Psychopharm

Journal of Psychopharmacology
20(6) (2006) 842–849

ISSN 0269-8811
SAGE Publications Ltd, London, Thousand Oaks, CA and New Delhi
10.1177/0269881106065907

Implications of mechanism-based inhibition of CYP2D6 for the pharmacokinetics and toxicity of MDMA

Jiansong Yang *Simcyp Limited, Sheffield, UK.*
Masoud Jamei *Simcyp Limited, Sheffield, UK.*
Amir Heydari *University of Sheffield, Sheffield, UK.*
Karen R. Yeo *Simcyp Limited, Sheffield, UK.*
Rafael de la Torre *Institut Municipal d'Investigació Mèdica, Barcelona, Spain.*
Magí Farré *Institut Municipal d'Investigació Mèdica, Barcelona, Spain.*
Geoffrey T. Tucker *University of Sheffield, Sheffield, UK.*
Amin Rostami-Hodjegan *University of Sheffield, Sheffield, UK.*

Abstract

The aim of this study was to model the *in vivo* kinetic consequences of mechanism-based inhibition (MBI) of CYP2D6 by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). A model with physiologically-based components of drug metabolism was developed, taking account of change in the hepatic content of active CYP2D6 due to MBI by MDMA. Based on the *in vitro* information, plasma concentration–time profiles of MDMA after various doses were computed and compared with reported observations. The analysis suggested that a typical recreational MDMA dose could inactivate most hepatic CYP2D6 within an hour, and the return to a basal level of CYP2D6 could take at least 10 days. Thus, the genetic polymorphism of CYP2D6 and co-administration of CYP2D6 inhibitors may have less impact on MDMA pharmacokinetics and the risk of acute toxicity than previously thought. This is consistent with clinical observations that indicate no obvious link between inherited CYP2D6 deficiency and acute MDMA intoxication.

Keywords

ecstasy, inactivation, MDMA, mechanism-based inhibition, modelling

Introduction

MDMA (3,4-methylenedioxymethamphetamine), commonly known as 'ecstasy', is a synthetic amphetamine derivative. Its increasing popularity as a recreational drug has raised concern about its acute and long-term effects on users. Acute MDMA toxicity manifests as an increase in heart rate and blood pressure, and hyperthermia, potentially leading to rhabdomyolysis, kidney and liver failure, and death (Henry *et al*., 1992). Chronic use is linked to changes in serotoninergic function (Green *et al*., 2003). *In vitro* studies (Tucker *et al*., 1994; Kreth *et al*., 2000; Ramamoorthy *et al*., 2002) have shown that CYP2D6 is the main enzyme responsible for the demethylenation of MDMA, its major metabolic pathway (Fig. 1). Therefore, subjects genetically deficient with respect to CYP2D6 ('poor metabolizers' (PMs), who represent about 7–10% of Caucasians (Ingelman-Sundberg *et al*., 1999)), were postulated to be more susceptible to the acute toxic effects of MDMA (Tucker *et al*., 1994; Delaforge *et al*., 1999). Conversely, since long-term neurotoxicity is believed to be mediated by metabolites formed after methylendioxyphenyl ring-opening by CYP2D6 (de la Torre and Farre, 2004; Jones *et al*., 2005), PMs might be protected against chronic toxicity. However, several reports indicate no obvious link between inherited CYP2D6 deficiency and acute MDMA intoxication (O'Donohoe *et al*., 1998; Schwab *et al*., 1999; Gilhooly and Daly, 2002).

Corresponding author: Dr Jiansong Yang, Simcyp Limited, Blades Enterprise Centre, John Street, Sheffield S2 4SU, UK. *Email*: J.Yang@simcyp.com

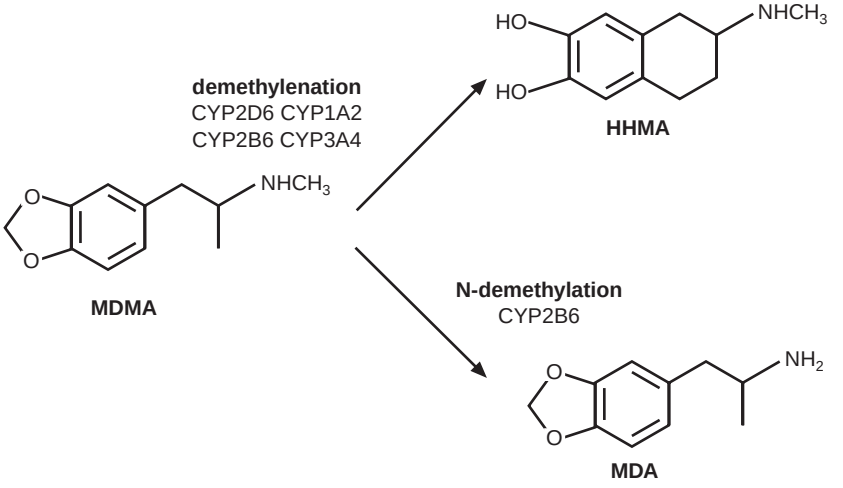


Figure 1 Principle metabolic pathways of MDMA. Tucker *et al*. (1994) showed that about two-thirds of the demethylenation is mediated by CYP2D6 in human liver microsomes. Kreth *et al*. (2000) confirmed this and indicated the involvement of other CYPs in the main pathways (HHMA = 3,4-dihydroxymethamphetamine; MDA = 3,4-methylenedioxyamphetamine).

