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Regular Article

Changes in CYP1A2 Activity in Humans after 3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy) Administration Using Caffeine as a Probe Drug

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Summary: 3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) is a ring-substituted amphetamine widely used for recreational purposes. MDMA is predominantly O-demethylenated in humans by cytochrome P450 (CYP) 2D6, and is also a potent mechanism-based inhibitor of the enzyme. After assessing the inhibition and recovery of CYP2D6 in a previous study, the aim of this work was to study in humans the activity of CYP1A2 *in vivo* after CYP2D6 had been inhibited by MDMA, using caffeine as a probe drug. Twelve male and nine female recreational MDMA users were included. In session 1, 100 mg of caffeine was given at 0 h. In session 2, a 1.5 mg/kg MDMA dose (range 75–100 mg) was given at 0 h followed by a 100 mg dose of caffeine 4 h later. Aliquots of plasma were assayed for caffeine (137X) and paraxanthine (17X) and statistically significant differences were assessed with a one-way ANOVA. There were significant gender differences at basal condition, which persisted after MDMA administration. CYP1A2 activity was higher in both genders after drug administration, with an increase in 40% in females and 20% in males. Results show an increase in CYP1A2 activity when CYP2D6 is inhibited by MDMA in both genders, being more pronounced in females.

Keywords: CYP1A2; CYP2D6; mechanism based inhibition; MDMA; caffeine; clinical pharmacokinetics; drug interactions

Introduction

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is an amphetamine derivative widely used by young people due to its euphoric and emphatic effects. There are concerns about the neurotoxicity of MDMA and other amphetamine derivatives, and although the mechanisms underlying MDMA toxic effects remain to be elucidated, it has been postulated that MDMA-induced neurotoxicity in humans is mediated *via* the formation of bioactive metabolites (catecholthioether adducts).[1–4)]

As shown in **Figure 1**, there are two main metabolic steps in MDMA disposition: O-demethylenation and O-methylation.[5–7)] The O-demethylenation step is regulated by

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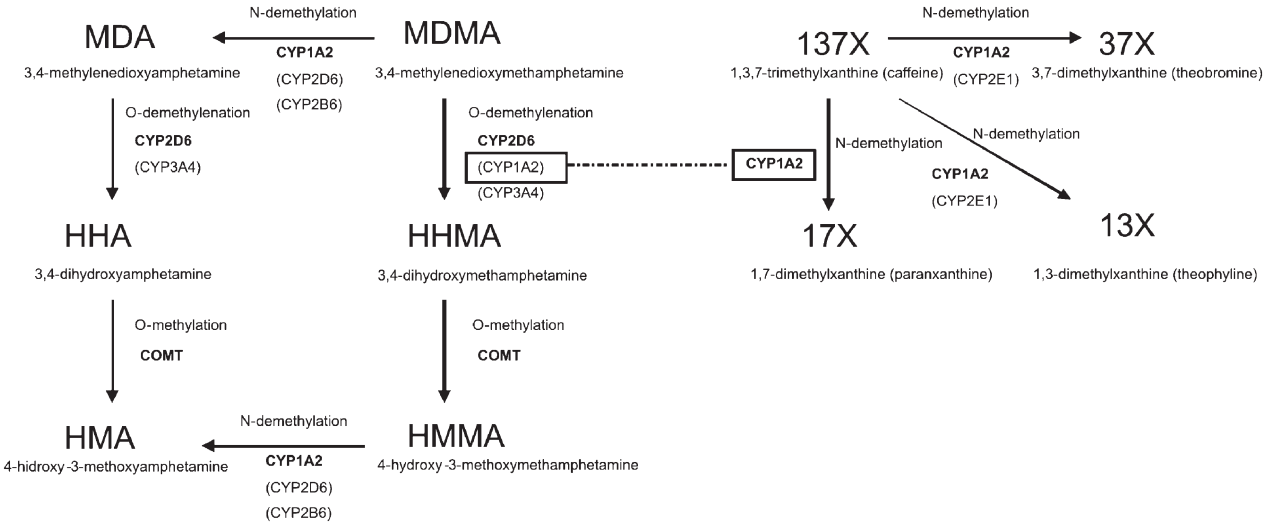

