An Investigation into the use of an Empirical Scaling Strategy for the Prediction of *In Vivo* Aldehyde Oxidase Clearance

Helen Humphries, Karen Rowland Yeo, Zoe Barter and Iain Gardner

Simcyp Ltd (a Certara company)

Blades Enterprise Centre, Sheffield, S2 4SU

BACKGROUND

- There is an increasing awareness of the importance of aldehyde oxidase (AO) to drug metabolism [1,2].
- *In vitro* assays and *in vitro-in vivo* extrapolation (IVIVE) strategies for AO are less robust than available for P450 and there is a need for further research and refinement [2,3].
- An under-prediction of *in vivo* clearance is often seen using *in vitro* human liver data from cytosol (HLC), S9 (HLS9) or hepatocytes (HHEP) [3,4,5].
- Absolute AO protein abundance data for human liver cytosol (HLC) have recently been published [6]. However, the importance of extrahepatic AO to drug metabolism is still unclear.
- mRNA and relative protein abundance data indicate widespread distribution including liver, kidney, respiratory system and adrenal gland [7,8].

STUDY AIMS

- To assess published literature for AO substrates with available *in vitro* liver AO intrinsic clearance ($CL_{int,AO}$) and clinical intravenous and/or oral clearance (CL_{IV} or CL_{PO}) data.
- To compare *in vivo* AO clearance prediction accuracy from *in vitro* liver data obtained using HLC, HLS9 and HHEP systems.
- To investigate the benefits of an empirical scaling strategy to improve *in vivo* clearance prediction accuracy using *in vitro* liver data for AO substrates.

METHODS

- *In vitro* $CL_{int,AO}$ data were used to predict *in vivo* hepatic AO blood clearance ($CL_{H,AO}$) using the well-stirred liver model and a simulated healthy volunteer population n=1000 (Simcyp V13).

Incorporation of inter-individual variability in scaling factors, geometric mean (90% CI): 79 (49-117) mg cytosolic protein per gram liver, 114 (77-169) mg S9 protein per gram liver, 109 (77-155) x10^6 cells per gram liver, 1597 (1206-2116) g liver weight and 86 (74-100) L/h liver blood flow (Q_H).

- Observed *in vivo* $CL_{H,AO}$ values were obtained from CL_{IV} and CL_{PO} data, accounting for the fraction metabolised by AO (fm_{AO}) and any renal or biliary excretory clearance ($CL_{excretory}$)

$$CL_{H,AO} = \frac{CL_{IV} - CL_{excretory}}{B:P} \bullet fm_{AO} \qquad CL_{H,AO} = \frac{(CL_{PO} \bullet fa \bullet F_G \bullet F_H) - CL_{excretory}}{B:P} \bullet fm_{AO}$$

- **CL_{PO} data:** fa and F_G assumed to be 1 due to a lack of data. F_H assumed to be 1 except where $CL_{H,AO}$ ≥ 2-fold higher than Q_H (Table 1)
- Comparison of predicted and observed $CL_{H,AO}$ in order to assess if there is an empirical relationship.
- 3 approaches were assessed:
  - ***1. CL_{IV} data only***
  - ***2. All CL_{IV} and CL_{PO} data***
  - ***3. Test set of CL_{PO} data (using CL_{IV} relationship)***

RESULTS

Table 1. Observed *in vivo* clearance data for twelve AO substrates from clinical data

	CL_{IV} (L/h)	CL_{PO} (L/h)	$f_{m,AO}$	$CL_{H,AO}$ (L/h)
BIBX1382	156	2447	1.00	156
O6-benzylguanine	58	-	0.83	54
Carbazeran	154	7382	0.52	114
DACA	78	-	0.65	59
6-deoxypenciclovir	-	626	1.00	86[a]
FK3453	-	2777	1.00	86[a]
PF-4217903	-	25	1.00	28
PF-945863	-	454	1.00	86[a]
RS-8359	-	33	1.00	27
XK-469	0.15	-	1.00	0.26
Zaleplon	60	188	0.63	51
Zoniporide	96	-	0.60	49

from CL_{IV}

from CL_{PO}

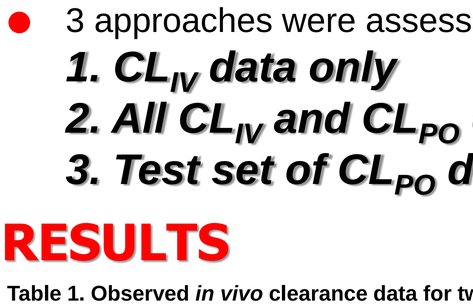
[a] Limited at 86 L/h. Assumption of F_H = 1 resulted in $CL_{H,AO}$ value >3-fold higher than Q_H