As well as being metabolized by CYP2D6, MDMA is also a potent mechanism-based inhibitor of the enzyme *in vitro* (Wu *et al*., 1997; Delaforge *et al*., 1999; Heydari *et al*., 2004), inactivating the enzyme by the formation of a metabolic inhibitory complex. Auto-inhibition by this phenomenon may explain dose- and time-dependence observed in the *in vivo* kinetics of MDMA (de la Torre *et al*., 2000). In this study, we have used the *in vitro* data on metabolism and mechanism-based inhibition (MBI) to simulate the *in vivo* kinetics of MDMA, and to assess whether MBI might explain its non-linear kinetics and the lack of association between the poor metabolizer genotype for CYP2D6 and the risk of acute toxicity.

Methods

Pharmacokinetic model

A physiologically-based model of the *in vivo* kinetics of MDMA was developed (Fig. 2), with the following assumptions: (i) first-order oral absorption after a lag time, with no gut wall metabolism, (ii) a blood to plasma MDMA concentration ratio of one, (iii) a single-compartment distribution model and elimination by renal excretion and hepatic metabolism, (iv) CYP2D6, CYP1A2 and CYP2B6 are the major enzymes contributing to the *in vitro* metabolism of MDMA (Kreth *et al*., 2000), (v) only CYP2D6 is subject to MBI, (vi) CYP2D6 turnover described by a zero-order synthesis rate and a first-order degradation rate, and (vii) unbound MDMA concentration in blood as the driving force for metabolism in the liver.

Differential equations describing the model were as follows:

$$V_{PV} \cdot (dC_{PV}/dt) = C_{sys} \cdot Q_{PV} + ka \cdot fa \cdot D \cdot e^{-ka \cdot (t - t_{lag})} - C_{PV} \cdot Q_{PV} \quad (1)$$

$$V_{liver} \cdot (dC_{liver}/dt) = (C_{PV} \cdot Q_{PV} + C_{sys} \cdot Q_{HA}) - (fu_B \cdot C_{liver} \cdot CL_{int} + C_{liver} \cdot Q_H) \quad (2)$$

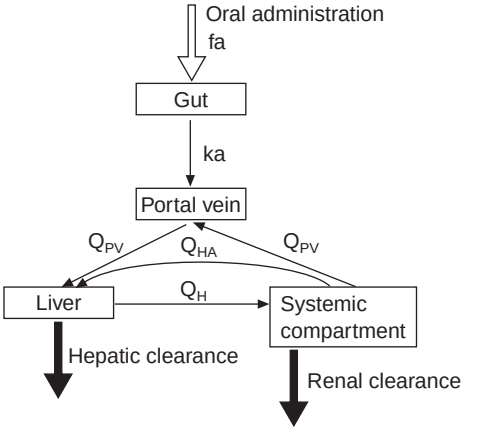


Figure 2 A physiologically-based pharmacokinetic model of MDMA kinetics. fa = fraction absorbed; ka = absorption rate constant; Q_{PV} = hepatic portal blood flow; Q_{HA} = hepatic arterial blood flow; Q_H = hepatic blood flow. The systemic compartment is comprised of all the other tissues apart from liver and blood. The distribution of MDMA within the systemic compartment is assumed to be instantaneous.

$$CL_{int} = V_{max,2D6} \cdot [E_{2d6}]_t/(K_{m,2d6} + fu_B \cdot C_{liver}) + V_{max,1A2} \cdot E_{1A2}/(K_{m,1A2} + fu_B \cdot C_{liver}) + V_{max,2B6} \cdot E_{2B6}/(K_{m,2B6} + fu_B \cdot C_{liver}) \quad (3)$$

$$d[E_{2D6}]_t/dt = k_{deg,2D6} \cdot ([E_{2D6}]_0 - [E_{2D6}]_t) - k_{inact} \cdot fu_B \cdot C_{liver} \cdot [E_{2D6}]_t/(K_I + fu_B \cdot C_{liver}) \quad (4)$$

$$V \cdot (dC_{sys}/dt) = C_{liver} \cdot Q_H - C_{sys} \cdot Q_H - C_{sys} \cdot CL_R \quad (5)$$

where: V_{PV}, V_{liver}, V are the volumes of portal vein, liver and the systemic compartment, respectively, and C_{PV}, C_{liver} and C_{sys} are the corresponding MDMA concentrations; ka and fa are the first-order

absorption rate constant and the fraction of MDMA dose absorbed from the gut lumen, respectively; t_{lag} is the time delay for absorption. D is the oral dose of MDMA; Q_H, Q_{PV} and Q_{HA} are blood flows for liver, portal vein and hepatic artery, respectively, fu_B is the unbound fraction in blood; CL_{int} is the hepatic intrinsic clearance; $V_{max,1A2}$, $V_{max,2B6}$, $V_{max,2D6}$ are the maximum rates of metabolism by CYP1A2, CYP2B6, CYP2D6, respectively; and $K_{m,1A2}$, $K_{m,2B6}$, $K_{m,2D6}$ are the corresponding Michaelis-Menten constants; $[E_{2D6}]_0$ and $[E_{2D6}]_t$ are the active amounts of CYP2D6 at baseline and at time t after MDMA dosage, respectively; E_{1A2} and E_{2B6} are the abundances of CYP1A2 and CYP2B6 in the liver, respectively; $k_{deg,2D6}$ is the degradation rate constant (turnover rate constant) of CYP2D6; k_{inact} is the maximum rate constant for inactivation of CYP2D6 by MDMA; K_I is the MDMA concentration which produces half the maximal rate of inactivation; and CL_R is renal clearance.

