Bias in Estimates of Metabolic Constants When Applying the Michaelis-Menten Equation to Drugs Exhibiting Atypical Enzyme Kinetics

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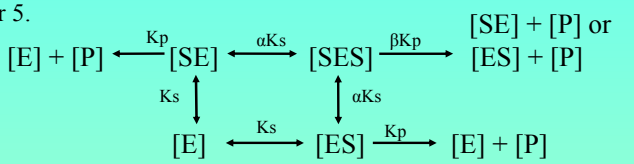
Introduction

Homotropic co-operativity in drug metabolism by CYP enzymes observed *in vitro* has minimal impact on *in vivo* clearance at therapeutic drug concentrations (Jamei 2005). Nevertheless, 'force fitting' of *in vitro* data that exhibit such behaviour by a simple Michaelis-Menten function may introduce bias when predicting *in vivo* clearance.

We have investigated the effects of ignoring atypical *in vitro* kinetics and using a simple Michaelis-Menten model to predict kinetic parameters.

Methods

A CYP3A4-mediated reaction showing atypical enzyme kinetics (substrate inhibition) at high concentrations is the 6β-hydroxylation of progesterone. A two-site binding model (Eq. 1) and associated values of α (13.2) and β (0.41) (Lin 2001), together with a range of each of these values (0.01, 0.1, 1, 10, and 20) for 25 virtual compounds, were used to simulate (Microsoft Excel®) rates of metabolism *vs* substrate concentration. The single point concentration data were then fitted with the Michaelis-Menten equation (Eq 2.) using the proportional weighting option in GraFit Ver 5.



A schematic of a two-site binding model.

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

$$v = \frac{V_{max}}{K_m + [S]} \qquad \text{Eq. 2}$$

Results

Using Eq. 1 and a set of α and β values, homotropic negative co-operativity (substrate inhibition) for a range of substrate concentrations was simulated and shown in Figure 1.

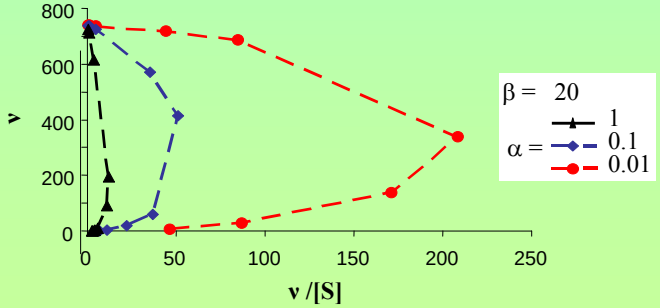


Figure 1 – Eadie-Hofstee graphs for different values of α and a constant β.

Figure 2 shows that fold errors in K_m prediction are dependent on α, particularly at β > 1. However, when β ≤ 1 the fold errors are almost insensitive to changes in α and the predicted K_m is about 10 fold less than the true value (see Figure 3).

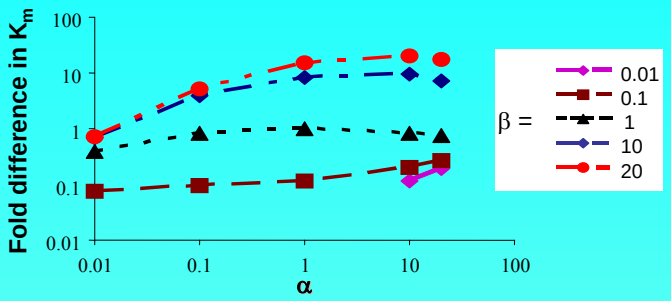


Figure 2 – The effect of α on the fold difference between apparent and true K_m.

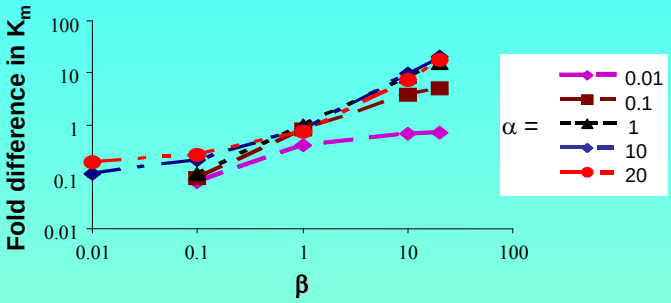


Figure 3 – The effect of β on the fold difference between apparent and true K_m.

Figure 4 illustrates that the fold differences between estimated and true V_{max} values are influenced only by β and α plays a minor role when it is less than 10.

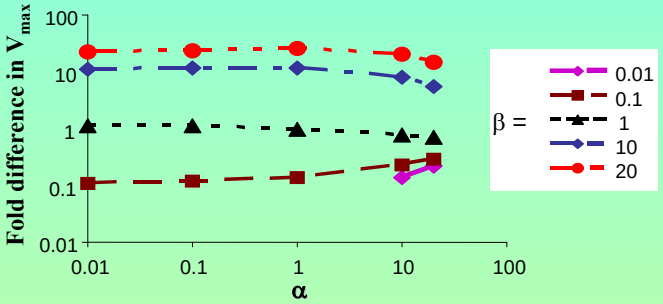


Figure 4 – The effect of α on the fold difference between apparent and true V_{max}.

Estimates of CL_{int} at very low, single point substrate concentrations (0.01 K_s) are insensitive to α at β < 1. However, at β > 1 values are mainly determined by α if it is less than 1.

Conclusions

The results confirm that bias (0.01 to 100 fold) in estimates of CL_{int}, V_{max} and K_m (K_s), and hence the prediction of drug clearance, can result if atypical *in vitro* enzyme kinetics are ignored and the data are fitted by simpler functions.

In vitro kinetics parameters should be estimated using the most appropriate model.

References

Jamei, M. *et al. Drug Metabolism Reviews,* 2006, **38**, Sup 1, 14.
Lin, Y. *et al. Drug Metabolism and Disposition, 2001,* **29**, 368-374.