Data are from an analysis of 15 clinical studies (n = 285)
Values are geometric mean
fm_{AO} values from [9] or clinical mass balance data

- $CL_{H,AO}$ ranged between 0.26 L/h (XK-469) and 156 L/h (BIBX1382)
- CL_{IV} data were available for 7 AO substrates

1. CL_{IV} data only

Figure 1. Observed *in vivo* $CL_{H,AO}$ versus liver blood flow for seven AO substrates with available CL_{IV} data



$CL_{H,AO}$ values are from clinical data (Table 1)

Numbers above data points are the number of individuals for which clinical CL_{IV} data were available

Q_H values were simulated using a healthy volunteer population (n = 1000). Solid horizontal line is geometric mean value of 86 L/h, dashed lines are 90% CI 74 – 100 L/h

- There was a need for more CL_{IV} data, *eg.,* $CL_{H,AO}$ values for carbazeran and zoniporide were from <10 clinical subjects
- BIBX1382 and carbazeran: $CL_{H,AO}$ was clearly > Q_H (81% and 33% difference of geometric mean, respectively)
- O6-benzylguanine and DACA: $CL_{H,AO}$ could be > Q_H for some individuals

1. CL_{IV} data only

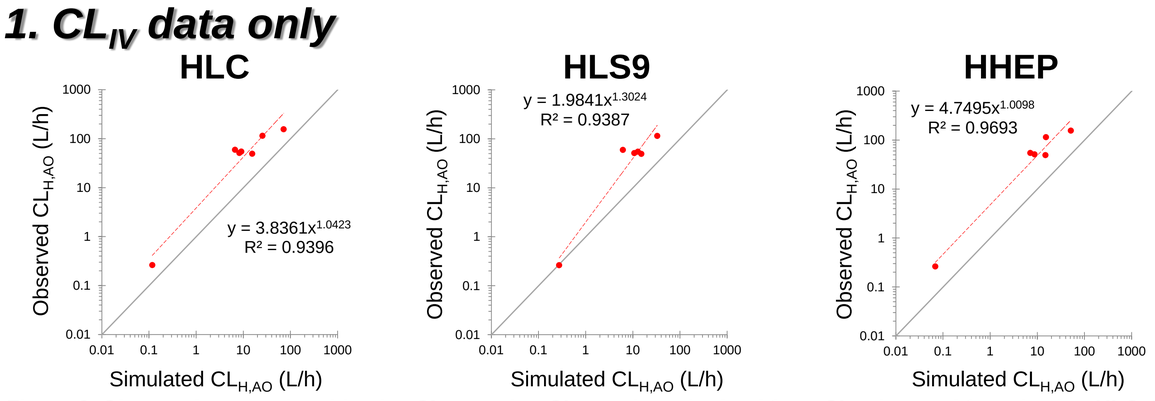


Figure 2. Observed vs simulated *in vivo* $CL_{H,AO}$ using $CL_{int,AO}$ data obtained from CL_{IV} data and from *in vitro* HLC, HLS9 and HHEP systems. Simulated data are geometric mean from a simulated population of healthy volunteers n = 100. Data points are (order of increasing Observed $CL_{H,AO}$) **HLC:** XK-469, Zoniporide, Zaleplon, O6-benzylguanine, DACA, Carbazeran, BIBX1382; **HLS9:** as HLC minus BIBX1382; **HHEP:** as HLC minus DACA. *In vitro* data from [3-5].

- With the exception of XK-469 (HLS9), an under-prediction of observed $CL_{H,AO}$ was seen for all compounds using all *in vitro* liver systems
- The extent of under-prediction ranged between 2-fold (BIBX1382) and 10-fold (DACA) and did not appear to be affected by choice of *in vitro* system
- Excluding XK-469, relationships were: **HLC:** $y = -0.025x^2 + 3.6083x + 23.833$ ($r^2 = 0.90$); **HLS9:** $y = 0.1645x^2 - 4.3962x + 80.32$ ($r^2 = 0.99$); **HHEP:** $y = -0.0458x^2 + 5.0947x + 15.285$ ($r^2 = 0.77$)