Parameter values and modelling procedure

Most of the model parameters were fixed based on prior knowledge (blood flows, *in vitro* metabolism, enzyme abundances and reported estimates of plasma binding and renal clearance) (Table 1). However, the values of fa, ka, t_{lag} and V were not available *a priori*. Simple pharmacokinetic models which do not account for time-dependent changes in MDMA clearance cannot be used to derive values of these parameters, and the direct estimation of V requires knowledge of the kinetics after intravenous administration. Studies involving intravenous administration of MDMA have not been carried out. To solve the problem required that estimates of the missing parameters be determined from the available experimental data obtained after oral administration of MDMA in healthy volunteers (de la Torre *et al*., 2000; Farre *et al*., 2004). Thus, the outcome of the modelling process was based partly on fitting of these data. Estimates of fa, ka, t_{lag}, and V were obtained by fitting the complete physiological model (Fig. 2) simultaneously to the average observed data at the 75, 100 and 125 mg dose levels (de la Torre *et al*., 2000; Farre *et al*., 2004). Initially we attempted to utilize commonly-used pharmacokinetics/pharmacodynamics PKPD packages for fitting the data. Thus, all differential equations of the model were incorporated into WinNonLin 4.0.1 (Pharsight Corporation, CA). However, when the Gauss-Newton algorithms (Levenberg and Hartley; Hartley) were applied, the fittings failed to converge; and when the Nelder-Mead algorithm was applied, the fitting results were not reproducible. Therefore, a genetic algorithm (GA) was applied as an alternative optimization approach for this highly non-linear system (see Appendix for details).

Briefly, GA is a global search and optimization approach that is based on natural selection, the process that drives biological evolution (Holland, 1975; Goldberg, 1989; Back *et al*., 1997). Whereas most stochastic search methods operate on a single solution to the problem at hand, GA operates on a population of solutions. Therefore, GA generally performs better than gradient search methods if the search space has many local optima (Goldberg, 1989; Back *et al*., 1997).

The sum of squared errors (eq. 6) was used as the objective function (Obj) for optimization process. The method was implemented in Matlab and Simulink (The MathWorks, Inc., MA, USA).

Table 1 Parameter values used in the modelling exercise

Parameter	Value	Reference
Molecular weight	193.25	
fu_B	1*	Heydari *et al*., 2004
$[E_{2D6}]_0$	7 pmol/mg protein**	Yeo *et al*., 2004
E_{1A2}	37 pmol/mg protein**	Yeo *et al*., 2004
E_{2B6}	7 pmol/mg protein**	Yeo *et al*., 2004
$V_{max,2D6}$	223 pmol/min/mg protein**	Kreth *et al*., 2000
$K_{m,2D6}$	2.2 μM	Kreth *et al*., 2000
$V_{max,1A2}$	745 pmol/min/mg protein**	Kreth *et al*., 2000
$K_{m,1A2}$	377 μM	Kreth *et al*., 2000
$V_{max,2B6}$	138 pmol/min/mg protein**	Kreth *et al*., 2000
$K_{m,2B6}$	1111 μM	Kreth *et al*., 2000
$k_{deg,2D6}$	0.01 /h	Renwick *et al*., 2000
k_{inact}	0.29 /min	Heydari *et al*., 2004
K_I	12.9 μM	Heydari *et al*., 2004
CL_R	11.8 L/h	de la Torre *et al*., 2000
Q_H	98 L/h	Valentin, 2002
Q_{PV}	74 L/h	Valentin, 2002
Q_{HA}	24 L/h	Valentin, 2002
V_{liver}	1.6 L	Valentin, 2002
V_{PV}	0.008 L	Valentin, 2002

* The fraction unbound in plasma is 1.0 (Heydari *et al*., 2004) and no uptake into hepatocytes was assumed.

** When calculating hepatic enzyme abundances and intrinsic clearance, 1 pmol/mg microsomal protein was equated to 0.053 μmol/liver (assuming a liver weight of 1.6 kg, and 33 mg microsomal protein per gram of human liver (Wilson *et al*., 2003)).

$$\text{Obj} = \Sigma \left(\frac{(C)_i - (\hat{C})_i}{(\hat{C})_i} \right)^2 \quad (6)$$

where C is the observed non-zero plasma drug concentration, and $\hat{C}$ is the corresponding estimated value.

Simulations

Using the physiologically-based model, MDMA concentration–time profiles in plasma were recovered for the following conditions: (i) single oral 50, 75, 100, 125 or 150 mg doses of MDMA in an average individual with functional CYP2D6 ('extensive metabolizer', EM); (ii) two successive oral doses of 100 mg MDMA staggered by 24 h in an average EM; (iii) a 100 mg oral dose of MDMA in an average individual without functional CYP2D6 (PM, $[E_{2D6}] = 0$); and (iv) a 100 mg oral dose of MDMA in average EM individuals with renal insufficiency (50% normal renal clearance) and renal failure (no renal clearance).

