Mechanism-Based Inactivation of CYP2D6 by Methylenedioxymethamphetamine (MDMA): Differences between Expressed Enzyme Systems and Human Liver Microsomes

L.M. Van*[1], J.A. Hargreaves[2], K. Rowland-Yeo[3], M.S. Lennard[1], G.T. Tucker[1,3] and A. Rostami-Hodjedan[1,3]

*** Correspondence: L.M.Van@sheffield.ac.uk**

[1]Academic Unit of Clinical Pharmacology, University of Sheffield, UK ; [2]AstraZeneca, DMPK, Alderley Park, Macclesfield, UK ;

[3]Simcyp Ltd, Blades Enterprise Centre, Sheffield, UK

INTRODUCTION

- The limited availability of human tissue has made the use of recombinantly expressed cytochrome P450 systems (rCYP) a practical alternative for assessing drug metabolism and enzyme inhibition.
- rCYP systems are now used routinely for drug metabolism studies in part, because of their compatibility with high-throughput protocols.
- Systematic evaluation of different rCYP systems in assessing mechanism-based enzyme inactivation (MBI) is lacking.

AIMS & OBJECTIVES

- To investigate the inactivation of CYP2D6 by 3,4-methylenedioxy-methamphetamine (MDMA) using different rCYP systems.
- To compare MBI kinetic parameters, k_{inact} (the maximum rate of inactivation) and K_I (the inactivation constant), in the different systems.

METHODS

- MDMA (0, 2, 5, 10 and 20 μM) was incubated with 30 pmol of enzyme from rCYP systems (CYPEX® bactosomes, *Saccharomyces Cerevisiae* (yeast) and BD Gentest® supersomes) expressing CYP2D6 or 0.2 mg human liver microsomes from a characterised liver sample (HLM6, *1/*1, 24 pmol of CYP2D6 per mg microsomal protein) at 37°C for 0, 2.5 and 5 min.
- This was followed by dilution (5-fold) into fresh NADPH-regenerating solution (0.4 μmol of NADP+, 4 μmol of glucose-6-phosphate (G6P), 2 μmol of $MgCl_2$ and 0.4 units of of G6P dehydrogenase) containing dextromethorphan (50 μM).
- The reaction was allowed to proceed for an additional 10 min and dextrorphan (DOR) was assayed by HPLC with fluorescence detection (λ_{ex}: 280 nm; λ_{em}: 310 nm) to assess remaining CYP2D6 activity.

DATA ANALYSIS

- Inhibition of DOR formation was expressed as a percentage of the time-matched control value without MDMA.
- Initial slopes of %LN (Enzyme Activity Remaining) *vs* preincubation time were used to determine inactivation rates ,k_{obs}.
- k_{inact} and K_I values were calculated from k_{obs} (weighted by variance) by non-linear least squares fitting of equation 1 (GraFit®, Erithacus Software Ltd):

$$k_{obs} = \frac{k_{inact} \times I}{K_I + I} \qquad \text{Equation 1}$$

- Mean values of k_{inact} and K_I observed using rCYPs and HLM were compared using the Z-test.

RESULTS

- Inactivation profiles of CYP2D6 by MDMA observed with the two eukaryotic rCYP (BD Gentest® supersomes and yeast) and HLM6 (Fig. 1) were consistent, giving broadly similar MBI kinetic values (Table 1).
- Kinetic parameter values could not be estimated with reasonable confidence using CYPEX® bactosomes (prokaryotic source).
- The CYPEX® system was associated with the highest turnover rate amongst the rCYPs.

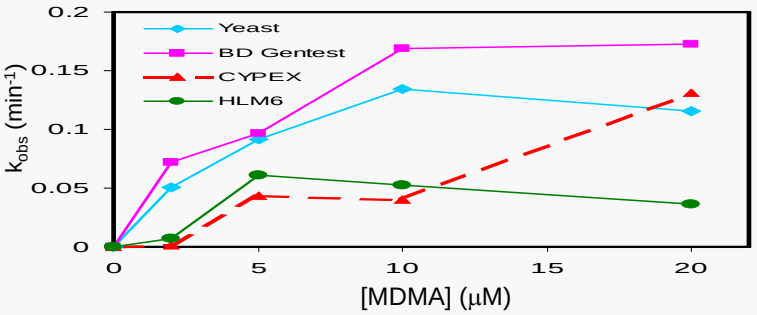

Figure 1. Relationship between inactivation rate constant (k_{obs}) and MDMA concentration. Values represent means of triplicate measurements.

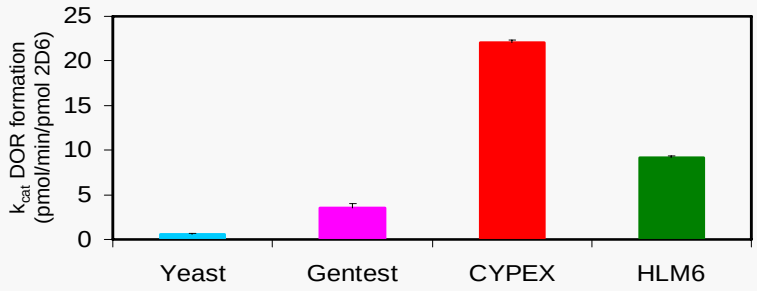

Figure 2. Turnover rates of the different systems with respect to the conversion of dextromethophan to dextrorphan. Value represent mean ± SE of triplicate measurements. *NB: HLM6 estimated to have 24 pmol 2D6/mg protein*.

Table 1: Comparison of kinetic parameters (mean ± SE; n = 3) characterising MBI of CYP2D6 by MDMA determined using different rCYP and HLM

Kinetic Data	Yeast (Microsomes)	BD Gentest® (Supersome)	CYPEX® (Bactosomes)	HLM6
k_{cat} (pmol min^{-1} pmol^{-1})	0.6 ± 0.08	3.5 ± 0.54	22 ± 0.31	9.2 ± 1.0
k_{inact} (min^{-1})	0.13 ± 0.009	0.22 ± 0.02	0.007 ± 80 [a]	0.05 ± 0.03
K_I (μM)	2.69 ± 0.82	4.01 ± 1.07	10.52 ± 292 [a]	2.01 ± 4.5
k_{inact}/K_I (mL min^{-1} nmol^{-1})	0.05	0.05	0.0007	0.02

(a) Values derived from double- reciprocal plots since fitting by non-linear regression did not converge.

DISCUSSIONS & CONCLUSIONS

- Disparity between HLM and some rCYP systems observed in this study has also been noted with regard to other compounds known to cause MBI (*e.g.* paroxetine (unpublished data); and cimetidine (Maderia *et al.*, 2004)).
- Quantitative prediction of the *in vivo* consequences of MBI will be compromised if kinetic values obtained from recombinant systems differ from those obtained with human liver.

REFERENCES

Maderia *et al*. (2004). *Drug Metab. Dispos*. **32**: 460-467.

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