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SHORT COMMUNICATION

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MDMA (ecstasy) pharmacokinetics in a CYP2D6 poor metaboliser and in nine CYP2D6 extensive metabolisers

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Introduction

MDMA (3,4-methylenedioxymethamphetamine, ecstasy) is a ring-substituted amphetamine widely abused by the youth for its euphoric and empathic effects. MDMA's metabolic disposition includes as its first step an *O*-demethylenation to 3,4-dihydroxymethamphetamine (HHMA), regulated by cytochrome P_{450} (CYP) isoform 2D6 [2]. HHMA is further metabolised to 4-hydroxy, 3-methoxymethamphetamine (HMMA) by catechol- *O*-methyltransferase (COMT). HMMA induces vasopressin secretion to a higher extent than MDMA and has been postulated to contribute to hyponatraemia observed in some MDMA acute intoxications [4]. The CYP2D6 gene is highly polymorphic and many variations affect the expression or activity of the enzyme. Approximately 7–10% of European Caucasians present a metabolic deficiency and are termed poor metabolisers (PM) [12]. Evidence that individuals possessing a compromise in CYP2D6 activity would be more susceptible to acute toxic effects of MDMA has been lacking. A previous metabolic bioactivation of MDMA, regulated partially by this enzyme, is needed to elicit neurotoxic effects in the central nervous system. It is postulated that the chemical species involved in serotonergic neurotoxicity are catechol thiol conjugate metabolites of the drug (HHMA) [8]. The functional polymorphisms in CYP2D6 could influence the development of MDMA neurotoxicity. During a trial designed to study the pharmacology of two consecutive doses of MDMA [3], one subject was found to possess the *CYP2D6*4/*4* genotype and was classified as a PM. The objective of this paper was to compare the results of this subject with those of nine others previously published. Furthermore, the paper includes pharmacokinetic data on MDMA metabolites not previously published.

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Materials and methods

A randomised, double-blind, cross-over, placebo-controlled trial was conducted in 10 healthy male subjects. The study was conducted in accordance with the Declaration of Helsinki and approved by the local Institutional Review Board (CEIC-IMAS). All volunteers gave their written informed consent before inclusion. MDMA 100 mg or placebo was administered in two successive doses separated by an interval of 24 h. Variables were measured throughout the 10 h following drug administration and again at 24 h following administration. A light meal was provided 6 h after MDMA administration. Tobacco smoking was permitted 6 h after drug administration.

A 5-ml venous blood sample was obtained from each subject and stored at −20°C for later transport on dry ice to the University of Sheffield, UK, for CYP2D6 genotyping. DNA was prepared with a QIAmp DNA Blood Midi Kit (Qiagen Ltd., Crawley, UK) and immediately analysed, according to published methods, for *CYP2D6*3*,**4* and **5* [15, 16] and **6* [12] alleles. If none of the mutations sought was detected, the wild-type *CYP2D6*1* allele was assigned.

The PM (*CYP2D6*4/*4*) subject in question was a 22-year-old male, with a weight of 67 kg and a height of

1.83 m. The demographics of the subjects and methods including physiological effects, subjective effects and plasma prolactin concentrations conducted during in the trial investigating two repeated doses of MDMA have been previously described [3]. Subjects were phenotyped for CYP2D6 activity using dextromethorphan as a drug probe. In summary, 30 mg dextromethorphan was administered, and 1-ml aliquots of the recovered urine (0–8 h) were stored at −20°C until further analysis. Dextromethorphan and dextrorphan concentrations were determined by means of high-performance liquid chromatography (HPLC/FLU) using an adapted method previously described [13] with levallorphan as internal standard. Extraction was carried out using a solid reversed-phase extraction column (C8) with cation exchange groups (benzene-sulphonic acid) (Bond Elut Certify, Varian, Palo Alto, CA, USA). Analysis was performed using a model 1090 liquid chromatograph coupled to a fluorescent detector model 1100 (Agilent Technologies, Palo Alto, CA) at excitation and emission wavelengths of 280 nm and 310 nm, respectively. The assay was validated in our laboratories for both urine and plasma. For all analytes, the inter-assay precision and accuracy were less than 14% and 9.8%, respectively. The limits of quantification for urine and plasma was 30 ng/ml for all analytes.