Results

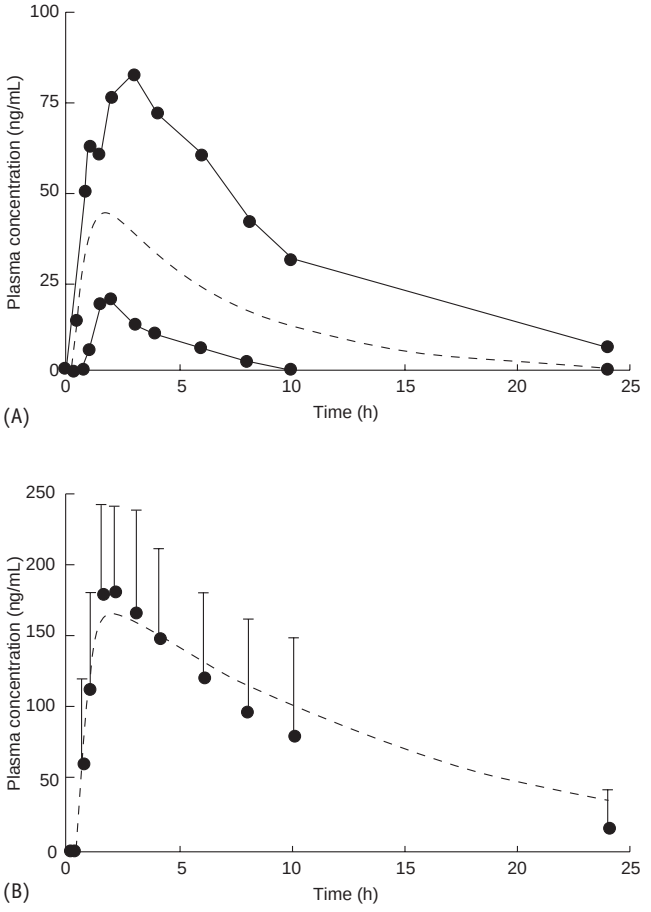
Estimated kinetic parameter values

Mean values for fa, ka, t_{lag} and V were 0.84 (± 0.03 SD), 2.3 (± 0.3) h^{-1}, 0.42 (± 0.01) h and 325 (± 11) L, respectively. Fixing the value of fa to 1 gave inferior fits to the data.

Comparison of model-derived and observed plasma MDMA concentrations

Given the issues around the modelling process described above, a reasonable agreement was apparent between the model-derived and observed plasma MDMA concentrations after single oral doses of 50, 75, 100, 125 or 150 mg (Fig. 3). The *in vivo* data for the 50 and 150 mg MDMA doses were not part of the optimization process because of the limited experimental data (two individuals at each dose level); nevertheless, the predicted concentration–time profiles seem to be consistent with the experimental data.

Fig. 4 shows predicted and observed plasma MDMA concentrations after two successive 100 mg oral doses. Also shown is the predicted second dose profile based on simple superposition of the first dose data. C_{max} and the area under the concentration vs time



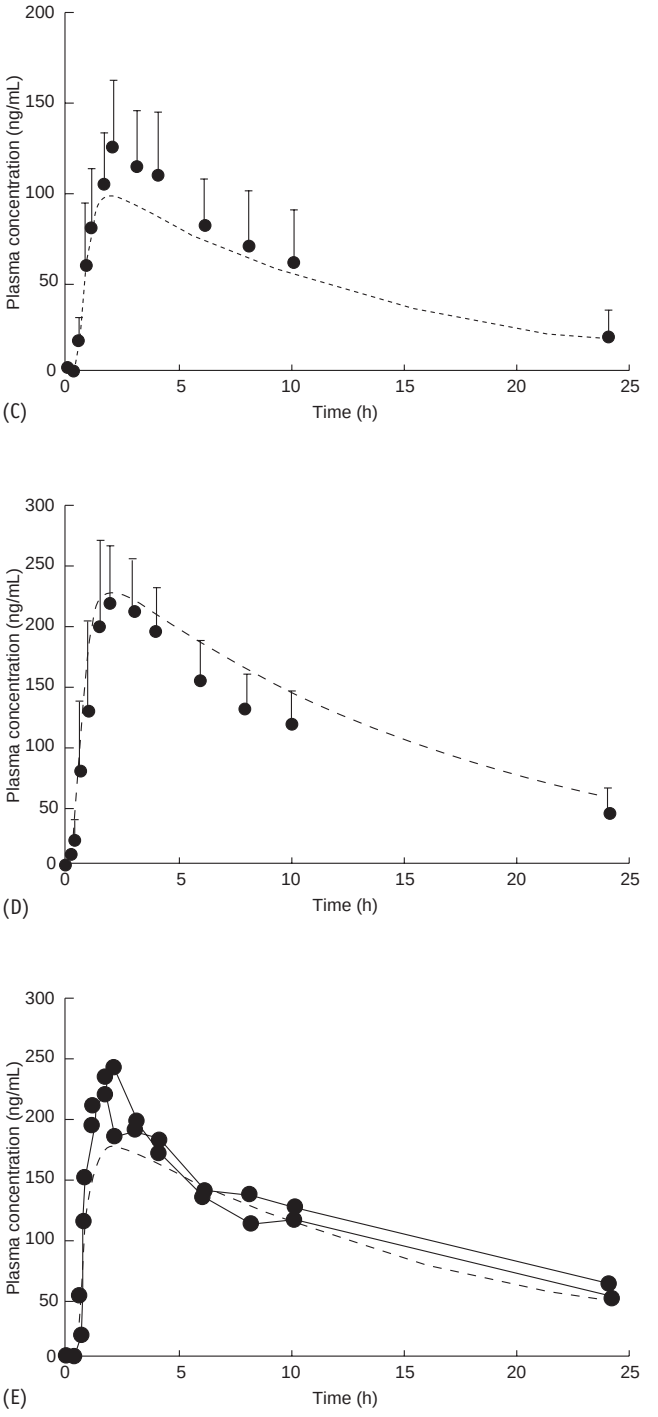


Figure 3 Observed (de la Torre *et al.*, 2000; Farre *et al.*, 2004) and model-derived plasma MDMA concentrations after single oral doses of 50 (A), 75 (B), 100 (C), 125 (D) or 150 mg (E) MDMA (n=2, 8, 9, 8 and 2, respectively). Solid circles represent observed values, and error bars represent standard deviations. For the 50 and 150 mg doses, individual observed data are shown; for the other doses mean observed data are shown. The broken lines indicate the model-derived profiles.

