An investigation into the prediction of *in vivo* clearance for a range of aldehyde oxidase substrates using a mechanistic population-based pharmacokinetic model

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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 intravenous clearance (CL_{iv}) 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 ($CL_{int,AO}$). 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 CL_{IV}: XK-469, O6-benzylguanine, zaleplon, DACA, zoniporide and carbazeran. **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)

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 ($CL_{int,AO}$) obtained from literature studies using HLC or HLS9. Preference for metabolite formation method (Vmax/Km).

Compound selection: Clinical data available and fraction eliminated 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 **CPPGL:** 5 literature sources (Cubitt *et al*., 2011) plus 3 unpublished sources (n = 135).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). r^2=0.75.

S9PPGL = CPPGL + MPPGL (mg cytosolic, S9 or microsomal protein per gram liver)

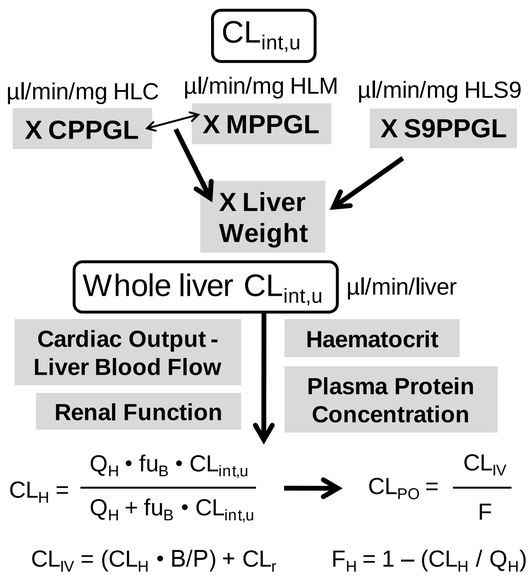

Figure 1. Incorporation of population-specific variability Indicated by grey boxes

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 $CL_{int,AO}$ than substrate depletion for 4 of 5 drugs (**Figure 2**). Range in difference from 11% (zoniporide) to **3-fold** (**DACA**).

HLS9: Higher $CL_{int,AO}$ than HLC for 6 of 8 drugs (**Figure 3**). Range in difference from 33% (zaleplon) to **6- fold** (**6-deoxypenciclovir**).

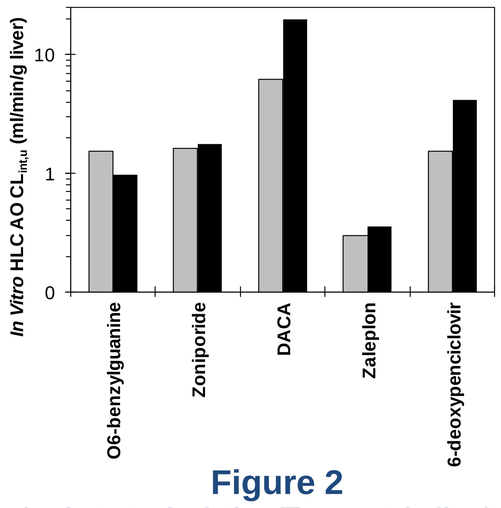

Figure 2

Impact of substrate depletion ■ vs metabolite formation ■ methods on $CL_{int,AO}$. Data from 8 *in vitro* studies

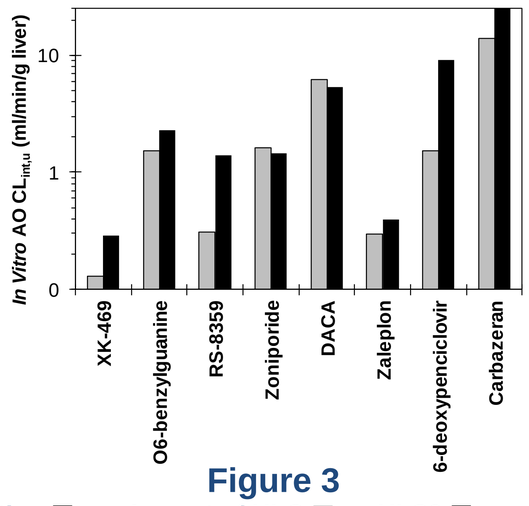

Figure 3

Impact of HLC ■ vs HLS9 ■

Data from Zientek *et al*., 2010

Data in Figures 2 and 3 scaled with average CPPGL and S9PPGL of 81 and 121 mg/g liver, respectively