Blood samples for determination of MDMA and its metabolites (HHMA and HMMA) were collected during each experimental session at baseline and at 20, 40, 60 and 90 min, and 2, 3, 4, 6, 8, 10 and 24 h after drug administration. Blood samples for determination of prolactin were collected during each experimental sessions at baseline and at 20, 40, 60 and 90 min, and 2, 3, 4 and 6 h after drug administration. Details of both analytical methods have been published previously [3, 9, 10, 14]. Oral temperature was recorded at −15 min and immediately before drug administration (time 0, baseline), and at 20, 40, 60 and 90 min, and 2, 3, 4, 6, 8, 10 and 24 h after each drug administration using a Dinamap 8100-T vital signs monitor (Critikon, Tampa, FL, USA).

Peak concentration (C_{max}), time to reach peak concentration (t_{max}) and area under the concentration–time curve from 0 h to 24 h after drug administration (AUC) were calculated for MDMA and metabolite plasma concentrations. Values for prolactin and oral temperature were transformed to differences from the baseline. C_{max}, t_{max} and AUC were calculated from 0 h to 6 h for prolactin and temperature because of postprandial effects on these variables in the following hours. The AUC values were calculated using the trapezoidal rule. All variables for *CYP2D6*1/*1* and *CYP2D6*1/*4* subjects were analysed by the Student's *t*-test for independent data with genotype as a factor. No *post-hoc* analysis was conducted due to restricted sample size. Data from the *CYP2D6*4/*4* subject were compared with other genotypic groups using the Dixon test for extreme values. If the Dixon tabulated value, Q_{tab}, for an alpha error of 0.05 or 0.01 was less than the calculated value, Q_t, then the *CYP2D6*4/*4* subject's response was considered an outlier (equivalent to a $P<0.05$ or $P<0.01$, respectively). When the Student's *t*-test produced a significant difference between *CYP2D6*1/*1* and *CYP2D6*1/*4* subjects, the two groups were split and separate Dixon tests were conducted comparing the *CYP2D6*4/*4* subject to each group ($n=7$ for *CYP2D6*1/*1* versus *CYP2D6*4/*4* and $n=4$ for *CYP2D6*1/*1* versus *CYP2D6*1/*4*).

Results

All prospective volunteers, but one, were extensive metabolisers (EMs) according to their urinary dextromethorphan/dextrorphan ratio. An antimode of 0.3 was used to distinguish the PM from the EM. The ratios for the EM ranged from 0.007 to 0.05 with a standard deviation of 0.014, and the ratio for the PM was 4.334. Although the sample number was small ($n=10$), the **4* allele was detected at a frequency of 25%, comparable to published data of other Caucasian populations [12]. One *CYP2D6*4/*4* individual and three *CYP2D6*1/*4* individuals were detected in the sample group. There was no significant difference between the dextromethorphan metabolic ratio of the *CYP2D6*1/*4* individuals and the *CYP2D6*1/*1* individuals.

In the *CYP2D6*4/*4* subject, the $AUC_{0–24\ h}$ value for MDMA was 3.25 times that observed in *CYP2D6*1/*1* subjects ($Q_t=0.76$ $Q_{tab}=0.64$, $\alpha=0.01$, $n=7$). The $AUC_{0–24\ h}$ and $C_{max\ 0–24\ h}$ of HHMA were 51% and 40% of those found in *CYP2D6*1/*1* subjects, respectively. The largest difference was observed for HMMA, with an $AUC_{0–24\ h}$ that represented only 12% of that observed for *CYP2D6*1/*1* subjects ($Q_t=0.67$, $Q_{tab}=0.64$, $\alpha=0.01$, $n=7$). In the *CYP2D6*1/*1* subjects, the $t_{max\ 0–24\ h}$ of HHMA preceded that of MDMA, suggesting a first-pass effect. This was not evident in the *CYP2D6*4/*4* subject.