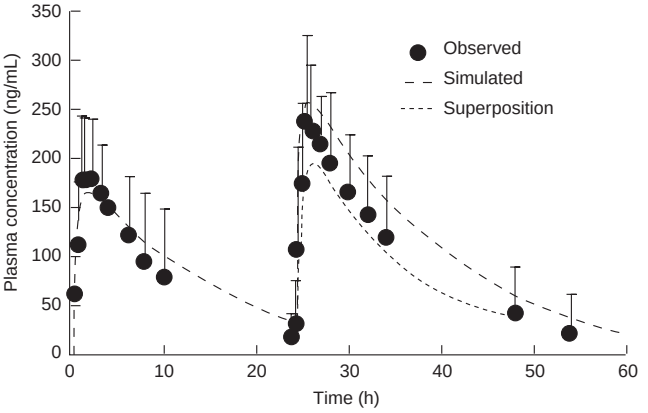


Figure 4 Observed (Farre *et al.*, 2004) and model-derived plasma MDMA concentrations after two oral doses of 100 mg MDMA given successively at 0 and 24 hours. Concentrations following the second dose predicted from the first dose by superposition are also indicated.

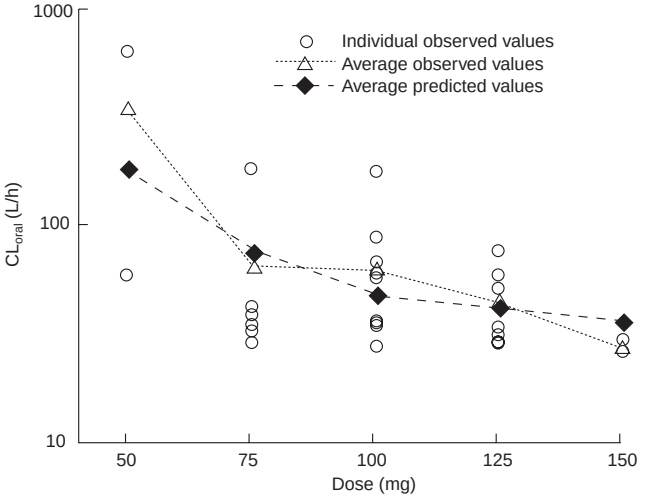


Figure 5 Individual and average observed values of the oral clearance of MDMA after various doses (from de la Torre *et al.*, 2000; Farre *et al.*, 2004) and average values predicted by the model.

curve (AUC) associated with the second dose were overpredicted by the model but underpredicted by superposition, although in both cases the mean predictions were within the range of observed values in individual subjects.

Individual observed values of oral clearance (dose/AUC) at each dose level are shown in Fig. 5, along with mean observed values and model-derived values. Both observed and model-derived mean values show a trend for decrease in clearance with increase in dose.

Active CYP2D6 content in the liver after oral dosage of MDMA

The model indicated that after single typical recreational doses of MDMA, CYP2D6 activity decreases rapidly (within an hour) as a consequence of MBI and the return to a basal level of CYP2D6 could take at least 10 days (Fig. 6). Above a dose of 100 mg the differences in the enzyme activity profiles are minimal.

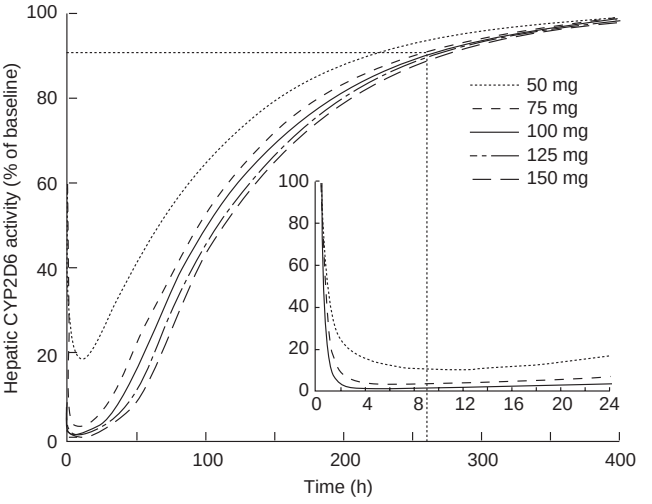


Figure 6 Predicted active CYP2D6 in the liver after oral doses of 50, 75, 100, 125 and 150 mg MDMA.

The effect of CYP2D6 expression on MDMA kinetics

The plasma MDMA concentrations in EM and PM subjects predicted by the model indicated that the genetic absence of functional CYP2D6 would be associated with a 36% increase in C_{max} and a 70% increase in AUC (Fig. 7).

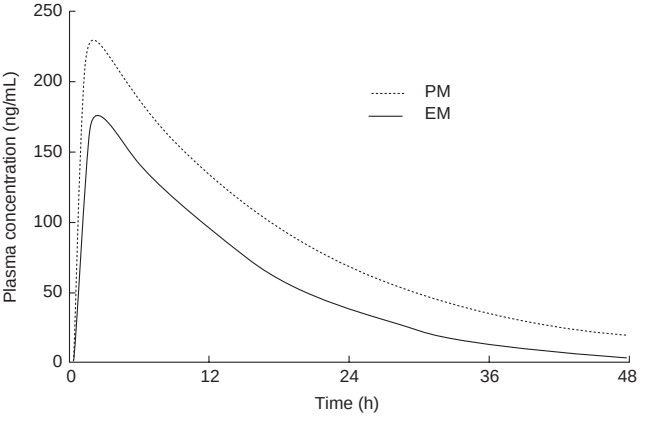


Figure 7 Model-derived plasma concentrations of MDMA after an oral dose of 100 mg MDMA in a PM and EM with respect to CYP2D6 activity.

