BACKGROUND

There is a growing interest in the importance of aldehyde oxidase (AO) metabolism; partly due to increased efforts avoiding extensive P450 metabolism and also using kinase inhibitors as therapeutic targets (Garattini and Terao, 2011; Pryde et al., 2010). In vitro assays and in vitro-in vivo extrapolation (IVIVE) strategies for AO are less robust than available for P450. Indeed, Zientek et al., (2010) reported that predicted intravascular clearance (CLv) values for four compounds partially metabolised by AO were 3- to 32- fold lower than observed. The authors developed a correlation approach to estimate in vivo AO intrinsic clearance (CLint,u). However, there is a need to refine in vitro and IVIVE methods, especially for substrates eliminated by multiple different pathways (where Human Liver Cytosol (HLC) and Human Liver Microsomal (HLM) data will be combined). In addition, no absolute AO protein abundance data are available and the importance of extrahepatic metabolism is unknown. Relative protein abundance data indicate widespread tissue distribution including liver, kidney and respiratory system (Moriwaki et al., 2001).

STUDY AIMS

Rationalise under-prediction of CLv, XK-469, O6-benzylguanine, zaleplon, DACA, zoniporide and carbarzner. Assessment of impact of:

1) Variable in vitro assay conditions in the literature - Metabolite formation vs substrate depletion. Human Liver Cytosol (HLC) vs Human Liver S9 (HLS9) (20 trials, 50 individuals, total n = 1000)
2) Additional in vitro and clinical data to Zientek et al., (2010)
3) Presystemic metabolism

METHODS

In vitro data selection:
Unbound intrinsic clearance values via AO (CLint,u) obtained from literature studies using HLC or HLS9. Preference for metabolite formation method (Vmax/Km).

Compound selection: Clinical data available and fraction formed by AO metabolism (fm AO) ≥ 20%.

IVIVE: Well-stirred liver model (Figure 1).
Simcyp® Population-based Simulator (V11) 20 trials, 50 individuals (n = 1000). CPPGL: 5 literature sources (Cubitt et al., 2011) plus 3 unpublished sources (n = 135).

Correlation to MPPGL (Barter et al., 2007, 2008). F0 = 0.75.

RESULTS

1) Variable in vitro assay conditions: Number of donors: 3-13, incubation time: 2-240 mins, protein concentration: 0.2-6 mg/ml, pH 7.0-7.8.

All studies used HLC. Zientek et al., (2010) also used HLS9.

Metabolite formation method: Higher CLint,u than substrate depletion for 4 of 5 drugs (Figure 2). Range in difference from 11% (zoniporide) to 3-fold (DACA).

HLS9: Higher CLint,u than HLC for 6 of 8 drugs (Figure 3). Range in difference from 33% (zaleplon) to 6-fold (6-deoxypenciclovir).

2) Additional elimination data: CLint,u, O6-benzylguanine, DACA, zaleplon, zoniporide (9 metabolite formation studies). CLint,u, O6-benzylguanine, DACA, carbarzner (3 clinical studies). fm OA: (Table 1) Comparable to Zientek et al., (2010) values for XK-469, DACA, carbarzner and zoniporide. However, fm OA values for O6-benzylguanine were 2-fold and 17% lower in the current study, respectively.

Table 1: Contribution of CLv, CLint,u and CLpo to total elimination

<table>
<thead>
<tr>
<th>Drug</th>
<th>CLint,u (%)</th>
<th>CLpo (%)</th>
<th>fm OA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DACA</td>
<td>0.2</td>
<td>50.6</td>
<td>45.6</td>
</tr>
<tr>
<td>Carbarzner</td>
<td>0.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>0.0</td>
<td>60.4</td>
<td>40.4</td>
</tr>
<tr>
<td>Zoniporide</td>
<td>17.7</td>
<td>77.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Accuracy of predicted CLint,u (Figure 4) Observed CLv was under-predicted by in vitro CLint,u by 2.2- to 5.4- fold using HLC data (zoniporide and O6-benzylguanine) and by 13% to 11-fold using HLS9 data (XK-469 and DACA).

3) Presystemic metabolism: Clinical data for both CLiv and CLpo were available for carbarzner and zaleplon and F was calculated as 0.02 and 0.32, respectively. CLiv for 5 of 6 drugs with available CLiv data was >90% of Qr. For carbarzner and zoniporide, CLiv was 7% and 2-fold higher than Qr, respectively. An apparent CLiv that is > Qr indicates a contribution from presystemic metabolism (lun) and/or additional systemic metabolic clearance (kidney).

CONCLUSIONS

- A significant improvement of CLiv and CLiv was predicted using it carefully selecting and combining additional sources of elimination data
- However, there is still a significant under-prediction of CLiv

Potential reasons for under-prediction of clearance:
- In vitro assay. Metabolite formation method
- Need for further assay development of incubation conditions
- Extrahepatic metabolism by AO, eg., Lung, kidney and gut
- CPPGL and SPPGL are not corrected for loss of protein during preparation
- Limited clinical data
- Lack of information on other elimination routes
- Biliary clearance (efflux transport), eg. for XK-469 (Anderson et al., 2005)
- Other metabolism (P450, xanthine oxidase etc.)
- There is a need for further evaluation of absolute abundance, in order to accurately assess population variability and drug-drug interaction potential.

REFERENCES

Anderson, L. W. et al. (2005). Cancer Chemother Pharmacol. 56; 351
Barter, Z. et al. (2007). CCM, 8: 33
Moriwaki, Y. et al. (2001). Histol Histopathol, 16; 745