$AUC_{0–24\ h}$ of MDMA for the *CYP2D6*1/*4* subjects was 2.5 times that of *CYP2D6*1/*1* subjects ($P<0.0005$, $t=-13.123$, $n=9$) and 79% of that observed for the *CYP2D6*4/*4* subject ($Q_t=0.78$, $Q_{tab}=0.77$, $\alpha=0.05$, $n=4$). The $AUC_{0–24\ h}$ and $C_{max\ 0–24\ h}$ of HHMA were 80% and 51% of those found in *CYP2D6*1/*1* subjects, respectively. The $AUC_{0–24\ h}$ for HMMA also differed significantly from that of *CYP2D6*1/*1* subjects (63%, $P=0.002$, $t=5.118$, $n=9$) but did not reach significance in the Dixon test comparing AUCs of *CYP2D6*1/*4* and *CYP2D6*4/*4* subjects despite being 5.17 times that of the *CYP2D6*4/*4* subject ($Q_t=0.72$, $Q_{tab}=0.77$, $\alpha=0.05$, $n=4$).

There was a lack of prolactin response in the *CYP2D6*4/*4* subject following both doses of MDMA ($AUC_{0–6\ h}$ −10.09 ng h ml^{-1} and −11.21 ng h ml^{-1}, respectively). Slight temperature increases were seen in all subjects, but temperature increased more in the *CYP2D6*4/*4* subject than in the *CYP2D6*1/*1* subjects with $AUC_{0–6\ h}$ values of 2.867°C/h and 0.404°C/h, respectively.

Table 1 Pharmacokinetics of MDMA and metabolites based on CYP2D6 genotype following two doses of 100 mg MDMA administered 24 h apart (standard deviations in *italics*)

m	AUC_{0-24} (ng/ml h^{-1})	AUC_{24-48} (ng/ml h^{-1})	$C_{max\ 0-24}$ (ng/ml)	$C_{max\ 24-48}$ (ng/ml)	$t_{max\ 0-24}$ (h)	$t_{max\ 24-48}$ (h)	$t_{1/2\ (0-24)}$ (h)	$t_{1/2\ (24-48)}$ (h)
MDMA								
*1/*1	955.6*	2215.7****	163.3***	214.2 ****	1.75	25.50	6.0	8.1
	(*243.8*)	(*650.3*)	(*26.3*)	(*29.9*)	(*0.27*)	(*0.32*)	(*1.98*)	(*1.50*)
*1/*4	2446.0	3261.3	214.3	269.1	2.00	25.50	9.1	10.1
	(*94.8*)	(*414.2*)	(*9.3*)	(*30.8*)	(*0.29*)	(*0.29*)	(*<0.01*)	(*0.52*)
*4/*4	3113.9	4256.4	256.8	327.2	2.00	25.75	8.7	14.2
HHMA[a]								
*1/*1	2065.9	1580.9	200.4	170.3	1.00	25.50	9.4	10.4
	(*752.3*)	(*829.6*)	(*95.7*)	(*85.1*)	(*0.29*)	(*1.61*)	(*1.56*)	(*2.70*)
*1/*4	1664.3	1696.6	104.1	95.0	1.00	24.66	25.5	35.1
*4/*4	1063.4	924.4	79.8	65.2	3.00	26.00	15.1	19.5
HMMA								
*1/*1	3230.1**	2304.0****	362.1***	158.6****	1.75	26.50	8.3	20.7
	(*428.3*)	(*382.2*)	(*122.7*)	(*38.4*)	(*0.68*)	(*0.92*)	(*1.96*)	(*13.06*)
*1/*4	2033.8	1545.6	182.6	106.0****	1.88	26.75	12.7	14.9
	(*268.7*)	(*153.2*)	(*18.4*)	(*12.9*)	(*<0.01*)	(*0.58*)	(*1.81*)	(*2.85*)
*4/*4	393.2	345.9	57.6	44.5	2.00	26.88	14.8	15.0

