Biokinetic and PBPK modelling to support the rotenone and strobilurin read across case studies (CS4)

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Certara UK and Cyprotex* Limited and CS4 partners.

Overview

PBPK and biokinetic models were used to support the two read across exercises in case study 4 (rotenone/deguelin and strobilurins). **Plasma** and **brain** concentrations of the compounds after various exposure scenarios in both species were predicted.

Human PBPK models were constructed using:
- physicochemical data, human plasma protein, human blood to plasma ratio
- the rate of metabolism in human hepatocytes measured *in vitro*.

For the **rat** PBPK models
- clearance was predicted by allometric scaling of the predicted human value.
- in the absence of measured data protein binding was predicted from *in silico* approaches and by assuming the same affinity for rat and human proteins and then accounting for differences in protein content between the species.

The distribution of compounds between cells and media in the various *in vitro* toxicology assays was predicted using the VIVD biokinetic model and compared for exemplar compounds with measured data.

A reverse dosimetry approach was used to predict oral doses of the compounds that would achieve the concentrations in the target organ (brain) equivalent to those causing toxicity *in vitro*.

Model details

Biokinetic predictions were made using VIVD (SIVA version 3). PBPK modelling was conducting in Simcyp V17 or 18 using a full body PBPK model. Clearance of compounds by metabolism was assumed to be linear with dose, non-linear changes in fa due to compound solubility were considered in the strobilurin PBPK models. There was uncertainty in the values of protein binding for rotenone (0.00365 – 0.043) and deguelin (0.00326 – 0.063) in human plasma. Sensitivity analyses were conducted to assess the impact of varying the values of fu across the range of reported/predicted values. Dermal exposure was predicted using the MPML-Mechderma model within Simcyp V18 using default QSAR prediction options.

Biokinetic model results

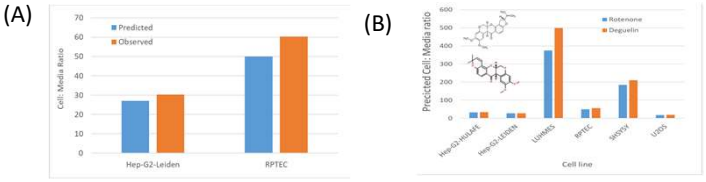
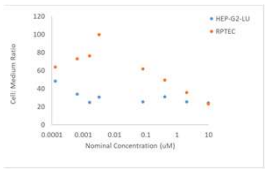


Figure 1 (A) Measured (mean of all data) and predicted cell: media ratios of rotenone in HEP G2 and RPTEC cells. (B) Predicted cell:media ratios of rotenone and deguelin in five different cell lines.



The effect of rotenone nominal concentration on measured cell:medium ratio (each data point is the mean of 3 replicates)

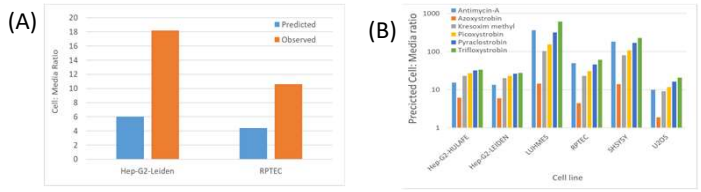


Figure 2 (A) Measured (mean of all data) and predicted cell: media ratios of azoxystrobin in HEP G2 and RPTEC cells. (B) Predicted cell: media ratios of the strobilurin compounds in five different cell lines.

PBPK model results – rotenone and deguelin

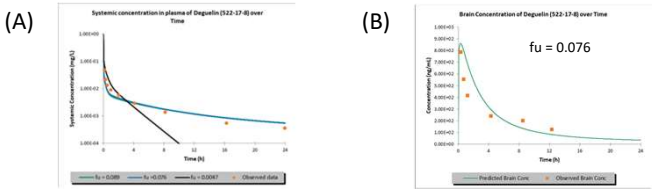


Figure 3 Predicted and observed pharmacokinetics of deguelin in (A) **rat** plasma and (B) **rat** brain following an IV bolus dose of 0.25 mg/kg. Different predicted values of plasma protein binding for deguelin in the **rat** were used in the plasma simulations in the absence of a measured value. Observed data were taken from Udeani et al. Cancer Chem Pharm, 2001, 47, 263

