Application of PBPK Modelling for Prediction of FMO Metabolism Using Benzydamine as a Probe for FMO3

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BACKGROUND

- Benzydamine *N*-oxidation is often used as a probe reaction for characterisation of FMO3 activity *in vitro* (Fig. 1). However, there are a lack of validated methods for extrapolating *in vitro* hepatic CL_{int} for FMO to *in vivo* clearance (IVIVE).
- Fisher *et al.* (2002)[1] have previously shown an over-estimation of *in vivo* FMO3 clearance using *in vitro* human liver microsomal (HLM) or human hepatocyte (HHEP) CL_{int} for benzydamine.

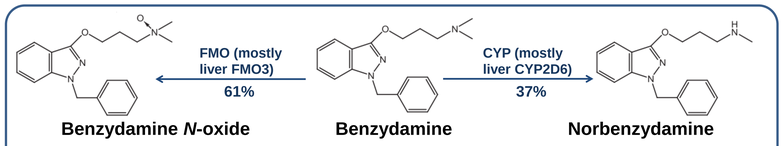

Figure 1. Benzydamine *N*-oxygenation and *N*-demethylation pathways mediated by HLM Adapted from Taniguchi-Takizawa *et al.,* 2015[11]. Fraction metabolised values calculated using data from the same study assuming 2% renal excretory elimination (CL_R)

- Several rare loss-of-function variants of *FMO3* have been associated with an inability to metabolise trimethylamine and a characteristic 'fish-odour syndrome'. However, it is thought that variability in drug metabolism may be more likely to be affected by altered, but functional FMO3[2].
- The impact of individual *FMO3* variants on drug metabolism *in vivo* is not clear. However, the *cis*-linked variants Glu158Lys and Glu308Gly appear to contribute to reduced FMO3 activity when expressed together but not individually (benzydamine *N*-oxidation activity was 0.6-fold of wild-type activity *in vitro*)[2,3].
- A study with 179 Caucasian volunteers has indicated that the Glu158Lys and Glu308Gly variants are expressed together at a haplotype frequency of 16.5%[4].

AIMS

- To assess via IVIVE the ability to predict *in vivo* benzydamine FMO3 metabolism using *in vitro* data from 3 literature sources and thereby expand the work of Fisher *et al.* (2002)[1].
- To develop a PBPK model to assess the pharmacokinetics of benzydamine and the potential impact of phenotype differences in benzydamine *N*-oxidation FMO3 activity based on the Glu158Lys and Glu308Gly variants.

METHODS

Prior metabolic, protein binding and physicochemical data for benzydamine were obtained from the literature and incorporated into a minimal PBPK model with a 1st order absorption model using Simcyp Population-based Simulator V14 Release 1.

Static Prediction of Benzydamine in vivo clearance

- Inter-individual variability was incorporated into the static IVIVE for FMO3 in a similar way as described for CYP metabolism[5] using individual values for FMO3 hepatic abundance (weighted mean 71 pmol FMO3 per mg HLM, CV 60%, n=11)[6,7] and assuming an Inter-System Extrapolation Factor (ISEF) of 1.
- Variability in benzydamine CYP2D6 metabolism was incorporated using the Sim-Healthy Volunteer library file in Simcyp V14 Release 1, which incorporates a complete loss of CYP2D6 activity for a poor metaboliser (PM) at a frequency of 8.2% of the population.

PBPK model for Benzydamine

- Vss was predicted using the method reported by Rodgers, T. and Rowland, M (2006)[8] and a Kp Scaler of 0.2 was needed to accurately recover the *in vivo* C_{max}.
- Benzydamine *N*-oxidation CL_{int} (µl/min/pmol) ratio for the Glu158Lys and Glu308Gly variants was calculated from an *in vitro* study using an *E. Coli* recombinant system as 0.60 : 0.72 : 1.00 (both Glu158Lys and Glu308Gly variants : Glu158Lys variant only : wild-type, respectively)[3]. This ratio was incorporated into the PBPK model, assuming the same activity ratio *in vivo* and no impact of additional variants.
- The model assumed that all FMO metabolism was by liver FMO3.

