Use of a PBPK modelling approach for aldehyde oxidase substrates

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Background

Understanding **Aldehyde oxidase (AO)** clearance of compounds has become increasingly important in many drug discovery projects as compounds with less propensity for metabolism and interaction of CYP isozymes are being synthesised. However the less extensive knowledge about AO activity and abundance has led to **poor prediction of *in vivo* clearance (CL)** and consequently to clinical failure of some AO substrates [1,2].

In this work we aimed to assess the prediction of intravenous (IV) clearance of six AO substrates from *in vitro* data.

Methods

The involvement of AO in the metabolism of **O6-benzylguanine, BIBX1382, carbazeran, zaleplon, zoniporide and ziprasidone** was investigated.

AO activity was measured in human liver cytosol (HLC) (from Corning Life Sciences,Ultrapool of 150 donors) using 1 mg/ml of final matrix concentration. After a pre-incubation period of 5 min at 37°C experiments were initiated by addition of test compound with a final concentration of 0.1 µM. HLC were incubated for 2 hours with sampling time at 0, 10, 30, 60, 90 and 120 min.

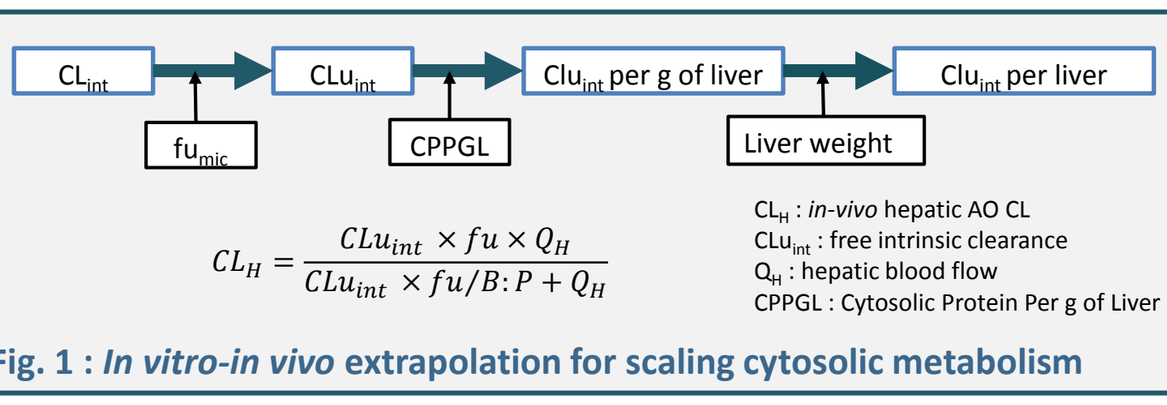

Fig. 1 : *In vitro-in vivo* extrapolation for scaling cytosolic metabolism

The IVIVE scaling approach uses the **well-stirred liver model** with a simulated average cytosolic protein per gram of liver (**CPPGL**) yield of 76.97 mg/g and a liver weight of 1570 g (Fig. 1).

The **blood-to-plasma ratio (B:P)**, fraction unbound in plasma (**fu**) and in microsomes (fu_{mic}) were also measured and used to develop **physiologically based pharmacokinetic (PBPK) models** for each compound. Physicochemical properties were gathered and minimal PBPK models with a predicted volume of distribution from the Rodgers and Rowlands method were used [3].

The contribution of **microsomal metabolism, renal and biliary excretion** to clearance was added to the PBPK models when applicable.

All simulations were performed using **Simcyp V17R1** and the healthy volunteer population with an age range of 30-50 years and a proportion of 50% female.

For comparison the CL_{IV} were also predicted with $CL_{int,AO}$ values obtained from the literature. Finally the AO scaling approach was verified by comparing the predicted to observed CL_{IV}.

