THE IMPACT OF STUDY DESIGN ON THE ESTIMATION OF PARAMETERS DESCRIBING MECHANISM-BASED ENZYME INHIBITION

Heydari A[1], *Rowland-Yeo K[2], Lennard MS[1], Tucker GT[1,2], Rostami-Hodjegan[1,2]

*** Correspondence: k.r.yeo@simcyp.com**

1- Academic Unit of Clinical Pharmacology, Royal Hallamshire Hospital, University of Sheffield,
2- Simcyp Limited, Blades Enterprise Centre, John Street, Sheffield, UK

Introduction

Mechanism-based inhibition (MBI) is associated with irreversible or quasi-irreversible loss of enzyme activity. This type of inhibition may be a cause of particularly profound and long-lasting drug-drug interactions, requiring synthesis of new enzyme for return of normal enzyme function. Description of the effects of MBI is based on the *in vitro* estimation of two key parameters, namely k_{inact} (the maximum rate constant for inactivation) and K_I (the concentration of inhibitor which produces half the maximal rate of inactivation). The experimental procedure involves two steps (Silverman, 1995):

1- pre-incubation of enzyme with inhibitor (enzyme inactivation),
2- incubation with a probe substrate (measurement of residual enzyme activity).

To prevent further inactivation during step 2, enzyme and inhibitor should be diluted into a solution containing a high concentration of probe substrate. In this preliminary study we have investigated the impact of dilution and 3 different probe substrate concentrations on estimates of k_{inact} and K_I for 3,4-methylenedioxymethamphetamine (MDMA), an inhibitor of CYP2D6. Although recommendations have been made for the optimal design of MBI studies (Silverman, 1995), to our knowledge, the quantitative effects of experimental variables on k_{inact} and K_I have not been investigated systematically.

Experimental Methods

Details of the MBI experiments are given elsewhere (Heydari *et al* 2004). In brief, the pre-incubation mixture contained MDMA (2 – 40 µM and 0.625 – 12.5 µM for experiments with and without dilution, respectively), KCL, an NADPH-generating system, potassium phosphate buffer (pH 7.4). All reactions were started by addition of yeast microsomes expressing CYP2D6 (20 pmol), and were carried out at 37°C. In the first experimental scheme, the CYP2D6 probe, dextromethorphan (DEX) (5, 10 or 20 µM), was added directly to the pre-incubation mixture after different pre-incubation times (1 – 5 min) (no dilution). In the second scheme, aliquots (62 µl) were taken from the pre-incubation mixture at different times (1 – 5 min) and added to incubation tubes containing DEX solution (final DEX concentration 20 µM ; final volume 250 µl ; a 4 fold dilution of the MDMA in the pre-incubation mixture). The reactions were stopped after 5 min by the addition of perchloric acid.
The inhibition of CYP2D6 was determined by the conversion of DEX to dextrorphan in the presence and absence of MDMA.

Data Analysis

Inactivation constants (k_{obs}) were calculated from the % inhibition of the conversion of DEX to dextrorphan (Ito *et al.* 1998). Hence, k_{inact} and K_I values were estimated using the following equation (Heydari *et al.* 2004) and non-linear regression.

$$k_{obs} = \frac{k_{inact} \times [I]}{K_I + [I]}$$

Statistical inferences were made using the z-test.

Results

Figure 1 shows the model fits to the relationship between k_{obs} and MDMA concentration obtained from experiments with no dilution and using different DEX concentrations. Increasing concentrations of DEX were not associated with statistically significant differences in estimates of K_I or k_{inact} values ($p > 0.05$).

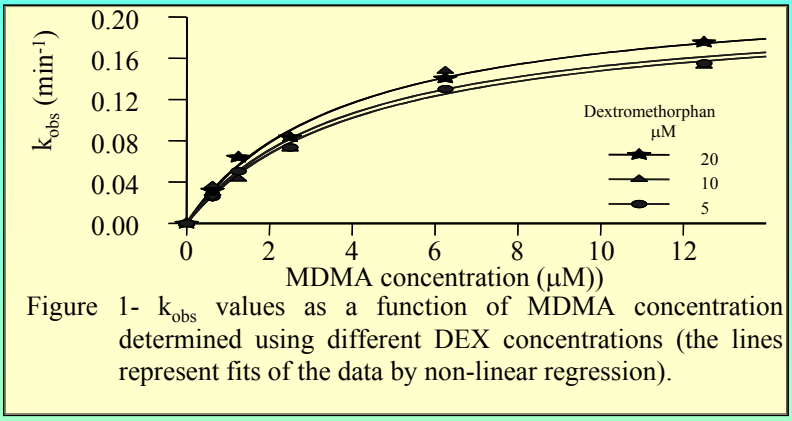

Figure 1- k_{obs} values as a function of MDMA concentration determined using different DEX concentrations (the lines represent fits of the data by non-linear regression).

A 4-fold dilution of the pre-incubation mixture resulted in higher values of k_{inact} and K_I relative to those observed without dilution (0.29 ± 0.03 SE vs 0.21 ± 0.02 min^{-1} ; $p < 0.02$ and 12.9 ± 3.6 vs 3.5 ± 0.8 µM ; $p < 0.01$; respectively).

Discussion

The observations indicate that probe substrate concentrations in excess of K_m (≈ 1.7 µM for DEX) will not have a major impact on K_I and k_{inact} values. Dilution after pre-incubation minimises any additional MBI that may occur during the subsequent incubation period and increases the value of K_I. However, the fold dilution that fully prevents inactivation during the incubation stage requires further evaluation as the literature is inconsistent in this regard (Ghanbari *et al*. in preparation)
These preliminary data emphasise the importance of study design in the accurate characterisation of the kinetics of MBI, with implications for *in vitro-in vivo* extrapolation of the extent of MBI-based drug-drug interactions.

References

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