RESULTS

Static Prediction of Benzydamine in vivo clearance

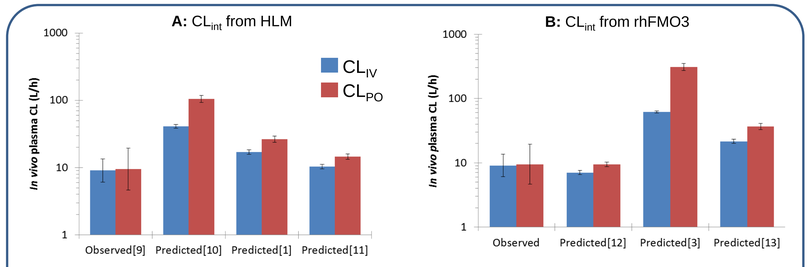

Figure 2. Predicted *in vivo* clearance for benzydamine in comparison to observed using FMO3 CL_{int} data from **A:** 3 HLM and **B:** 3 recombinant human (rh) FMO3 studies. Blue bars are systemic CL_{IV} and red bars are CL_{PO}. Data points are the geometric mean. Error bars are 95% confidence intervals.

- Predicted benzydamine CL_{IV} was comparable to observed (14% error) using *in vitro* CL_{int} from a HLM pool of 200 donors[11] (Fig. 2A). The CL_{IV} was over-predicted by 4.5- [10] and 2-fold[1] for the other two HLM studies (n=35[10] and unknown[1]).
- Predicted CL_{PO} was 11- [10], 3- [1] and 1.5-fold [11] higher than observed using the 3 sets of *in vitro* HLM data (Fig. 2A).
- Predicted benzydamine CL_{IV} and CL_{po} was comparable to observed (<25% error) using *in vitro* CL_{int} from a commercial baculovirus rhFMO3 system (Fig. 2B)[12].
- Predicted CL_{IV} was 7- [3] and 2-fold [13] higher and CL_{PO} was 33- [3] and 4-fold [13] higher than observed (Fig. 2B) using *in vitro* CL_{int} from 2 other rhFMO studies. These rhFMO3 systems were not commercially available and were *E. Coli*[3] and baculovirus[13] systems.
- ISEF values were estimated as 1.68[12], 0.02[3] and 0.20[13] for the 3 rhFMO3 studies. These values could be used to improve the prediction accuracy of other FMO3 substrates using rhFMO3 *in vitro* data and the corresponding *in vitro* assay.

PBPK model for Benzydamine

CL_{int} data from the study by Taniguchi-Takizawa *et al*., 2015[11] were selected for use in the PBPK model (unbound HLM CL_{int} values of 9.94 and 6.93 µl/min/mg for FMO and CYP, respectively) as this study:

- Used a pool of HLM from a large number of donors (n=200) that should be representative of a general population;
- Obtained CL_{int} values that gave a good prediction of *in vivo* clearance;
- Generated both FMO and CYP CL_{int} in the same laboratory.

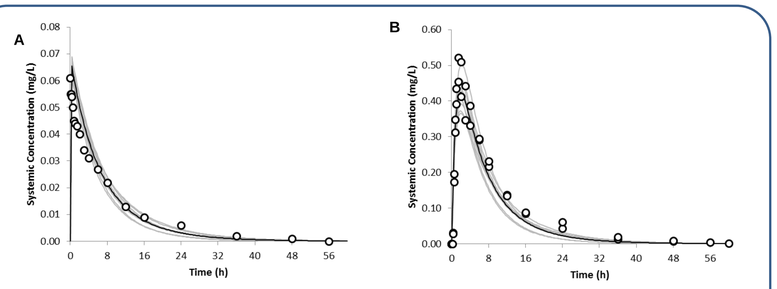

Figure 3. Simulated plasma concentrations for benzydamine using HLM CL_{int} data[11] in comparison to observed[9] (open circles are mean data) **A:** Single IV dose of 5 mg (5 min infusion). Observed data: mean from 6 males, age 41-51 years. **B:** Single PO dose of 50 mg. Observed data: mean from 6 males and 6 females, age 18-51 years. Grey lines are 10 trials of 10 simulated individuals and solid line is mean (total n=100).

	IV DOSE		PO DOSE	
	C_{max} (mg/L)	AUC (mg/L.h)	C_{max} (mg/L)	AUC (mg/L.h)
Mean	**0.070**	**0.49**	**0.43**	**3.88**
Trial 1	0.071	0.43	0.43	3.31
Trial 2	0.064	0.55	0.37	3.76
Trial 3	0.071	0.48	0.47	4.13
Trial 4	0.074	0.52	0.43	4.01
Trial 5	0.067	0.49	0.40	3.67
Trial 6	0.070	0.48	0.44	3.90
Trial 7	0.070	0.49	0.42	3.95
Trial 8	0.073	0.53	0.50	4.50
Trial 9	0.073	0.56	0.47	4.49
Trial 10	0.067	0.42	0.38	3.06
Observed	**0.068**	**0.54**	**0.50**	**4.95**

Table 1. Simulated C_{max} and AUC in comparison to observed. Observed data[9]: n=6 (IV) and 12 (PO). Simulated data are mean from 10 trials of 10 simulated individuals (total n=100).