2. All CL_{IV} and CL_{PO} data

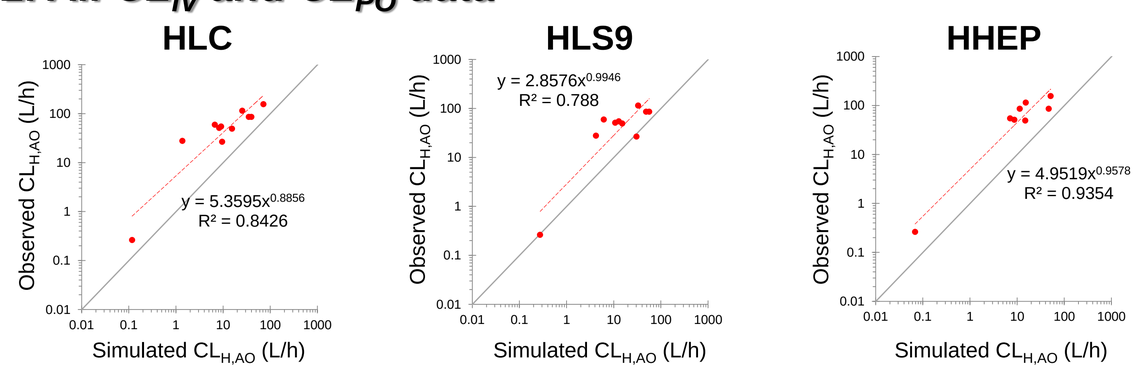


Figure 3. Observed vs simulated *in vivo* $CL_{H,AO}$ using $CL_{int,AO}$ data obtained from CL_{IV} and CL_{PO} data and from *in vitro* HLC, HLS9 and HHEP systems. Simulated data are geometric mean from a simulated population of healthy volunteers n = 100. Data points are (order of increasing Observed $CL_{H,AO}$) **HLC:** XK-469, RS-8359, PF-4217903, Zoniporide, Zaleplon, O6-benzylguanine, DACA, Carbazeran, BIBX1382, 6-deoxypenciclovir, PF-945863; **HLS9:** as HLC minus BIBX1382; **HHEP:** as HLC minus RS-8359, PF-4217903, DACA and PF-945863 plus FK3453. *In vitro* data from [3-5].

- With the exception of XK-469 and RS-8359 (HLS9), an under-prediction of observed $CL_{H,AO}$ was seen for all compounds using all *in vitro* liver systems
- **HLC:** Range in extent of under-prediction was 2-fold (BIBX1382) to 20-fold (PF-4217903). This was not affected by choice of *in vitro* system except for PF-4217903 and RS-8359 (3-fold better prediction using HLS9 vs HLC)
- Excluding XK-469, relationships were: **HLC:** $y = -0.0025x^2 + 1.8656x + 31.689$ ($r^2 = 0.82$); **HLS9:** $y = -0.0083x^2 + 1.4186x + 34.789$ ($r^2 = 0.38$); **HHEP:** $y = 0.0042x^2 + 1.2135x + 54.862$ ($r^2 = 0.49$)

3. Test set CL_{PO} data (using CL_{IV} relationship)

Table 2. Impact of using an empirical relationship from CL_{IV} data (Section 1.) for compounds where only CL_{PO} values were available (Section 3).

	Fold Under-Prediction From HLC Data	
	Minus Empirical Relationship	Plus Empirical Relationship[a]
6-deoxypenciclovir	2.4	0.7
PF-4217903	20	1.0
PF-945863	2.1	0.7
RS-8359	2.8	0.5

[a] Empirical relationship for HLC excluding XK-493: $y = -0.025x^2 + 3.6083x + 23.833$ where y = Observed $CL_{H,AO}$ and x = Predicted $CL_{H,AO}$

- Prediction of observed $CL_{H,AO}$ was improved by the use of the empirical relationship for the limited test set of four compounds

CONCLUSIONS

- $CL_{H,AO}$ can be significantly > Q_H, which suggests that extrahepatic AO metabolism is important
- A preferred scaling strategy would incorporate extrahepatic AO abundance and activity. There is currently a lack of these data
- In the meantime, the above relationships could be used to assess a potential range in predicted *in vivo* AO clearance for new compounds in development
- However, there is a need for more *in vitro* and clinical AO data in order to improve the accuracy and validate the empirical scaling strategy before implementation in the simulator

Can Consortium Members help with this?
Please email: h.humphries@simcyp.com

REFERENCES

[1] Pryde, D. C. *et al.,* (2010). J Med Chem. 53. 8441
[2] Hutzler, M. *et al.,* (2013). EODMT. 9 (2): 153
[3] Zientek, M. *et al.,* (2010). DMD, 38 (8): 1322
[4] Hutzler, M. *et. al.,* (2012). DMD. 40: 467-275
[5] Akabane *et al.,* (2012). Xenobiotica. 42 (9): 863-871
[6] Fu, C. *et al.,* (2013). DMD, [Epub ahead of print]
[7] Moriwaki, Y. *et al.,* (2001). Histol Histopath, 16: 745
[8] Nishimura and Naito (2006). DMPK. 21 (5): 357-374
[9] Strelevitz, T. J. *et al.,* (2012). DMD. 40: 1441-1448