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

L.M. Van†1, J.A. Hargreaves2, K. Rowland-Yeo3, M.S. Lennard1, G.T. Tucker1,3 and A. Rostami-Hodjedan1,3

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

1Academic Unit of Clinical Pharmacology, University of Sheffield, UK; †AstraZeneca, DMPK, Alderley Park, Macclesfield, UK; 3Simcyp 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-methylene dioxy 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 MgCl₂ and 0.4 units 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): $$k_{obs} = \frac{k_{inact} \times I}{K_I + I}$$ 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.

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.

ACKNOWLEDGEMENT

L.M. Van is supported by an AstraZeneca PhD Studentship.