The effect of renal impairment on MDMA kinetics

The model indicated that a progressive decrease in the renal clearance of MDMA should have an appreciable effect on plasma MDMA concentrations (Fig. 8). Although the effect on C_{max} was small, the impact on AUC was up to a 110% increase in complete renal failure. This effect would be even greater if CYPs 1A2, 2B6 and 3A4 were subject to MBI in addition to CYP2D6.

Discussion

The fitting procedure utilized a GA approach. Although a comparison between GA and conventional optimization methods was not the primary objective of this study, the outcome supports the further application of GA in complex PKPD modelling. GA has been already applied successfully in various fields, including chemistry, biology and many engineering disciplines. However, to our knowledge its application to PKPD modelling has been limited. It is well-known that GA performs better than gradient search methods if the search space has many local optima (Goldberg, 1989). Considering the growing complexity of physiologically-based PKPD models, the use of conventional optimization techniques and software in PKPD analysis becomes less appropriate. In the current application, commonly-used optimization algorithms, as implemented in WinNonLin, were unable to provide a modelling solution.

The physiologically-based pharmacokinetic model recovered observed plasma MDMA concentrations reasonably well with respect to both the underlying dose- and time-dependence of the kinetics (Fig. 3). Simple superposition underestimated concentrations after a second dose (Fig. 4). A degree of overprediction using the model may be due to several factors. For example, the conventional experimental protocol (Silverman, 1988) used for estimating the parameters describing MBI (k_{inact} and K_I) of MDMA may introduce bias (Yang *et al*., 2005), and estimates of the V_{max} and K_m values of a mechanism-based inactivator may be confounded by the inactivation of the enzyme (Yang and Rostami-Hodjegan, 2004). Simulation of the turnover of hepatic CYP2D6 was based on limited data from studies of the rate of loss of enzyme activity in human liver slices, indicating an average half-life of about 70 h (Renwick *et al*., 2000).

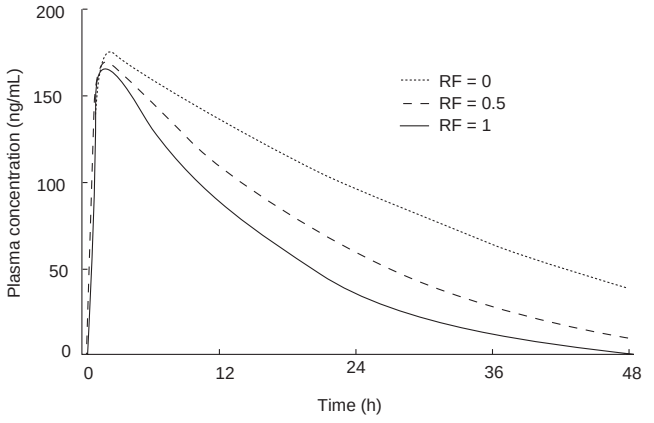


Figure 8 Model-derived plasma concentrations of MDMA after an oral dose of 100 mg MDMA in subjects with impaired renal clearance (RF=renal function relative to normal).

Despite the above caveats, the modelling exercise suggests some novel implications with regard to understanding the kinetics and toxicity of MDMA. Effectively, because of rapid MBI during 'first-pass' through the liver, the importance of CYP2D6 as the main enzyme involved in the metabolism of MDMA *in vitro* may not be extrapolated directly to *in vivo* kinetics after recreational doses. Although there are no prospective studies comparing the kinetics of MDMA in CYP2D6 PM and EM individuals, the results of an unpublished study (de la Torre *et al*.) indicate only a two-fold difference in AUC between the only PM and the other nine individuals receiving a single 100 mg oral dose of MDMA. This is in agreement with the model-derived difference of 1.7-fold (Fig. 7). Several further implications follow from this:

1 Genetic deficiency in CYP2D6 may not be a significant risk factor for acute toxicity to MDMA since, at recreational doses, the CYP2D6 activity of EM individuals may approach that of PMs. This suggestion is consistent with several reports that fail to make a link between PM status and acute toxicity (O'Donohoe *et al*., 1998; Schwab *et al*., 1999; Gilhooly and Daly, 2002).
2 According to the simulations, the majority of hepatic CYP2D6 is inactivated within an hour after a recreational dose of MDMA (Fig. 6). Therefore, the pharmacokinetics of MDMA after a normal recreational dose are unlikely to be markedly affected by co-administered CYP2D6 inhibitors, unless they also inhibit other elimination pathways (i.e. renal clearance, and metabolism by CYP1A2, CYP2B6 and, possibly, CYP3A4). This is supported by a modest (30%) increase in the AUC of MDMA when co-administered with paroxetine (Segura *et al*., 2005), a potent mechanism-based inactivator of CYP2D6 (Bertelsen *et al*., 2003). Reports on life-threatening interactions between MDMA and CYP2D6 inhibitors (e.g. ritonavir (Henry and Hill, 1998)) explained these interactions by inhibition of CYP2D6. However, this seems unlikely based on our findings. Thus, ritonavir inhibits not only CYP2D6 (von Moltke *et al*., 1998), but also CYP2B6 (Hesse *et al*., 2001) and CYP3A4 (von Moltke *et al*., 2000).
3 Because of the inactivation of CYP2D6 by MDMA, its renal clearance would be expected to assume much more importance as a route of elimination after recreational doses. Therefore, subjects with compromised renal function and those taking other drugs that can impair renal function (e.g. ritonavir (Deray *et al*., 1998)) may be more susceptible to intoxication. In addition, dehydration and MDMA intoxication itself (associated with hepatic and renal impairment) may also impair the elimination of the drug. Moreover, since MDMA is a relatively lipophilic weak base it is expected that

variation in urine pH will influence its urinary excretion. An alkaline urine pH would be expected to promote tubular reabsorption and decrease renal clearance.