* $P<0.05$ between the three genotypes; ** $P<0.05$ between *1/*1 and *1/*4, and between *1/*1 and *4/*4; *** $P<0.05$ between *1/*1 and *4/*4; **** $P<0.05$ between first and second doses

[a] For HHMA determination $n=3$ (*CYP2D6*1/*1* subjects), $n=1$ (*CYP2D6*1/*4* subject) and $n=1$ (*CYP2D6*4/*4* subject)

Discussion

The fortuitous inclusion of a CYP2D6 PM was the basis for examining the pharmacology of MDMA from the perspective of *CYP2D6* genotype. The number of subjects of each genotype limits the study, as does the limited number of *CYP2D6* alleles analysed. Alleles monitored covered between 95% and 99% of PM as well as most heterozygotes for non-functional alleles. Reduced activity alleles such as **9*,**10*, **17* or **41* were not investigated. Therefore, there is the possibility that some subjects assigned the EM phenotype could be intermediate metabolisers. However, the dextromethorphan metabolic ratio did not differ widely in this group and the low frequency of the alleles not investigated does not suggest possession of such a phenotype in the group studied.

MDMA acute toxicity has been associated with elevated plasma concentrations [6]. The elevated $AUC_{0-24\ h}$ and $C_{max\ 0-24\ h}$ values of MDMA found in the *CYP2D6*4/*4* subject could increase the risk of acute toxicity. Following the second dose of MDMA, differences in phenotype were no longer apparent as CYP2D6 was being inhibited and *CYP2D6*1/*1* and *CYP2D6*1/*4* subjects behaved similarly to the *CYP2D6*4/*4* subject administered single doses. This suggests a tendency towards the phenomenon of phenocopying following repeated doses. Changes seen in MDMA metabolite $AUC_{0-24\ h}$ and $C_{max\ 0-24\ h}$ values could also influence the risk of neurotoxicity. Higher $AUC_{0-24\ h}$ values of HHMA in the *CYP2D6*1/*4* and *CYP2D6*1/*1* may be translated to an increased risk of neurotoxicity for these subjects.

MDMA has been observed to increase prolactin secretion following both a single dose and two consecutive doses 24 h apart [3, 9]. It is proposed that the lack of response is associated with the CYP2D6 genotype and deserves further study. The lack of prolactin response in the *CYP2D6*4/*4* subject might be explained by presently undescribed conditions of hypoprolactinemia, differences in drug use history among volunteers including recency of drug exposure and coincident cannabis or amphetamine use (not observed), and possession of a polymorphism in the serotonin transporter which affects prolactin response [5, 7, 11].

Temperature increases were more apparent in the *CYP2D6*4/*4*. The female Dark Agouti rat, a model of CYP2D6 poor metabolism, is more susceptible to the hyperthermic effects of MDMA [1]. It is hypothesised that the increase observed in the *CYP2D6*4/*4* subject in this study is related to the higher $AUC_{0-24\ h}$ and $C_{max\ 0-24\ h}$ values of MDMA in plasma; thus, *CYP2D6*4/*4* subjects may be under increased risk of hyperthermia caused by MDMA.

It is concluded that MDMA and metabolite pharmacokinetics differ according to CYP2D6 genotype. This has consequences for the development of acute and long-term toxicity. Whether changes in other pharmacological variables in response to MDMA administration are related to CYP2D6 deficiency is subject to debate. A more thorough study of this phenomenon is required (Table 1).

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