Estimation of the Kinetic Parameters of Mechanism-Based Inhibition of CYP2D6 by Methylenedioxymethamphetamine (MDMA) in Cryopreserved Human Hepatocytes

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INTRODUCTION

- Human liver microsomes (HLM) and recombinant systems expressing human cytochromes P450 (rCYP) are commonly used to characterise mechanism-based inactivation (MBI) of these enzymes, but few studies have used hepatocytes for this type of investigation.
- A recent report has evaluated time-dependent inactivation (TDI) in hepatocytes but kinetic estimates were not defined (Zhao *et al*, 2005).
- Previous work in our laboratory has shown that MDMA is a potent MBI of CYP2D6 in both rCYP and HLM microsomes (Heydari *et al*, 2004).

AIMS & OBJECTIVES

- To determine MBI kinetic parameter values for the inhibition of CYP2D6 by MDMA in cryopreserved human hepatocytes.
- To compare MBI kinetic parameter values obtained from cryopreserved human hepatocytes with those using HLM.

METHODS

- MDMA was pre-incubated (0, 2, 5, 10, 20, and 50 μM) with 2.5 × 10^5 cells (female individual) in Leibovitz (L15) medium at 37°C supplemented with 5% CO_2.
- After 0, 15, 30 and 60 min, the pre-incubation mixture was diluted 5-fold in fresh L15 medium containing dextromethorphan, DEX (100 μM).
- Reactions proceeded for an additional 30 min before being quenched with an equal volume of acetronitrile containing 0.2 μg/μL of levallorphan as an internal standard.
- Samples were centrifuged and supernatants analysed by LC/MS/MS for dextrophan, DOR (258 → 201) and levallorphan (284 → 201).
- HLM experiments were similar to those reported by Heydari *et al*. (2004) and was carried out in a single *1/*1 genotype.

DATA ANALYSIS

- The relative inhibition of DOR formation expressed as percentage of the time-matched control without MDMA was calculated.
- Initial slopes from the %LN(Enzyme Activity Remaining) vs the preincubation time was used to determine inactivation rate at a given MDMA concentration, k_{obs} (Excel®).
- The values of k_{obs} (weighted by their variance) were used to obtain k_{inact} and K_I as part of non-linear fitting of all data together (GraFit®, Erithacus Software Ltd) according to equation 1:

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

- The mean values of k_{inact} and K_I and their standard errors (SE), as reported by non-linear fitting procedure, were used to compare HLM and hepatocytes results (Z-test implemented in Excel®) ; p values < 0.05 were considered to indicate statistically significant differences.

RESULTS

According to the results MDMA is an MBI of CYP2D6 in hepatocytes (see inactivation trends in Figure 1 (a)). Non-linear fitting to k_{obs} values defined the kinetic parameters, k_{inact} and K_I, in hepatocytes (Figure 1 (b)). K_I values in hepatocytes were not different from those derived from HLM experiments although MDMA appeared to be 10-fold less efficient in hepatocytes compared to HLM according to k_{inact} and inactivation efficiency (k_{inact}/K_I) (Figure 2).

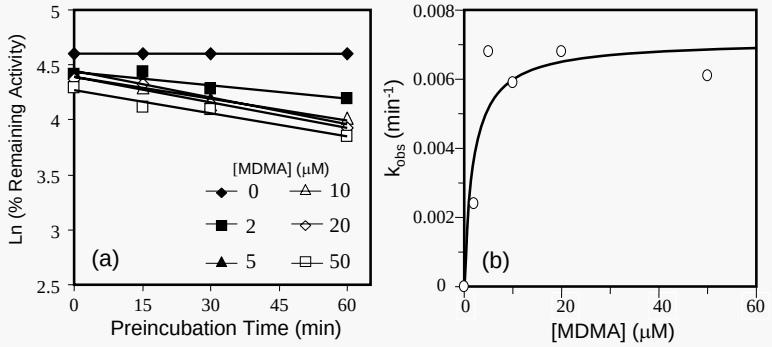

Figure 1. The effect of various MDMA concentrations and preincubation times on CYP2D6 activity in human hepatocytes (a). Non-linear regression of the inactivation rate (average of duplicate values of k_{obs}) vs MDMA concentrations to calculate kinetic parameter values (b).

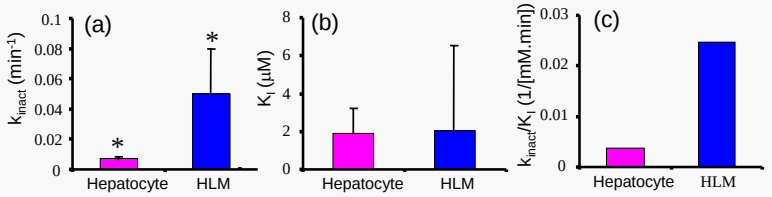

Figure 2. Comparison of k_{inact} (a), K_I (b) and inactivation efficiency (c). of MDMA between hepatocytes and HLM. Mean values and SE, as obtained from non-linear fitting, are marked by an asterisk if a statistically significant difference exists between hepatocytes and HLM.

DISCUSSIONS & CONCLUSIONS

- Hepatocytes more closely represent the liver than cell free systems, but limited availability of tissue has made the use of rCYPs a more practical alternative. To allow informed choice, comparison of the behaviour of MBIs in different *in vitro* systems is important.
- Current results using human hepatocytes confirmed previous observations that MDMA is an MBI of CYP2D6.
- The differences observed in the kinetic parameters from the different *in vitro* systems could be due to several factors including variation of metabolic consumptions in hepatocytes, and presence of active efflux transport.
- Inter-individual differences could also account for the discrepancy in results, since different donors were used for the hepatocytes and HLM. Further study using larger donor numbers is required to confirm inter-system differences.

REFERENCES

Heydari, A. *et al* (2004). *Drug Metab Dispos*. 32: 1213-1217

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