Evaluation of *In Vitro-In Vivo* Extrapolation (IVIVE) of the induction potential for known CYP 2C9 inducers

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Abstract

- The aim of this study was to assess the relationship between mRNA and activity data for known inducers of CYP2C9 in human hepatocytes *in vitro* and to apply these data for prediction of the magnitude of CYP2C9-mediated drug-drug interactions (DDIs) *in vivo*.
- There was a strong correlation (r^2 = 0.96) between *in vitro* induction markers for CYP2C9 (mRNA *vs.* Activity).
- Utilising a combination of IVIVE and physiologically based pharmacokinetic (PBPK) modelling (Simcyp, V16, UK), the induction potential of rifampicin (RIF), phenobarbital (PBT) and ritonavir (RTV) was evaluated using 2C9 substrates [tolbutamide (TBT), S-warfarin (WFN), phenytoin (PHN), glimepiride (GMP)].
- Using the measured *in vitro* 2C9 induction parameters (weighted mean; activity data), with the exception of PBT [6], both RIF and RTV models markedly under-predicted the clinical interaction (up to ~3-fold) [comparing predicted and observed geometric mean ratio (GMR) for AUC or CL] for 2C9 substrates [1-4, 7].
- For RIF, the optimised induction parameters for 2C9 (Ind_{max}=6; $IndC_{50}$=0.1 µM) using the clinical data [1] predicted the clinical outcome reasonably well [2-5] (GMR within 2-fold for AUC and CL). For RTV (Ind_{max}=3.33; $IndC_{50}$=0.07 µM) or PBT (Ind_{max}=6.25; $IndC_{50}$=43.9 µM), the calibrated induction parameters using the RIF induction data was able to capture the clinical interaction well (GMR within 2-fold for AUC or CL).
- These results warrant further investigation of the *in vitro* data, and verification of the 2C9 induction model with additional clinical DDI studies.

Background

- Induction of CYP2C9 by xenobiotics is a potential source of variation in the elimination of 2C9 substrates, which may result in therapeutic failure [8]. Differences in induction efficacy and potency derived from mRNA *versus* activity data can have an impact on the predicted magnitude of interaction when this information is utilised in PBPK models.
- In this study we assessed the relationship between mRNA and activity data for known inducers of CYP2C9 *in vitro* and applied these data for predicting the magnitude of CYP2C9-mediated DDIs *in vivo*.

Methods

- The *in vitro* CYP2C9 induction parameters (Ind_{max}, maximum fold induction; $IndC_{50}$, concentration that gives half maximal fold induction) based on changes in mRNA (TaqMan® Assay Kit) and activity (formation of 4-hydroxy tolbutamide or hydroxy diclofenac) in human hepatocyte incubations were collated (based on n=5 studies and n=11 lots of hepatocytes) from the literature [1, 9-11] for known CYP inducers (RIF, PBT, RTV).
- The Simcyp Simulator (V16.1, UK) was used to simulate the time course of "victim" drugs (2C9 substrates; TBT or WFN or PHN or GMP) and "perpetrator" (2C9 inducers; RIF or PBT or RTV) concentrations in plasma. Study design and characteristics of the virtual subjects were matched closely to that of clinical studies [1-7]. For each simulation, 10 separate trials were generated to assess variability across groups.
- Except for GMP, all compound models used in this study were taken from Simcyp library files (V16). The performance verification of these drugs had been verified using the observed data. GMP is predominantly metabolised by CYP2C9 [5]. The GMP model was developed as part of this study, and the contribution of 2C9 to the overall metabolism was verified using 2C9 inhibitors [12].

