Drug metabolites as contributors to clinical drug-drug interactions

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AstraZeneca Gothenburg, Sweden

2400 employees, including more than 300 scientists from nearly 50 countries
Drug metabolites as contributors to clinical DDIs

BACKGROUND INFORMATION

Metabolites present at 25% of the parent AUC or 10% of total drug-related exposure should trigger further characterization with regard to DDI risk (EMA & FDA guidance).

Metabolites of some CYP inhibitors have significant impact in addition to parent drug.

Metabolites as main perpetrators of DDI: Limited examples: bupropion, amiodarone, sertraline, gemfibrozil.
AZD1981: An oral CRTh2 receptor antagonist

LogD$_{7.4}$ = -0.12

Solubility > 1 mM

Human plasma protein binding = 97.5%

CACO2 AB Papp = 1 x 10$^{-6}$ cm/s (efflux ratio = 2)

CYP inhibition: all > 20 µM (no time dependent CYP inhibition detected)

Active uptake into rat and human hepatocytes detected

Metabolic stability high (human hepatocyte CL$_{int}$ < 3 µL/min/million cells)

Low lipophilicity, high solubility molecule with low/moderate in vitro permeability & no concerns over drug-drug interaction risk at Candidate Drug nomination
AZD1981 metabolites identified in human hepatocyte incubations

AZD1981 acyl glucuronide

AZD1981 N-deacetylated amino acid

LogD$_{7.4}$ = 0.22

AZD1981 sulphoxide

More lipophilic metabolite than parent identified (N-deacetylated AZD1981) plus acyl glucuronide - but levels of all very low in vitro

Metabolite detection inconsistent due to low levels formed in vitro (2 hour suspension incubation) & no indication of which metabolite(s) may be considered major in a clinical setting
A primary human hepatocyte assay for Low CL_{int}

HµREL primary human hepatocyte/stromal cell co-cultures allow definition of statistically significant CL_{int} to 0.2 µL/min/million cells – unfortunately not available when AZD1981 was nominated.
Improved metabolite identification performance

Hultman, Vedin, Abrahamsson, Winiwarter & Darnell, 2016, Mol Pharm., 13:2796
Early clinical excretion balance study with $[^{14}\text{C}]-\text{AZD1981}$

**URINE:** AZD1981 (15%), acylgluc (11%), sulphoxide (10%)

**FAECES:** AZD1981 (20%), N-deacetylated AZD1981 plus other metabolites (< 5% each)

AZD1981 comprised 60% of the drug-related material (radioactivity) in plasma

Most abundant metabolite present in plasma was N-deacetylated AZD1981 (9% of total radioactivity)
Investigation of DDI risk for N-deacetylated AZD1981

Metabolite approximately 40x more potent a reversible inhibitor of CYP2C9
CYP2C9 time dependent inhibition analysis

AZD1981: no TDI

N-deacetylated AZD1981: $K_I = 10 \, \mu M$, $k_{\text{inact}} = 0.02 \, \text{min}^{-1}$

Very weak TDI ($k_{\text{inact}}/K_I$ ratio of 2.3 $\mu \text{L.min}^{-1}$ nmoles$^{-1}$ approximately 50-fold lower inactivation efficiency than tienilic acid)
In vitro human hepatocyte uptake studies

AZD1981 & N-deacetylated AZD1981 subject to active hepatic uptake
In vitro hepatic uptake data analysis: parameter estimation

\[ K_{p,uu} = \frac{CL_{int,up} + CL_{int,diff}}{CL_{int,met} + CL_{int,diff}} \]

<table>
<thead>
<tr>
<th></th>
<th>AZD1981</th>
<th>N-deacetylated AZD1981</th>
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<tbody>
<tr>
<td></td>
<td>( \mu \text{L/min/million cells} )</td>
<td></td>
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<tr>
<td>active uptake (( CL_{int,up} ))</td>
<td>77</td>
<td>36</td>
</tr>
<tr>
<td>passive diffusion (( CL_{int,diff} ))</td>
<td>11</td>
<td>2.8</td>
</tr>
<tr>
<td>metabolism (( CL_{int,met} ))</td>
<td>0.24</td>
<td>0.77</td>
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\[ K_{p,uu} = 8 \quad 12 \]

Unbound \( C_{\text{max}} \) plasma concentrations multiplied by \( K_{p,uu} \) (free drug distribution coefficient) to estimate unbound drug concentration in liver for DDI prediction
Prediction of S-warfarin drug-drug interaction

Fahmi et al., 2009, DMD, 37, 1658.

fmCYP = fraction of S-warfarin clearance due to the inhibited CYP2C9 (set to a value of 0.9 for S-warfarin)

\[
\text{AUC}_{\text{inhibited}} / \text{AUC} = \frac{1}{A \times B \times f_{\text{mCYP}} + (1 - f_{\text{mCYP}})}
\]

\[
A = \frac{k_{\text{deg}}}{[K_{\text{inact}} \times [I]_{H,u} / (K_i + [I]_{H,u})] + k_{\text{deg}}}
\]

\[
B = \frac{1}{1 + ([I]_{H,u} / K_i)}
\]

\[k_{\text{deg}}\] and \(K_i\) are the maximal rate of CYP inactivation & the concentration of AZD1981 N-deacetylated amino acid taken to elicit half-maximal inactivation

\(k_{\text{deg}}\) is the rate constant describing the rate of CYP2C9 degradation in vivo (defined as 0.0001 min\(^{-1}\))

\(K_i\) is the reversible inhibition constant. \(K_i\) was calculated from \(IC_{50}/2\) assuming competitive inhibition

\([I]_{H,u}\) is the unbound hepatic concentration of AZD1981 N-deacetylated amino acid (\(C_{\text{max},u} \times K_{p,uu}\))

100 mg AZD1981 predicted to cause 2-fold increase of S-warfarin AUC

400 mg AZD1981 predicted to cause 3-fold increase of S-warfarin AUC
The Warfarin interaction study: N-deacetylated metabolite accounted for 12 - 20% of the total drug exposure.
S- but not R-Warfarin exposure increased by AZD1981

100 mg AZD1981

100 & 400 mg AZD1981 increased of S-warfarin AUC 1.4 & 2.4-fold
Summary messages

N-deacetylated AZD1981 is added to the small list of drug metabolites reported as sole contributors to clinical DDIs, with weak time-dependent inhibition exacerbated by efficient hepatic uptake being the cause.

Prediction of which metabolites may be defined as major in a clinical setting cannot be made with any degree of certainty from simply assessing the relative concentrations from in vitro metabolite identification studies.

Regulatory guidance states that metabolites present at greater than 10% of total drug-related exposure in humans, or 25% of the parent exposure, should trigger further investigation of their inhibitory potency.

Even minor risk-associated metabolites with borderline exposure risk (based on Regulatory guidance) should be explored very early in clinical development, making use of the clinical pharmacokinetic data coupled with in vitro DDI information to investigate interaction risk.

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