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 ($K_{m} = 3.6 \, \mu M$) and weak autoinhibition ($K_{i} = 449 \, \mu M$) 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 ($Cl_{int} = 7 \, vs \, 11 \pm 2 \, \mu l/min/mg$) due to 2-fold lower catalytic activity ($V_{max}$) compared to the wild-type ($V_{max} = 73 \, vs \, 140 \pm 31 \, pmol/min/mg$).

A single enzyme model with substrate inhibition best fitted expressed CYP2C8 kinetic data ($K_{i} = 149 \, \mu M$). 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.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying, unless permission has been granted by the University to restrict access for a period of time.

Adelaide, March 2015

Muhammad Suleman Khan
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To my family, thank you for your prayers and support.

To my wife, thank you for your love and care.
List of Abbreviations

**ABL** Abelson murine leukaemia oncogene

**AUC** Area under the plasma concentration-time curve

**BCR** Break point cluster region gene

**°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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAB-2C8</td>
<td>Monoclonal antibody inhibitor of human CYP2C8</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram(s)</td>
</tr>
<tr>
<td>µl</td>
<td>Microliter(s)</td>
</tr>
<tr>
<td>µM</td>
<td>Micromolar</td>
</tr>
<tr>
<td>mg</td>
<td>Milligrams(s)</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter(s)</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram(s)</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NDIM</td>
<td>N-desmethyl imatinib</td>
</tr>
<tr>
<td>p</td>
<td>Probability</td>
</tr>
<tr>
<td>Ph⁺</td>
<td>Philadelphia chromosome</td>
</tr>
<tr>
<td>pmol</td>
<td>Picomole</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>rs</td>
<td>Reference SNP ID number</td>
</tr>
<tr>
<td>S</td>
<td>Substrate concentration</td>
</tr>
<tr>
<td>S₉</td>
<td>Supernatant fraction from liver (centrifuging at 9000 x g)</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>Plasma half life</td>
</tr>
<tr>
<td>TKI</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UGT</td>
<td>Uridine glucuronyl transferase</td>
</tr>
<tr>
<td>ν</td>
<td>Reaction rate</td>
</tr>
<tr>
<td>Vd</td>
<td>Volume of distribution</td>
</tr>
<tr>
<td>Vₘₐₓ</td>
<td>Maximum formation rate</td>
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