Results

- The overall Ind_{max} and $IndC_{50}$ values (derived from mRNA or activity) for CYP2C9 ranged from 3.6-5.6 fold and 0.1-1.5 µM, respectively, for RIF, 3.2-8.2 fold and 68-849 µM, respectively, for PBT, 2.4-3.9 fold and 1.9-7.5 µM, respectively, for RTV [1, 9-11].
- For 2C9 induction, mRNA data showed broadly similar level of induction efficacy and potency relative to activity data for RIF and PBT (Fig 1A). For RTV, mRNA data exhibited higher efficacy (1.63-fold higher Ind_{max}) and lower potency (mean 3.95-fold higher $IndC_{50}$) (Fig 1A).
- When the ratio of Ind_{max} : $IndC_{50}$ for CYP2C9 (mean ± SD) across three inducers was compared between mRNA and activity, it was variable, but broadly similar for RIF (0.93 ± 0.33) and PBT (1.64 ± 1.58) (Fig 1B), and lower for RTV (0.41; data is based on n=1 study only).
- Also, a comparison of the ratios (Ind_{max}/$IndC_{50}$) for these inducers based on mRNA *versus* activity data indicated that there was a strong correlation (r^2 = 0.96) between these markers (Fig 2).
- The *in vitro* CYP2C9 induction data (weighted mean; based on activity; collated from the literature) [1, 9-11] for RIF (Ind_{max}=4.01; $IndC_{50}$=0.93 µM), PBT (Ind_{max}=5.27; $IndC_{50}$=320 µM), and RTV (Ind_{max}=2.4; $IndC_{50}$=1.9 µM) were used in the model for predicting CYP2C9 induction mediated DDIs *in vivo*.
- Utilising the measured *in vitro* 2C9 induction data (weighted mean; activity data), with the exception of PBT [6], both RIF and RTV models significantly under-predicted the magnitude of interaction for 2C9 substrates [ratio of predicted and observed GMR of AUC ranged from 1.3 to 3-fold] compared to clinical outcome [1-4, 7].
- The IVIVE of induction potential using the *in vitro* 2C9 mRNA data [1, 9-11] was also investigated in this study and the predicted magnitude interaction was comparable with that of activity data (data not shown).

Results (Contd)

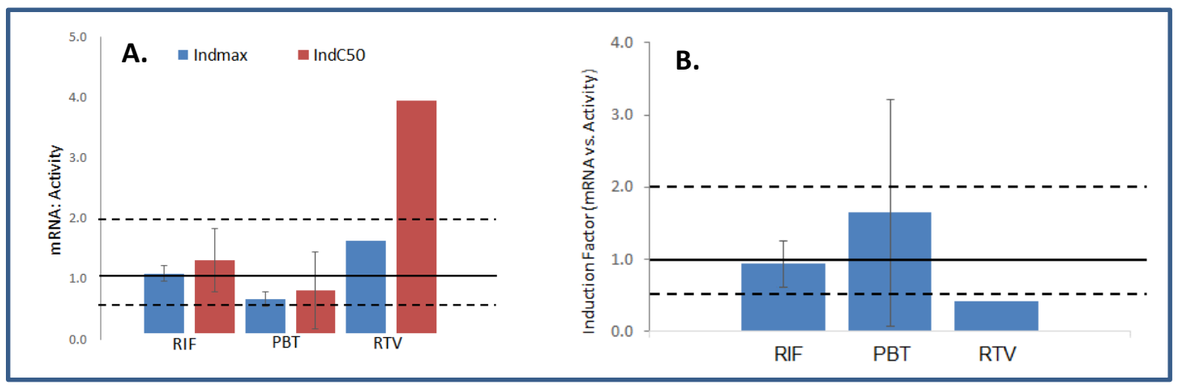

Fig 1: The fold difference in Ind_{max} (blue) and $IndC_{50}$ (red) values determined from mRNA and activity data (A), and the fold difference in the induction factor (Ind_{max}: $IndC_{50}$) between mRNA and activity (B) for CYP2C9. The line of unity and +/- 2-fold are shown for reference. Data are plotted as mean +/- SD for RIF and PBT. N=1 data for RTV.