Mean AUC and C_{max} were within 10% and 25% of observed for the IV and PO studies, respectively (Table 1).

A 40% reduction in *in vitro* CL_{int} for the linked E158K-E308G variants in comparison to wild-type FMO3 corresponded to a 31% and 169% increase in mean simulated AUC of benzydamine for CYP2D6 EM and PM, respectively (Fig. 4).

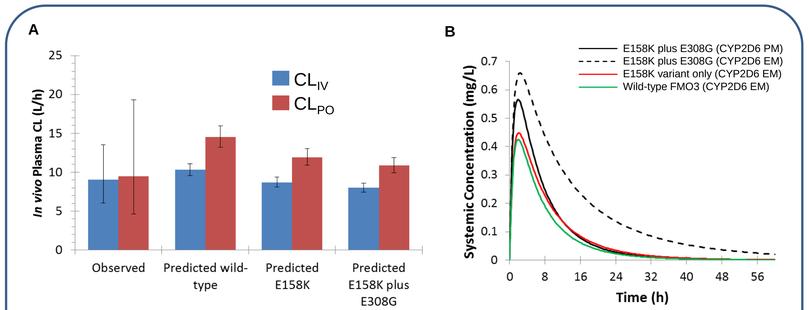

Figure 4. Predicted pharmacokinetics of benzydamine in comparison to observed using HLM CL_{int} data[11] and ratio of CL_{int} of 0.60 : 0.72 : 1.00 for both Glu158Lys and Glu308Gly variants : Glu158Lys variant only : wild-type, respectively[3]. Observed data from Baldock et al., 1991[9]. **A. Static predictions of CL_{IV}** (red) **and CL_{PO}** (blue) **and impact of the Glu158Lys and Glu308Gly FMO3 variants for CYP2D6 extensive metabolisers (EM)**. Data points are the geometric mean. Error bars are 95% confidence intervals. **B. Mean simulated plasma concentrations after a single PO dose of 50 mg.** All lines are mean of 10 trials of 10 simulated individuals (total n=100).

CONCLUSION

- Selection of a recently published source for *in vitro* CL_{int} has allowed the development of a 'bottom-up' PBPK model to predict the pharmacokinetics of Benzydamine, a probe substrate for FMO3.
- There is a tendency for over-prediction of *in vivo* benzydamine CL using *in vitro* HLM and rhFMO3 although in some cases a good prediction was seen. The model can potentially be used in the future to research:
  - *In vivo* FMO3 metabolism using *in vitro* data for other substrates of FMO3 (assuming the same ISEF values and/or variant : wild-type CL_{int} ratio)
  - *In vivo* DDI involving potential inhibitors of FMO3
- There is a need for:
  - Further assay development of incubation conditions for FMO3 to understand the inter-study differences seen.
  - More data on the impact of additional allelic variants for FMO3 on drug metabolism *in vitro* and *in vivo.*
  - More data for absolute FMO3 abundance in HLM (only available for 11 donors so far).
  - Information on extrahepatic FMO3 abundance.

REFERENCES

[1] Fisher, M. *et al.*, 2002. DMD. 30: 1087-1093
[2] Park, C. *et al.,* 2002. Pharmacogenetics. 12: 77-80
[3] Shimizu, M. *et al.,* 2007. DMD. 35: 328-330
[4] Cashman, J. *et al.*, 2001. DMD. 29: 1629-1637
[5] Cubitt, H. *et al.*, 2011. Xenobiotica. 41 (8): 623-38
[6] Haining, R. *et al.,* 1997. DMD. 25 (7): 790-797
[7] Overby, L. *et al.,* 1997. Chem-Bio Int. 106: 29-45
[8] Rodgers, T. & Rowland, M. 2006. J Pharm Sci. 95 (6): 1238-1257
[9] Baldock, G. *et al.,* 1991. Biopharm Drug Dispos. 12: 481-492
[10] Störmer, E. *et al.,* 2000. BJCP. 50: 553-561
[11] Taniguchi-Takizawa, T. *et al.*, 2015. DMPK. 30: 64-69
[12] Lickteig, A. *et al.,* 2009. DMD. 37: 1987-1990
[13] Lang, D. and Rettie, A. 2000. BJCP. 50: 311-314