2) Additional elimination data $CL_{int,AO}$: O6-benzylguanine, DACA, zaleplon, zoniporide (9 metabolite formation studies). **CL_{iv}:** O6-benzylguanine, DACA, carbazeran (3 clinical studies). **CL_r:** Zaleplon, O6-benzylguanine (3 clinical studies). **fm_{AO}:** (**Table 1**) Comparable to Zientek *et al*., (2010) values for XK-469, DACA, carbazeran and zoniporide. However, fm_{AO} values for **O6-benzylguanine** and **zaleplon** were 2-fold and 17% lower in the current study, respectively.

Table 1. Contribution of CL_r, CL_{bil} and CL_{AO} to total elimination

After inclusion of the additional studies
f_e = fraction by non-metabolic CL_r
F_{bil} = fraction via CL_{bil}
f_m = fraction metabolised
– represents no data available,
[a] assumed equal metabolism CYP and AO

	f_e (%)	f_{bil} (%)	fm (%) AO	fm (%) other
O6-benzylguanine	0.2	-	50 [a]	50 [a]
Carbazeran	0.0	0.0	100	0.0
DACA	-	-	100	0.0
XK-469	2.0	-	98	0.0
Zaleplon	0.0	-	60	40
Zoniporide	17	1.0	77	5.0

Accuracy of predicted CL_{iv}: (Figure 4) Observed CL_{iv} was under-predicted by *in vitro* $CL_{int,AO}$ 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).

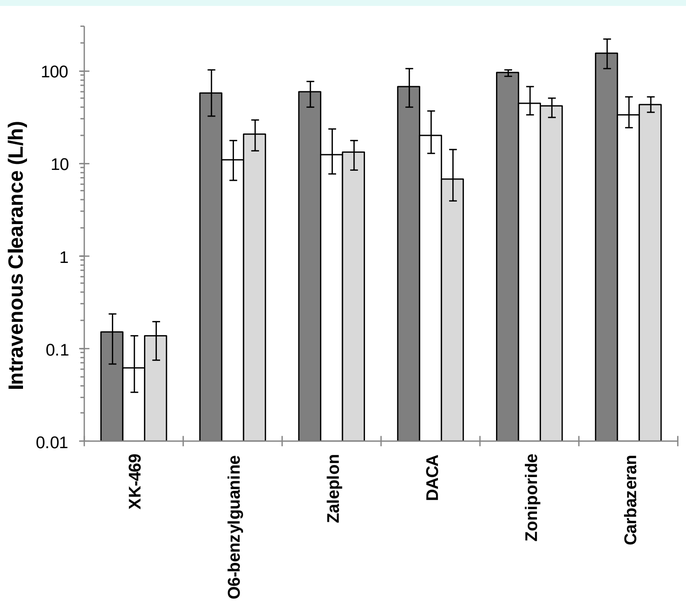

Figure 4.
Accuracy of predicted CL_{IV}

Observed CL_{iv} ■ and
Simulated CL_{iv} using HLC □ and HLS9 ■

Data points: Geometric mean CL_{IV}

Simulated data: Scaled with individual scaling factors as Figure 1 (20 trials, 50 individuals, total n = 1000)

Error bars: 90% confidence intervals.

3) Presystemic metabolism Clinical data for both CL_{IV} and CL_{PO} were available for carbazeran and zaleplon and F was calculated as 0.02 and 0.32, respectively. CL_H for 5 of 6 drugs with available CL_{IV} data was >90% of Q_H. For carbazeran and zoniporide, CL_H was 7% and 2-fold higher than Q_H, respectively. An apparent CL_H that is > Q_H indicates a contribution from presystemic metabolism (lung) and / or additional systemic metabolic clearance (kidney).

CONCLUSIONS

- A significant improvement of CL_{IV} and CL_{PO} prediction was seen by carefully selecting and combining additional sources of elimination data
- However, there is still a significant under-prediction of CL_{IV}

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 S9PPGL 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.

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