Results

Using newly generated *in vitro* data the average **CL_{IV} fold difference was 4.1** and ranged from 1.6 to 6.2 (Fig. 2). The best prediction was obtained for BIBX1382 (1.6 fold) and the biggest difference for zaleplon (6.2 fold). Assuming an fu_{mic} of 1 the average CL_{IV} fold difference was increased to 5.2 with a range from 2.1 to 8.6.

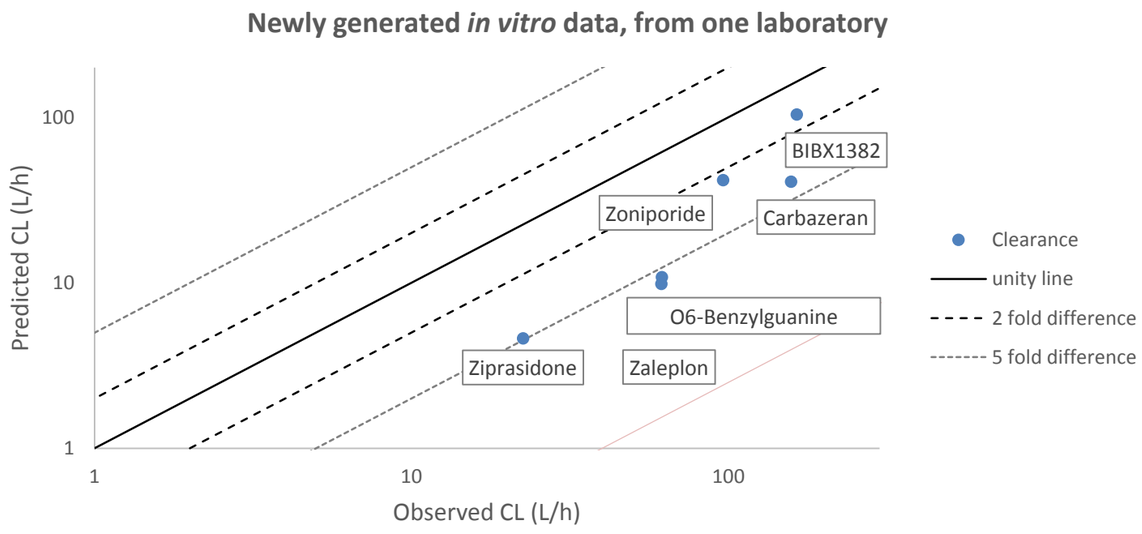

Fig. 2 : Predicted CL_{IV} of 6 AO substrates scaled from in house data

Results (con't)

The predicted fraction metabolised by AO were ranked as followed carbazeran > BIBX1382 > O6-benzylguanine > zaleplon > zoniporide > ziprasidone.
There was no correlation between the fraction metabolised (fm) by AO and the underprediction of CL (Fig. 3).