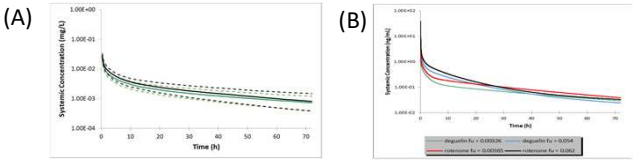


Figure 4 (A) Median simulated plasma concentrations of rotenone (black solid line) and deguelin (Green solid line) following administration of an intravenous 10mg dose to 100 **human** subjects (age 20-50; 50% female). The protein values of 0.062 and 0.054 were used for rotenone and deguelin respectively. The 5th and 95th percentiles of the population are shown by dashed lines for rotenone (black) and deguelin (green), (B) Simulated mean plasma exposure of unbound compound after an intravenous dose of 10mg of deguelin or rotenone to a population of 100 **human** individuals aged 20-50 (50% female).

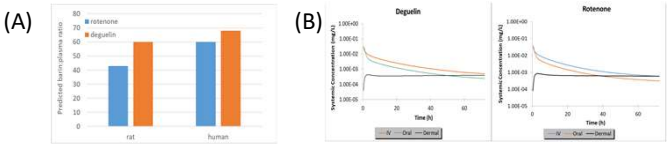


Figure 5 (A) Predicted brain to plasma ratio in **rat** and **human** for rotenone and deguelin. (B) Simulated mean plasma exposure to compound after an intravenous dose of 10mg of deguelin or rotenone to a population of 100 **human** individuals aged 20-50 (50% female) by IV, oral or dermal exposure route. Plasma protein binding values of 0.076 and 0.054 were used for deguelin and of 0.087 and 0.062 for rotenone in rat and human respectively.

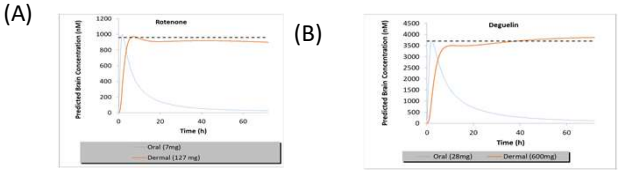


Figure 6 Predicted oral and dermal doses in **human** that achieve the same predicted mean maximal concentration in the brain as the predicted cell concentration (dashed line) causing a 50% decrease in neurite area in the LUHMES cell line (A) rotenone and (B) deguelin.

PBPK model results – strobilurins

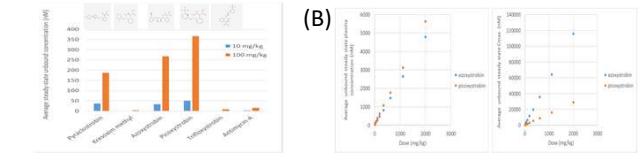


Figure 7 (A) Predicted average steady state **rat** exposure (total and unbound) for the strobin case study compounds at an oral doses of 10 and 100 mg/kg and (B) predicted unbound steady state (left panel) and Cmax (right panel) concentrations of azoxystrobin and picoxystrobin in the **rat** after various oral doses.

Discussion

ROTENONE AND DEGUELIN DATA
- The predicted cell concentrations for rotenone agreed closely with the measured values in two different cell lines (figure 1A). The predicted cell concentrations of rotenone and deguelin were similar in all cell lines (figure 1B).
- Reasonable predictions of deguelin plasma and brain concentrations were achieved with the PBPK model (figure 3).
- Despite uncertainty in the true value of fraction unbound in human plasma, sensitivity analysis showed that the unbound plasma concentration was similar across the range of protein binding estimates (figure 4) and was similar for rotenone and deguelin at the same dose.
- The predicted brain:plasma ratio for rotenone and deguelin was similar in rat and human (figure 5A).
- The maximum plasma exposure was predicted to be much lower after dermal exposure than after oral or IV exposure (figure 5B) .
- The equivalent doses giving predicted brain concentrations equal to the *in vitro* cell concentration where a 50% reduction in neurite area was observed were 7 and 28 mg (oral) and 127 and 600 mg (dermal) for rotenone and deguelin respectively (figure 6).

STROBILURIN DATA
- The predicted cell concentrations for azoxystrobin were about 3-fold lower than the measured values in two different cell lines (figure 2A). The predicted cell concentrations of azoxystrobin were the lowest for the set of azoxystrobin compounds (figure 2B).
- The average unbound concentration of azoxystrobin was in the range of the other strobin compounds (figure 7), although the predicted free Cmax at high doses was higher than the other compounds.

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www.eu-toxrisk.eu/