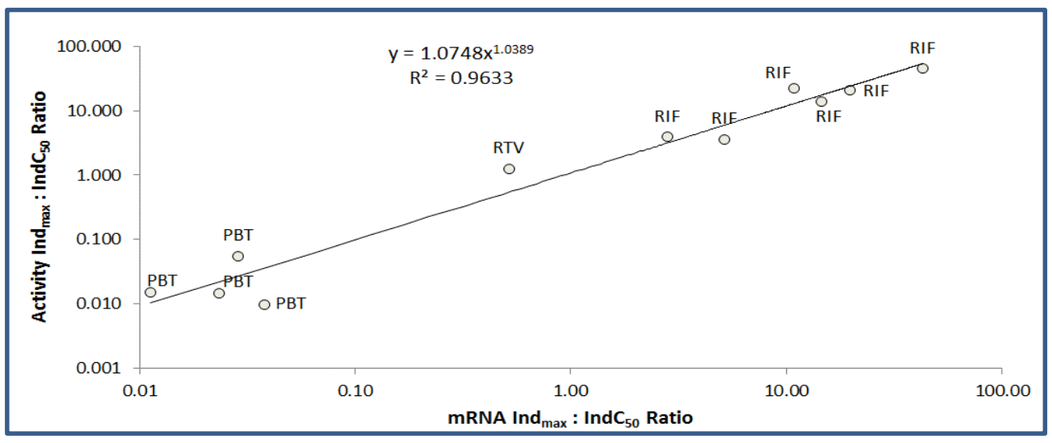

Fig 2: The correlation of the ratio of Ind_{max} : $IndC_{50}$ (open circles) derived from mRNA and activity data for CYP2C9 inducers.

- Considering the high variability in the measured *in vitro* 2C9 induction data and significant under-prediction of IVIVE of the induction potential, the induction parameters were optimised (using the Automated Sensitivity Analysis tool, Simcyp V16) for RIF (Ind_{max}=6; $IndC_{50}$=0.1 µM) using the clinical data [1] and the refined RIF model captured the clinical interaction reasonably well for TBT or WFN or PHN or GMP [2-5] (within 2-fold of GMR for AUC or CL) (Table 1).
- For RTV (Ind_{max}=3.33; $IndC_{50}$=0.07 µM) or PBT (Ind_{max}=6.25; $IndC_{50}$=43.9 µM), the calibrated induction parameters using the RIF induction parameters was able to capture the clinical interaction reasonably well for TBT or WFN (within 2-fold GMR for AUC or CL) (Table 1).

Table 1. Predicted and observed magnitude of interaction [Geometric Mean Ratio (GMR) for AUC or CL] for CYP2C9 inducers.

Perpetrator	Victim	AUC Ratio			CL Ratio		
		Pred. GMR (90% CI)	Obs. GMR (90% CI)	Pred./Obs.	Pred. GMR (90% CI)	Obs. GMR (90% CI)	Pred./Obs.
RIF	TBT	0.44 (0.41-0.47)	0.48 [2]	0.92	2.29 (2.14-2.44)	2.26 [2] (2.07-2.46)	1.01
	WFN	0.47 (0.45-0.49)	Not Reported		2.13 (2.04-2.23)	2.1 [3]	1.01
	PHN	0.50 (0.47-0.53)	Not Reported		2.02 (1.90-2.14)	2.09 [4]	0.97
	GMP	0.42 (0.39-0.44)	0.66 [5]	0.64	2.41 (2.26-2.56)	1.51 [5]	1.60
PBT	WFN	0.34 (0.31-0.37)	Not Reported		2.96 (2.69-3.25)	1.7 [6] (1.24-2.27)	1.74
RTV	TBT	0.54 (0.52-0.57)	0.5 [1] (0.43-0.58)	1.08	1.84 (1.77-1.91)	2.0 [1] (1.73-2.31)	0.92
	WFN	0.85 (0.82-0.87)	0.76 [7] (0.69-0.85)	1.12	1.18 (1.15-1.21)	Not Reported	

Conclusions

- There was a strong correlation (r^2 = 0.96) between *in vitro* induction markers for CYP2C9 (mRNA *vs.* Activity).
- Using the measured *in vitro* induction data as inputs, with the exception of PBT, both RIF and RTV models significantly under-predicted the induction potential for 2C9 substrates.
- Optimisation of induction parameters using the clinical DDI data was needed for RIF to predict the 2C9 induction potential *in vivo*, and for RTV and PBT models, the calibration of induction data improved DDI predictions.
- While these results are encouraging, considering the high inter-donor/inter-lab variability in the *in vitro* induction data, future investigation with robust *in vitro* data and verification of the model with additional clinical DDI studies would be beneficial for better understanding of IVIVE of induction potential for CYP2C9.

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14th European ISSX Meeting, 26-29 June, Gürzenich Köln, Cologne, Germany **Poster # 51**