A CRITICAL EVALUATION OF THE EXPERIMENTAL DESIGN IN REPORTS OF MECHANISM BASED INHIBITION (MBI) STUDIES AND THE ASSESSMENT OF THEIR IMPLICATION FOR IN VITRO-IN VIVO EXTRAPOLATION (IVIVE)

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INTRODUCTION

A mechanism-based inhibitor is metabolised by an enzyme to form a metabolic intermediate, which binds to the same enzyme, most often covalently, leading to irreversible inactivation of the enzyme and consequently to loss of protein function (Silverman, 1964). The *in vitro* characterization of a mechanism-based inactivator includes determination of the maximum inactivation rate constant (k_{inact}), the inactivator concentration that produces half-maximal rate of inactivation (K_I), and the partition ratio (r). Determination of the kinetics of MBI involves a pre-incubation followed by an incubation stage, although experimental design differs considerably between laboratories. The aim of the present study was to explore the variation in experimental conditions and data analysis used to determine inhibition constants for MBI of cytochromes P450 (CYPs). In addition, assuming that the reported kinetic values in these studies were correct, we attempted to assess the *in vivo* impact of the inhibitor on the specific metabolic pathway.

METHODS

Two electronic databases, ISI Web of Science, PUBMED and personal files were searched for published reports of MBI in the literature; 265 relevant papers were identified. Of these, 73 reported *in vitro* experiments on MBI and only 57 contained k_{inact} and K_I values. The latter were investigated for the following:

• The relative length of time for incubation and pre-incubation; fold-dilution of pre-incubation to the incubation mixture; and concentration of the probe substrate. The occurrence of simultaneous MBI and competitive inhibition during the incubation period is dependent on each of these factors.

• Normalization for time-dependent changes in enzyme activity in the absence of any inhibitor

• Methods used for calculation of the kinetic constants

• Clarity of the description of the methodology used

Among the mechanism based inhibitors investigated in this analysis therapeutic compounds were chosen for estimating the fold reduction in CYP intrinsic clearance (Cl_{int}) using the following equation (Rostami-Hodjegan and Tucker 2004)

$$\text{Fold Reduction in CL}_{int,j} = \frac{k_{deg} + \dfrac{[I] \times k_{inact}}{[I] + K_I}}{k_{deg}}$$

where [I] represents the therapeutic concentrations of the inhibitor at the active site of enzyme (considered to be equal to unbound concentration in plasma).

RESULTS

The results of this evaluation are shown in Figures 1 to 3. A wide variability in the timing of the first sample during the "pre-incubation" stage (Range: 0.5 – 6 min; Mode: 2 min), the dilution factor (Range: no dilution – 100 fold; Mode: 20 fold), and the incubation time for measurement of remaining enzyme activity (Range: 2 – 20 min; Mode: 10 min) was apparent.
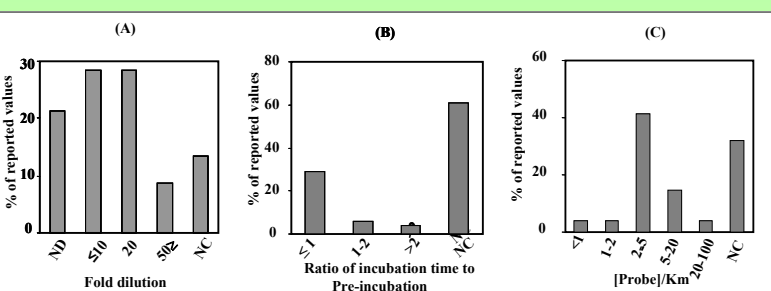

Figure 1. Assessment of methodology used to prevent the occurrence of MBI and competitive inhibition during incubation; (A) fold dilution of pre-incubation mixture to the incubation (ND; No Dilution, NC; Not Clear); (B) ratio of the incubation time to the pre-incubation as long as the inactivation rate is linear, (C) ratio of probe concentration to its K_m.

