Theoretical Assessment of a New Experimental Protocol for Determining Kinetic Values Describing Mechanism (Time)-Based Enzyme Inhibition

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Introduction

We have shown that the conventional experimental protocol (CEP) used to determine the kinetic values for mechanism-based inhibition (MBI) may introduce substantial bias in parameter estimation (Yang et al., 2005). A further concern with the CEP is that it only determines 3 parameters, namely k_{inact} (the maximum rate of inactivation), K_I (the concentration of inhibitor that produces half-maximal inactivation), and r (the partition ratio), and ignores reversible inhibition, as defined by K_i (the reversible inhibition constant). In taking account of the above issues, we now propose a novel, mechanistically-based experimental protocol (MEP) as an alternative to the CEP, and evaluate it theoretically by *in silico* simulation.

Methods

The MEP comprises three independent parts *viz.* assessment of the metabolism of the mechanism-based enzyme inactivator (MBEI), of its ability to participate in competitive inhibition and its ability to cause time-dependent inhibition (Fig. 1). Virtual experiments were run with 16 reported MBEIs of varying inactivation potencies using Matlab® and Simulink® (The MathWorks, Inc., MA, USA). These were 'real' compounds for which k_{inact}, K_I, r, and K_i values have been reported in the literature. Experimental error was incorporated into the simulations by adding normally distributed random errors (5% CV) to the simulated concentrations. The kinetic parameters (k_{inact}, K_I, r and K_i) were then determined by solving the kinetics simultaneously using a Genetic Algorithm, and compared with the starting values to evaluate the performance of the MEP.

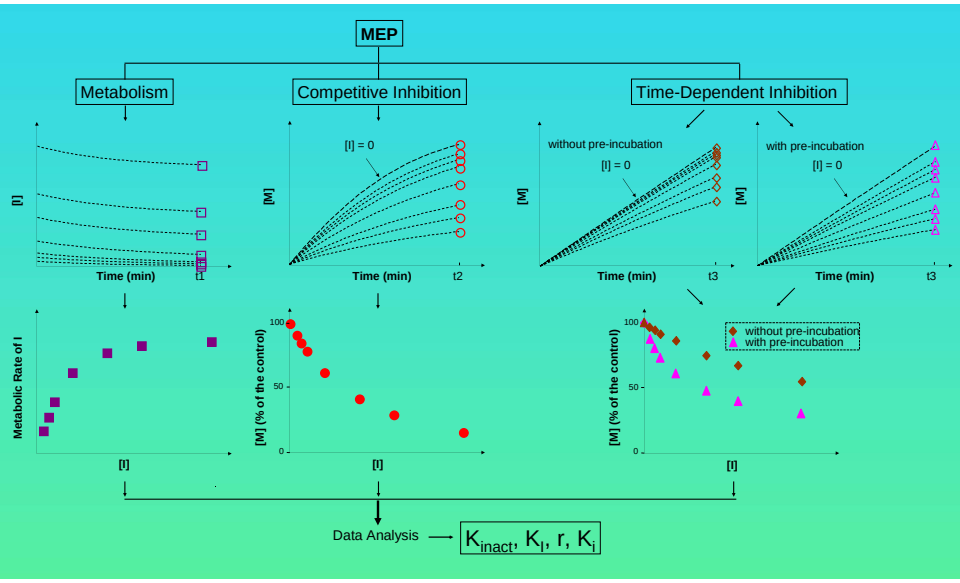
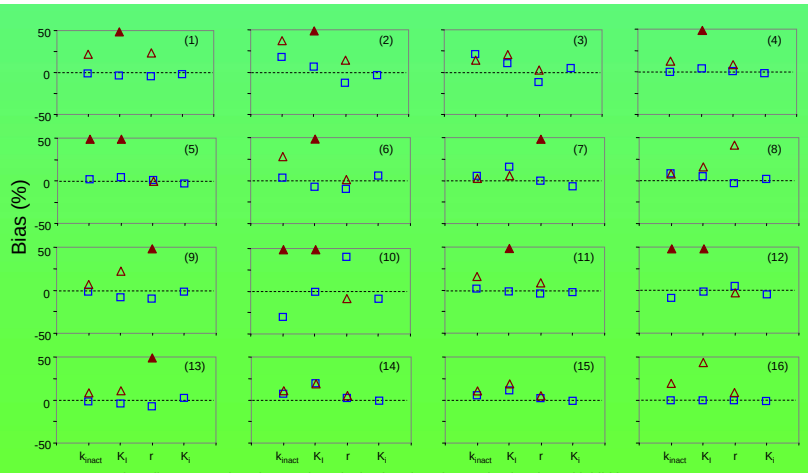


Fig. 1. The three elements of the MEP: metabolism of the MBEI, competitive inhibition of the probe substrate, and time-dependent inhibition of the probe substrate (The plots refer to the simulation of experiments with (R)-(+)-menthofuran).

Results and Conclusion

In the absence of experimental error, the MEP yielded exact kinetic parameters for all 16 MBEIs (data not shown); In comparison, estimates obtained using the CEP were less accurate. In the presence of experimental error, the MEP still yielded accurate and precise estimates (Fig. 2). Thus, relative to the CEP, which was associated with biases greater than 50% for one or more parameters, the MEP reduced the bias for 11 test compounds. For 5 test compounds parameter estimates using the CEP were reasonably accurate.

On theoretical grounds and based on the results of simulation, the MEP is considered to be superior to the CEP with regard to accuracy, precision and efficiency. Its application may allow better prediction of the *in vivo* implications of MBI.



CEP with no experimental error (▲ias of over 50%)

MEP with 5% CV experimental error

Fig. 2. Bias in recovering starting kinetic parameter values of 16 reported MBEIs by simulation. Bias for MEP with no experimental error for all 16 MBEIs are all less than 1% (not shown on the figure; (1) (R)-(+)-menthofuran; (2) 17-α-ethynylestradiol (2B6); (3) 17-α-ethynylestradiol (3A4); (4) delavirdine; (5) diltiazem; (6) EMTPP; (7) gestodene; (8) L-754,394; (9) mibefradil; (10) phencyclidine; (11) PPP; (12) suprofen; (13) TA; (14) ticlopidine; (15) tTEPA; and (16) verapamil.

Ref.

Yang J, Jamei M, Yeo KR, Rostami-Hodjegan A and Tucker GT (2005) Kinetic values for mechanism-based inhibition: Assessment of bias introduced by the conventional experimental protocol. *Eur J Pharm Sci* **26**:334-340.

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