4 The recovery of hepatic CYP2D6 after a recreational dose of MDMA is dependent on the turnover of CYP2D6. Since the average half-life of hepatic CYP2D6 turnover is estimated to be 70 hours (Renwick *et al*., 2000), this indicates that it should take about 260 hours to recover 90% of basal CYP2D6 activity after a normal recreational dose of MDMA (Fig. 6). Therefore, the administration of other drugs which are metabolized by CYP2D6 during this period may lead to their accumulation and possible toxicity.

5 The use of a 'microdosing' approach (Lappin and Garner, 2003) to avoid ethical issues around the study of MDMA kinetics in drug-naive subjects may not provide information that is valid for recreational doses.

6 The auto-inhibition of MDMA metabolism is time- and dose-dependent. This may explain the non-linear pharmacokinetics of MDMA (Fig. 5).

7 Since there may be a degree of dose-dependence in the MBI of CYP2D6 in the lower range of recreational doses of MDMA (Fig. 5), this may have subtle and counter-intuitive implications for the risk of any long-term neurotoxicity. Given that metabolites formed downstream of the ring-opening of MDMA by CYP2D6 (de la Torre *et al*., 2000) are proposed to mediate such toxicity, lower doses of MDMA, by not ablating CYP2D6 activity so effectively, may predispose to a greater risk than higher doses by generating greater amounts of neurotoxic metabolites. This would be particularly likely if the other CYPs that contribute to ring-opening are also subject to MBI, as the intermediate complex with the haem would be the same.

In summary, genetic polymorphism of CYP2D6 and co-administration of CYP2D6 inhibitors may have less impact on MDMA pharmacokinetics and the risk of acute toxicity than previously thought. The non-linear pharmacokinetics of MDMA may be explained by the MBI of hepatic CYP2D6 by MDMA.

Appendix

GA is a method for solving optimization problems based on Darwin's theory of evolution, the principles of which are summarized as follows:

- The personality of parents is passed on to offspring during reproduction.
- The fittest individuals are liable to have more offspring, and thus drive the population towards their favourable characteristics.
- Offspring's traits are inherited partly from their parents and partly generated through mutation.

The following table draws an analogy between an optimization problem (parameter estimation) and the evolution of a population:

Optimization	→	Evolution of a population
The problem	→	Environment
Potential solution	→	Individual in the population
Decision parameters	→	Traits
Superiority: how good (based on the objective function) is a solution in comparison with the others	→	Fitness: chances for survival to the next generation and reproduction

Generally, the algorithm starts with a randomly selected group of individuals (i.e. sets of model parameters) and repeatedly modifies the population (model parameters). At each step, GA selects individuals from the current population to be parents and uses them to produce offspring for the next generation. Over successive generations, the population is expected to evolve toward an optimal solution. Normally, there are three main steps in a GA to create the next generation from the current population: selection of parents, mating the selected parents by cross-over and mutation to produce offspring, and choosing the new population from the combination of parents and offspring.

The following table gives details of the GA parameters used in the present study:

Number of generations	250
Population size	50
Offspring size	30
Number of cross-overs	2
Cross-over probability	0.95
Mutation probability	0.05
Selection method	Goldberg linear scaling
Sampling method	Stochastic universal
Coding method	Gray coding
Length of chromosome for each parameter	20 bit

References

Back T, Fogel D B, Michalewics Z (1997) Handbook of Evolutionary Computation. Institute of Physics Pub., Oxford University Press, Bristol

Bertelsen K M, Venkatakrishnan K, von Moltke L L, Obach R S, Greenblatt D J (2003) Apparent mechanism-based inhibition of human CYP2D6 in vitro by paroxetine: comparison with fluoxetine and quinidine. Drug Metab Dispos *31*: 289–293

de la Torre R, Farre M (2004) Neurotoxicity of MDMA (ecstasy): the limitations of scaling from animals to humans. Trends Pharmacol Sci *25*: 505–508

de la Torre R, Farre M, Ortuno J, Mas M, Brenneisen R, Roset P N, Segura J, Cami J (2000) Non-linear pharmacokinetics of MDMA ('ecstasy') in humans. Br J Clin Pharmacol *49*: 104–109

Delaforge M, Jaouen M, Bouille G (1999) Inhibitory metabolite complex formation of methylenedioxymethamphetamine with rat and human cytochrome P450. Particular involvement of CYP 2D. Environ Toxicol and Pharmacol *7*: 153–158

Deray G, Bochet M, Katlama C, Bricaire F (1998) Nephrotoxicity of ritonavir. Presse Medicale *27*: 1801–1803

Farre M, de la Torre R, Oathuna M B, Roset PN, Peiro A M, Torrens M, Ortuno J, Pujadas M, Cami J (2004) Repeated doses administration of

MDMA in humans: pharmacological effects and pharmacokinetics. Psychopharmacology (Berl) *173*: 364–375

Gilhooly T C, Daly A K (2002) CYP2D6 deficiency, a factor in ecstasy related deaths? Br J Clin Pharmacol *54*: 69–70

Goldberg D E (1989) Genetic Algorithms in Search, Optimisation, and Machine Learning. Addison Wesley, Wokingham

