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

M. Jamei1, A. L. Finnoy2, G. T. Tucker1,2 and A. Rostami-Hodjegan1,2

1- Simcyp Ltd, Blades Enterprise Centre, John St, Sheffield, S2 4SU, UK
2- Academic Unit of Clinical Pharmacology, University of Sheffield, Sheffield, UK

Abstract

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.

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 $\alpha$ (13.2) and $\beta$ (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.

\[
\begin{align*}
[E] + [P] & \xrightarrow{K_p} [SE] \\
& \xrightarrow{K_s + \alpha K_p} [SES] \\
& \xrightarrow{K_s + \beta K_p} [ES] + [P] \\
& \xrightarrow{\alpha K_s} [E] \\
& \xrightarrow{K_p} [SE] + [P] \\
& \xrightarrow{\beta K_s} [ES] + [P] \\
& \xrightarrow{\alpha K_s} [E] \\
& \xrightarrow{K_p} [SE] + [P] \\
\end{align*}
\]

A schematic of a two-site binding model.

Results

Using Eq. 1 and a set of $\alpha$ and $\beta$ 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 $\alpha$ and a constant $\beta$.](image)

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

![Figure 2 - The effect of $\alpha$ on the fold difference between apparent and true $K_m$.](image)

Figure 3 – The effect of $\beta$ 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 $\beta$ and $\alpha$ plays a minor role when it is less than 10.

![Figure 4 - The effect of $\alpha$ on the fold difference between apparent and true $V_{max}$.](image)

Conclusions

The results confirm that bias (0.01 to 100 fold) in estimates of CL_int, $V_{max}$ and $K_m$, 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

Lin, Y. et al. Drug Metabolism and Disposition, 2001, 29, 368-374.