Co-operativity in the *in vitro* kinetics of cytochrome P450 (CYP) mediated drug metabolism will have minimal impact on *in vivo* metabolic clearance

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Introduction

Some CYPs exhibit atypical kinetics under *in vitro* conditions, and a two-site binding model has been proposed to accommodate negative and positive hetero- or homo-tropic co-operativity. The perfused rabbit liver with constant rate input of drug has been used to demonstrate possible *in vivo* consequences of this behaviour (Chen *et al.*, 2004), while there is no unequivocal evidence from *in vivo* studies in humans to support the importance of atypical metabolic kinetics.

In this study we have assessed the potential impact of homotropic co-operativity (substrate inhibition or activation), using the two-site binding model nested in a mechanistic physiologically-based pharmacokinetic (PBPK) model implemented in Simcyp®.

Methods

Figure 1 shows a schematic of the two-site binding model, where [E], [S] and [P] are enzyme, substrate and product concentrations, [SE], [SES] and [ES] are enzyme-substrate complex concentrations, α and β are the co-operativity parameters and K_s and K_p are dissociation and metabolic rate constants, respectively.

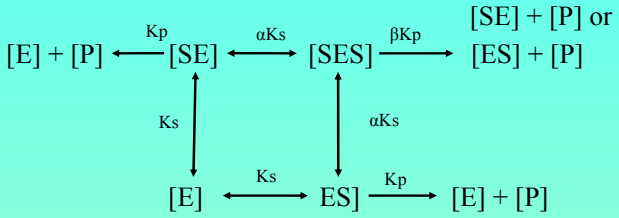
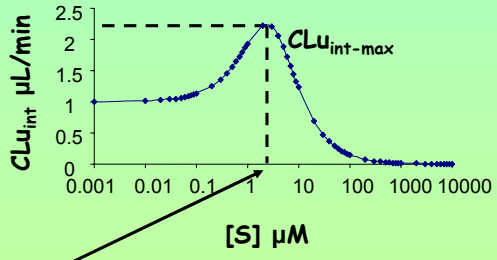


Figure 1 – A schematic of the two-site binding model.

The governing equations for this model is:

$$v = \frac{\frac{[S]}{K_s} + \frac{\beta [S]^2}{\alpha K_s^2}}{1 + \frac{2[S]}{K_s} + \frac{[S]^2}{\alpha K_s^2}} V_{max} \qquad CLu_{int} = \frac{v}{[S]}$$

where ν is the rate of metabolism, CLu_{int} is unbound intrinsic clearance and V_{max} is the maximum rate of metabolism. From the first-order differential the concentration at which maximum intrinsic clearance occurs can be determined. Figure 2 shows how CLu_{int} changes as a function of substrate concentration.



$$[S]_{optimal} = K_s \frac{-\alpha \pm \sqrt{\alpha^2 - \alpha\beta(2\alpha - \beta)}}{\beta} \qquad CL_{Ratio} = \frac{CLu_{int-max}}{\frac{V_{max}}{K_s}}$$

Solutions exist if $\alpha^2 \geq \alpha\beta(2\alpha - \beta)$

Figure 2 – The CLu_{int} as a function of [S].

Simcyp® version 6.1 was used to generate a virtual North European Caucasian population and their individual plasma drug concentration-time and hepatic and intestinal intrinsic clearance – time profiles.

In order to compare the effects of autoactivation on AUC and C_{max}, intrinsic clearances for all metabolic routes were calculated using Michaelis-Menten (MM) and two-site binding models.

Results

The effects on *in vivo* kinetics of a range of co-operativity numbers (α and β) reported from *in vitro* studies of different drugs were investigated. As a worse case scenario, the most extreme values of α and β (0.03-23.8) that have been reported (Lin *et al.*, 2001) were used in simulation studies to examine the *in vivo* implications of autoactivation.

The time-dependences of the hepatic CL_{int} of alprazolam and tolbutamide based on MM kinetics and the two-site binding model are compared in Figures 3 and 4.

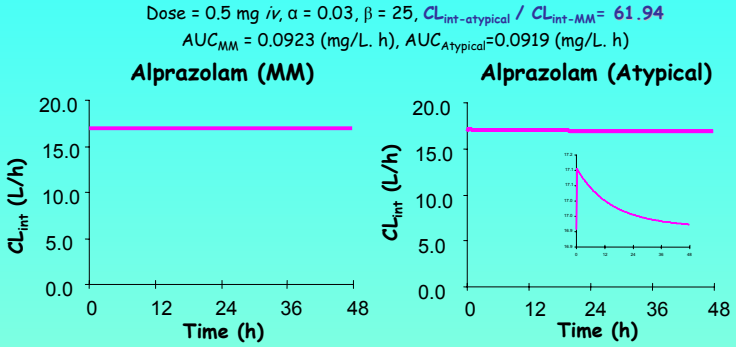


Figure 3 – Comparison of the time-dependence of the hepatic CL_{int} of alprazolam predicted by Michaelis-Menten and Two-Site Binding models,.

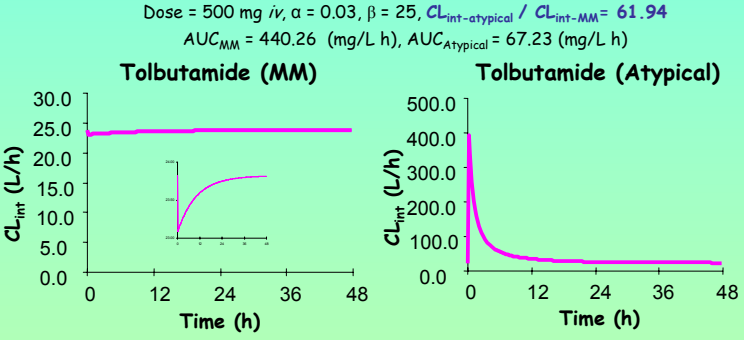


Figure 4 – Comparison of the time-dependence of the hepatic CL_{int} of tolbutamide predicted by Michaelis-Menten and Two-Site Binding models.

Discussion

The results indicate that for most drugs given in relatively low therapeutic doses (*e.g.* alprazolam) atypical enzyme kinetics are unlikely to be manifest in significant changes in *in vivo* clearance. Even with tolbutamide, given in relatively high doses, an impact is only evident when extreme values of the co-operativity numbers are applied. This is consistent with a lack of any discernable dose (concentration)-dependency in the *in vivo* kinetics of the drugs investigated in our study. PBPK modelling is a useful tool to clarify areas of *in vitro-in vivo* extrapolation where systematic *in vivo* studies on metabolic issues such co-operativity are lacking.

References

Chen, Q. *et al.*(2004), Effect of quinidine on the 10-hydroxylation of R-warfarin: species differences and clearance projection, *J Pharmacol Exp Ther* **311**, 307.

Lin, Y., *et al.* (2001), Substrate inhibition kinetics for cytochrome P450-catalyzed reactions. *Drug Metab Dispos*, **29,** 368.