Green A R, Mechan A O, Elliott J M, O'Shea E, Colado M I (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'). Pharmacol Rev *55*: 463–508

Henry J A, Hill I R (1998) Fatal interaction between ritonavir and MDMA. Lancet *352*: 1751–1752

Henry J A, Jeffreys K J, Dawling S (1992) Toxicity and deaths from 3,4-methylenedioxymethamphetamine (Ecstasy). Lancet *340*: 384–387

Hesse L M, von Moltke L L, Shader R I, Greenblatt D J (2001) Ritonavir, efavirenz, and nelfinavir inhibit CYP2B6 activity in vitro: potential drug interactions with bupropion. Drug Metab Dispos *29*:100–102

Heydari A, Yeo K R, Lennard M S, Ellis S W, Tucker G T, Rostami-Hodjegan A (2004) Mechanism-based inactivation of CYP2D6 by methylenedioxymethamphetamine. Drug Metab Dispos *32*: 1213–1217

Holland J H (1975) Adaptation in Natural and Artificial Systems. University of Michigan Press, Ann Arbor, MI

Ingelman-Sundberg M, Oscarson M, McLellan R A (1999) Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. Trends Pharmacol Sci *20*: 342–349

Jones D C, Duvauchelle C, Ikegami A, Olsen C M, Lau S S, de la Torre R, Monks T J (2005) Serotonergic neurotoxic metabolites of ecstasy identified in rat brain. J Pharmacol Exp Ther *313*: 422–431

Kreth K P, Kovar K A, Schwab M, Zanger U M (2000) Identification of the human cytochromes P450 involved in the oxidative metabolism of 'Ecstasy'-related designer drugs. Biochem Pharmacol *59*: 1563–1571

Lappin G, Garner R C (2003) Big physics, small doses: the use of AMS and PET in human microdosing of development drugs. Nature Rev Drug Discov *2*: 233–240

O'Donohoe A, O'Flynn K, Shields K, Hawi Z, Gill M (1998) MDMA toxicity: no evidence for a major influence of metabolic genotype at CYP2D6. Addiction Biol *3*: 309–314

Ramamoorthy Y, Yu A M, Suh N, Haining R L, Tyndale R F, Sellers E M (2002) Reduced(+/-)-3,4-methylenedioxymethamphetamine ('Ecstasy') metabolism with cytochrome P450 2D6 inhibitors and pharmacogenetic variants in vitro. Biochem Pharmacol *63*: 2111–2119

Renwick A B, Watts P S, Edwards R J, Barton P T, Guyonnet I, Price R J, Tredger J M, Pelkonen O, Boobis A R, Lake B G (2000) Differential maintenance of Cytochrome P450 enzymes in cultured precision-cut human liver slices. Drug Metab Dispos *28*: 1202–1209

Schwab M, Seyringer E, Brauer R B, Hellinger A, Griese E U (1999) Fatal MDMA intoxication. Lancet *353*: 593–594

Segura M, Farre M, Pichini S, Peiro A M, Roset P N, Ramirez A, Ortuno J, Pacific R, Zuccaro P, Segura J, de la Torre R (2005) Contribution of Cytochrome P450 2D6 to 3,4-methylenedioxymethamphetamine disposition in humans: use of paroxetine as a metabolic inhibitor probe. Clin Pharmacokinet *44***:** 649–660

Silverman R B (1988) Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology. CRC Press, Boca Raton, FL

Tucker G T, Lennard M S, Ellis S W, Woods H F, Cho A K, Lin L Y, Hiratsuka A, Schmitz D A, Chu T Y Y (1994) The demethylenation of methylenedioxymethamphetamine (ecstasy) by debrisoquine hydroxylase (CYP2D6). Biochem Pharmacol *47*: 1151–1156

Valentin J (2002) Basic Anatomical and Physiological Data for Use in Radiological Protection: Reference Values. Pergamon, Oxford

von Moltke L L, Durol A L B, Duan S X, Greenblatt D J (2000) Potent mechanism-based inhibition of human CYP3A in vitro by amprenavir and ritonavir: comparison with ketoconazole. Eur J Clin Pharmacol *56*: 259–261

von Moltke L L, Greenblatt D J, Grassi J M, Granda B W, Duan S X, Fogelman S M, Daily J P, Harmatz J S, Shader R I (1998) Protease inhibitors as inhibitors of human cytochromes P450: high risk associated with ritonavir. J Clin Pharmacol *38*: 106–111

Wilson Z E, Rostami-Hodjegan A, Burn J L, Tooley A, Boyle J, Ellis S W, Tucker G T (2003) Inter-individual variability in levels of human microsomal protein and hepatocellularity per gram of liver. Br J Clin Pharmacol *56*: 433–440

Wu D F, Otton S V, Inaba T, Kalow W, Sellers E M (1997) Interactions of amphetamine analogs with human liver CYP2D6. Biochem Pharmacol *53*: 1605–1612

Yang J, Rostami-Hodjegan A (2004) Metabolic kinetic constants when drugs cause mechanism-based inhibition of their own metabolism. Drug Metab Rev *36* (suppl. 1): 292

Yang J, Jamei M, Yeo K R, Rostami-Hodjegan A, Tucker G T (2005) Kinetic values for mechanism-based inhibition: assessment of bias introduced by the conventional experimental protocol. Eur J of Pharm Sci *26*: 334–340

Yeo K R, Rostami-Hodjegan A, Tucker G T (2004) Abundance of cytochrome P450 in human liver: a meta-analysis. Br J Clin Pharmacol *57*: 687–688