No correction was made for baseline decline in enzyme activity in 9% of studies and 14% of the reports used the slopes of the log in base 10 instead of the natural log (Ln) for calculating inactivation constants. The use of non-linear fitting was popular, though 55% of the data analyses were carried out using Kitz-Wilson plots.
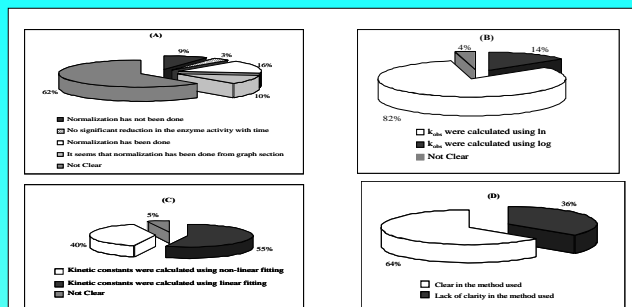

Figure 2. Assessment of 57 studies for : (A) normalization of time change in the absence of inhibitor, (B) method of calculating the rate of inactivation, (C) data analysis for calculating the kinetic values and (D) the clarity in describing the methods used.
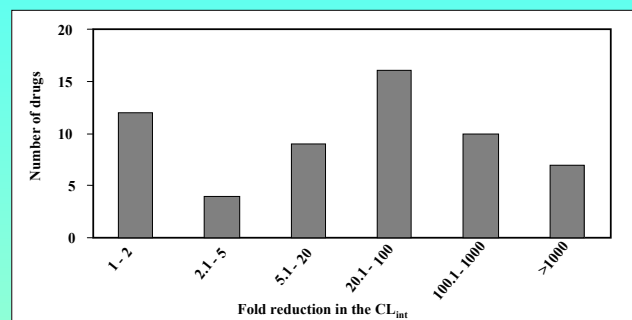

Figure 3. Fold reduction in CYP CL_{int} caused by mechanism based inhibitors.

The *in vivo* consequences of applying kinetic values from different reports (but for the same inhibitor) to IVIVE are demonstrated in Table 1. In this example, a range of parameter values are reported for the CYP3A inactivation by verapamil, which led to differences in the estimated values for fold reduction in the CL_{int} of CYP3A.

Table 1. Variation in estimated fold reduction in CYP3A4 CL_{int} caused by verapamil

Compound	CYP	System	k_{inact} (h^{-1})	K_I (μM)	Reference	Fold reduction in CYP3A CL_{int}
Verapamil	3A4	HLM	1.8	0.5	Ma et al 2000	45.6
Verapamil	3A4	HLM	9	2.9	Yeo & Yeo 2001	27.1
Verapamil	3A4	HLM	5.5	4.2	Polasek et al 2004	5.1

DISCUSSION

Estimates of the *in vitro* parameters k_{inact} and K_I are required for assessment of the *in vivo* consequences of MBI. A valid prediction requires a database containing accurate *in vitro* enzyme kinetics for the inhibitor and accurate *in vivo* pharmacokinetic data for substrates particularly with respect to the fractional metabolism via certain pathways (f_m). Thus improvements in designing MBI experiments to maximize the quality of the information obtained and careful assessment of literature reports are essential for increasing our confidence of *in vitro-in vivo* extrapolations. Calculation of the fold reduction in the CL_{int} could provide an estimate of the pharmacokinetic changes observed *in vivo*.

REFERENCES

Silverman, RB (1995) Mechanism-based enzyme inactivators. *Methods Enzymol* **249:**240-283.
Rostami-Hodjegan, A & Tucker, GT (2004) *Drug Discovery Today* **1**(4):441-448.
Ma, B *et al.* (2000) *Drug Metab Disp* **28**:125-130.
Yeo, KR & Yeo, WW (2001) *Br J Clin Pharmacol* **51**(5):461-70.
Polasek, TM *et al.* (2004) *J Pharmacol Exp Ther* **311**(3):996-1007.