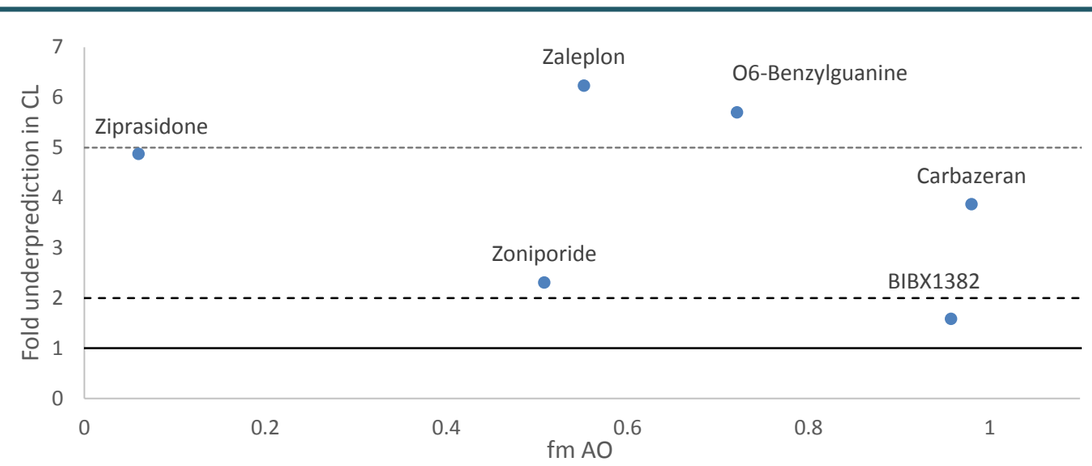

Fig. 3 : Impact of fm AO on the fold prediction in CL_{IV}

An **average fold difference of 2.9** (range: 1.5-4.4) was obtained with literature $CL_{int,AO}$ (Fig. 4). Adding the **renal metabolism** assuming a CPPGK of 40.58 mg/g and the same activity per mg of cytosol protein an average fold of **2.6** (range: 1.1-4.0) was observed.

The maximum change activity was observed for BIBX1382 and Carbazeran (+35% and 31%).

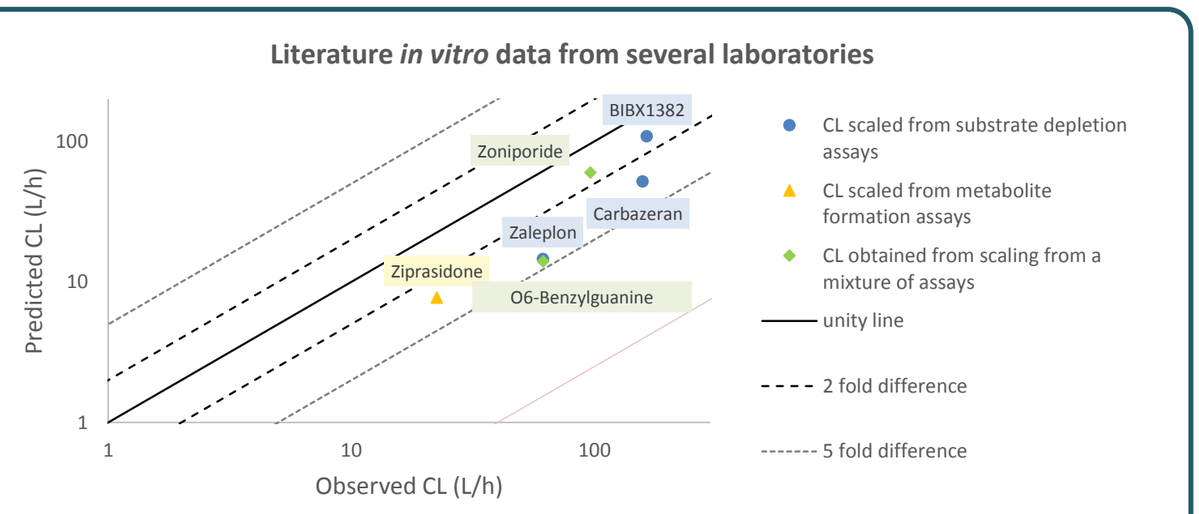

Fig. 4 : Predicted CL_{IV} of 6 AO substrates scaled from literature data

The average *in vitro* intrinsic clearances obtained from the literature were higher and gave better CL_{IV} prediction. It could be explained by different activity in AO in the different human liver cytosolic fractions, or to the difference in the experiment (*i.e.* incubation and sampling time).

Conclusions and discussion

Overall, using HLC, the intravenous CL was under estimated by up to 6 fold, although an improved predictability was observed in comparison to the 5- to 40- fold lower IV clearance reported by Zientek *et al*. 2010 [4].

Potential reason for underprediction of clearance:

- *In vitro* assays: non optimal assay conditions, i.e issue with AO stability
- Non Michaelis-Menten kinetics
- Limited clinical data for AO substrates with an fm > 0.8
- Lack of robust information for other elimination routes
- Extrahepatic metabolism in kidney and lung or other organs [5,6]

References

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