600 mg twice daily), a CYP3A4 inhibitor (itraconazole 100 mg twice daily, except first dose 200 mg) and their combination on imatinib and N-DMI pharmacokinetics after a single dose of imatinib 400 mg on day 3 (C and D) and multiple doses of imatinib 400 mg once daily (E and F), in 30 North European Caucasian subjects.
### Tables

**Table 1**

Enzyme kinetic parameters of imatinib metabolism determined on the basis of recombinant P450 enzyme studies and prediction of the hepatic clearance of imatinib by use of the static ISEF model.

<table>
<thead>
<tr>
<th>P450 isoform</th>
<th>Pathway</th>
<th>CL_{int} (µl/min/pmol)</th>
<th>V_{max} (pmol/min/pmol)</th>
<th>K_{m} (µM)</th>
<th>f_{umic}</th>
<th>ISEF^a</th>
<th>ISEF- and P450 abundance adjusted CL_{int} (µl/min/mg protein)^b</th>
<th>Scaled CL_{int}</th>
<th>CL_{H} (l/h)</th>
<th>% total CL_{H}</th>
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This article has not been copyedited and formatted. The final version may differ from this version.
Abbreviations: $f_{u,mic}$, unbound fraction of imatinib in microsomal incubations.

a Intersystem extrapolation factors used in the Simcyp compound file and in the static model calculations; 'BD Sup' indicates default values within Simcyp for incubations conducted with microsomes from BD Biosciences, whereas 'User' denotes in house values; 1.16 for CYP2C8, 0.09 for CYP3A4, and 0.06 for CYP3A5. Because the P450 activity between different recombinant enzyme lots may vary, in house ISEF factors for the key enzymes in imatinib metabolism were preferred. Furthermore, the best accuracy in interaction predictions was obtained using these values. The in house ISEF factors are based on $V_{\text{max}}$ for amodiaquine N-deethylation and midazolam 1'-hydroxylation in recombinant CYP2C8, CYP3A4, CYP3A5, and in pooled HLM.

b The average P450 hepatic abundance values used were 52 pmol/mg protein for CYP1A2, 17 pmol/mg protein for CYP2B6, 24 pmol/mg protein for CYP2C8, 8 pmol/mg protein for CYP2D6, 137 pmol/mg protein for CYP3A4, and 103 pmol/mg protein for CYP3A5 (these are average values of the North European Caucasian population within Simcyp).

c Calculated contributions of each P450 enzyme to the total hepatic clearance of imatinib.

d The unbound fractions of imatinib had previously been determined (Filppula et al., 2012), and vary between 0.8 and 0.9 depending on the protein concentration used in the experiment (0.5 or 0.1 mg/ml, respectively). The CLtot values for ‘other metabolites’ were calculated as the difference between $CL_{\text{int,u, ima dep}}$ and $CL_{\text{int,u, N DMI form}}$ and a $f_{u,mic}$ of 1 was entered in Simcyp in this case.
Table 2
Enzyme kinetic parameters N-DMI metabolism determined on the basis of recombinant P450 enzyme studies and prediction of the hepatic clearance of N-DMI by use of the static ISEF model

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<th>P450 isoform</th>
<th>Pathway</th>
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<th>f&lt;sub&gt;unmic&lt;/sub&gt;</th>
<th>ISEF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ISEF- and P450 abundance adjusted CL&lt;sub&gt;int&lt;/sub&gt; (µl/min/mg protein)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Scaled CL&lt;sub&gt;int&lt;/sub&gt; (l/h)</th>
<th>CL&lt;sub&gt;H&lt;/sub&gt; (l/h)</th>
<th>% total CL&lt;sub&gt;H&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
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<td>CYP1A2</td>
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<td>BD Sup</td>
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Abbreviations: f<sub>unmic</sub>, unbound fraction of N-DMI in microsomal incubations.

<sup>a</sup> Intersystem extrapolation factors used in the Simcyp compound file and in the static model calculations; 'BD Sup' indicates default values within Simcyp for incubations conducted with microsomes from BD Biosciences, whereas 'User' denotes in house values; 1.16 for CYP2C8, 0.09 for CYP3A4, and 0.06 for CYP3A5. Because the P450 activity between different recombinant enzyme lots may vary, in house ISEF factors for the key enzymes in imatinib metabolism were preferred. Furthermore, the best accuracy in interaction predictions was obtained using these values. The in house ISEF factors are based on V<sub>max</sub> for amodiaquine N-deethylation and midazolam 1'-hydroxylation in recombinant CYP2C8, CYP3A4, CYP3A5, and in pooled HLM.

<sup>b</sup> The average P450 hepatic abundance values used were 52 pmol/mg protein for CYP1A2, 24 pmol/mg protein for CYP2C8, 8 pmol/mg protein for CYP2D6, 137 pmol/mg protein for CYP3A4, and 103 pmol/mg protein for CYP3A5 (these are average values of the North European Caucasian population within Simcyp). Because the sum of the ISEF- and P450 abundance adjusted CL<sub>int</sub> values from recombinant P450 enzymes was equivalent to about 60% of the HLM CL<sub>int</sub> value (20 µl/min/mg), the model for N-DMI in Simcyp also included an additional HLM CL<sub>int</sub> value of 7.9 µl/min/mg.

<sup>c</sup> Calculated contributions of each P450 enzyme to the total hepatic clearance of N-DMI, assuming that the total CL<sub>H</sub> equals 1.7 l/h (calculated based on the HLM CL<sub>int</sub> value).
Figure 1

(A) Imatinib (% remaining after 30 min) for various CYP enzymes with 0.1 μM and 1 μM imatinib.

(B) N-DMI formation rate (pmol/min/μmol) for various CYP enzymes.

(C) N-DMI formation rate (% of control) for various compounds with 1 μM imatinib.

(D) N-DMI (% remaining after 30 min) with 0.1 μM N-DMI.
Figure 3

(A) Imatinib (ng/ml) vs. Time (h)

(B) N-DMI (ng/ml) vs. Time (h)

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Nikolova et al. (2004)