Impact of CYP2C8 single nucleotide polymorphisms on in-vitro metabolism of imatinib to N-desmethyl imatinib

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Abstract

Imatinib is a first line therapy for the treatment of chronic myeloid leukaemia (CML). Treatment with imatinib must be continuous and indefinite for most patients to maintain disease control. Despite excellent efficacy and tolerability, up to 50% of CML patients discontinue imatinib due to lack of efficacy and adverse events. Imatinib is metabolised to its main metabolite N-desmethyl imatinib by CYP3A4 and CYP2C8. *In vitro* human liver microsome (HLM) studies indicate imatinib autoinhibition of CYP3A4-mediated metabolism, suggesting a more significant role for CYP2C8 upon chronic dosing.

CYP2C8 is polymorphic and functional effects of the major *CYP2C8* polymorphisms *CYP2C8*3* and *CYP2C8*4* on N-desmethyl imatinib formation are unknown. It was hypothesised that *CYP2C8*3* and *CYP2C8*4* genetic polymorphisms will decrease imatinib metabolism to N-desmethyl imatinib in HLM. Therefore the aim of this study was to examine the impact of *CYP2C8*3* and *CYP2C8*4* on N-demethylation of imatinib in HLMs genotyped for *CYP2C8*1/*1* (n=5), *CYP2C8*1/*3* (n=4), *CYP2C8*1/*4* (n=2), in *CYP2C8*3/*3* pooled HLM, and in expressed CYP2C8 and CYP3A4 enzymes. Effects of CYP-selective chemical and antibody inhibitors on N-demethylation were also determined.

A single enzyme Michaelis-Menten model with substrate inhibition best fitted wild-type *CYP2C8*1/*1* HLM kinetic data (median ± SD K_i = 139 ± 61 µM). Three of four *CYP2C8*1/*3* HLMs showed single enzyme but no substrate inhibition kinetics. Binding affinity (K_m) was approximately 2-fold higher in *CYP2C8*1/*3* HLMs as compared to *CYP2C8*1/*1* (median ± SD K_m = 6 ± 2 *vs* 11 ± 2 µM, p=0.04). Intrinsic clearance (Cl_{int}) was higher in *CYP2C8*1/*3* HLMs compared to *CYP2C8*1/*1* (median ± SD Cl_{int} = 19 ± 8 *vs* 13 ± 2 µl/min/mg, p = 0.25).

*CYP2C8*3/*3* (pooled HLM) showed highest binding affinity (K_m = 3.6 µM) and weak autoinhibition (K_i = 449 µM) kinetics. N-desmethyl imatinib formation was below the limit of quantification in one *CYP2C8*1/*4* HLM, whereas the other *CYP2C8*1/*4* HLM showed lower intrinsic clearance (Cl_{int} = 7 *vs* 11 ± 2 µl/min/mg) due to 2-fold lower catalytic activity (V_{max}) compared to the wild-type (V_{max} = 73 *vs* 140 ± 31 pmol/min/mg).

A single enzyme model with substrate inhibition best fitted expressed CYP2C8 kinetic data (K_i = 149 µM). Expressed CYP3A4 showed two site enzyme kinetics with no evidence of autoinhibition. CYP2C8 inhibitors reduced N-demethylation in HLM by 47-75%, compared to 0-30% for CYP3A4 inhibitors. Two unidentified peaks M1 and M2 were found in expressed CYP3A4, whereas they were absent in expressed CYP2C8. These results indicate that *CYP2C8*3* may enhance CYP2C8 activity by influencing autoinhibition, and that *in vitro* the metabolism and autoinhibition of imatinib N-demethylation appears mainly mediated by CYP2C8 and not CYP3A4. *CYP2C8*4* appears a reduced functional allele for imatinib N-demethylation.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any University or other tertiary institution and, to the best of my knowledge and belief, contains no materials previously published or written by another person, except where due reference is made in the text.

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List of Abbreviations

ABL	Abelson murine leukaemia oncogene
AUC	Area under the plasma concentration-time curve
BCR	Break point cluster region gene
^{o}C	Degree Celsius
CI	Confidence interval
Cl_{int}	Intrinsic clearance
C_{max}	Maximum plasma concentration
CML	Chronic myeloid leukaemia
C_{trough}	Trough plasma concentration
CV	Co-efficient of variation
CYP450	Cytochrome P450
Cyt b5	Cytochrome b5
DL	Symbol for racemic mixture
DNA	Deoxyribonucleic acid
ER	Endoplasmic reticulum
h	Hill slope
HLM	Human liver microsomes
HLS	Human liver sample
HPLC	High performance liquid chromatography
HSA	Human serum albumin
IC_{50}	Half maximal inhibitory concentration
K_i	Substrate inhibition constant
K_m	Michaelis-Menten constant
LC-MS	Liquid chromatography mass spectrometry
LOQ	Limit of quantification
MAB-3A	Monoclonal antibody inhibitor of human CYP3A4

MAB-2C8	Monoclonal antibody inhibitor of human CYP2C8
µg	Microgram(s)
µl	Microliter(s)
µM	Micromolar
mg	Milligrams(s)
min	Minutes
ml	Milliliter(s)
mM	Millimolar
ng	Nanogram(s)
NADPH	Nicotinamide adenine dinucleotide phosphate
NDIM	N-desmethyl imatinib
p	Probability
Ph^+	Philadelphia chromosome
pmol	Picomole
QC	Quality control
rs	Reference SNP ID number
S	Substrate concentration
S_9	Supernatant fraction from liver (centrifuging at 9000 x g)
SD	Standard deviation
SNP	Single nucleotide polymorphism
$t_{1/2}$	Plasma half life
TKI	Tyrosine kinase inhibitor
UV	Ultraviolet
UGT	Uridine glucuronyl transferase
v	Reaction rate
Vd	Volume of distribution
V_{max}	Maximum formation rate