Fig. 1. Participation of CYP1A2 and other CYPs in MDMA and caffeine metabolism pathways

several isoforms of cytochrome P450, giving rise to 3,4-dihydroxymethamphetamine (HHMA) formation. The minor active N-demethylated MDMA metabolite, MDA (3,4-methylenedioxyamphetamine), is also O-demethylenated, giving rise to 3,4-dihydroxyamphetamine (HHA). Both HHMA and HHA are O-methylated in a reaction regulated by catechol O-methyltransferase (COMT) or form sulfate and glucuronide conjugates. A fraction of HHMA and HHA can undergo further auto-oxidation to the corresponding *ortho*-quinones, which are conjugated with glutathione (GSH) to form glutathionyl adducts.[8)]

The O-demethylenation step, which gives rise to HHMA, shows *in vitro* biphasic kinetics with high and low affinity components. The high affinity component is mainly regulated by the cytochrome P450 2D6 isoenzyme (CYP2D6), but several other P450 isozymes have the capacity to contribute to the microsomal oxidative metabolism of methylenedioxyamphetamines. The low affinity component is regulated by CYP1A2 and, to a lesser extent, by CYP2B6 and CYP3A4.[9)] In a more recent study, CYP2C19 was reported to contribute to the O-demethylenation of MDMA. A marked enantioselectivity towards the S-enantiomers for N-demethylation and O-demethylenation was observed for this isozyme.[10)] MDMA interacts with CYP2D6 both as substrate and inhibitor of its own metabolism by the formation of a complex between CYP2D6 and MDMA. This mechanism based inhibition has been observed *in vitro* and *in vivo* in both rats and humans.[11–14)] In clinical studies we have already shown that the pretreatment of subjects with CYP2D6 substrates (paroxetine) or with MDMA (two doses taken 24 h apart) profoundly alter MDMA clearance, increasing parent drug levels and impairing its metabolic disposition.[15,16)] Pre-clinical studies have extended this enzymatic inhibitory capacity of MDMA to other CYP isozymes. The relative potency of MDMA inhibition in human liver cells has been reported as follows: CYP2D6 > CYP3A4 ≫ CYP1A2.[17)] Contributions of minor CYP isozymes (CYP1A2, CYP3A4 and CYP2B6) to MDMA metabolism are expected to become relevant in acute intoxications or in the case of CYP2D6 inhibition (either by autoinhibition or interaction with a CYP2D6 substrate).

Data from a clinical trial using a mechanism-based CYP2D6 inhibitor such as paroxetine, showed that the contribution of CYP2D6 to MDMA metabolism was not higher than 30%.[16)] Concerning CYP3A4 activity, after MDMA exposure, we observed that there was a decrease in its activity.[18)] The ratio dextromethorphan/methoxymorphinan after dextromethorphan administration increased almost 3-fold after MDMA administration in both genders, showing that when CYP2D6 is impaired, CYP3A4 activity is apparently decreased in both genders. This observation would be in agreement with pre-clinical data examining the inhibitory capacity of MDMA on different CYP isozymes as previously described. In practice, MDMA, despite these enzymatic inhibitions, is essentially metabolically cleared as only 24% of the drug is excreted unaltered in urine.[19)] Taking into account the observations mentioned above, it was decided to examine the effect of MDMA on CYP1A2 activity once CYP2D6 was inhibited. Although CYP1A2 seems less sensitive to induced inhibition by MDMA, one would expect a lower activity of this isoenzyme following predictions from *in vitro* studies.

CYP1A2 is involved in the activation of environmental procarcinogens, such as arylamines, heterocyclic amines and aflatoxin B1. Its activity is considerably increased by xenobiotics and life style factors, including cigarette smoke and diet.[20)] Caffeine has been extensively used as a probe drug to determine CYP1A2 phenotype and to investigate gender and age differences in CYP1A2 activity. Although CYP2E1, CYP2A6, CYP2C8/9, and CYP3A4 are involved

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in caffeine metabolism, 90% of its clearance is thought to be regulated by CYP1A2.[21,22] Caffeine (1,3,7-trimethylxanthine; 137X) undergoes N-demethylation at three sites forming paraxanthine (1,7-dimethylxanthine; 17X: 84%), theophylline (13-dimethylxanthine: 4%), and theobromine (3,7-dimethylxanthine: 12%) (see **Fig. 1**). Its plasma clearance, and in particular paraxanthine-to-caffeine plasma concentration ratio, is widely used for the measurement of CYP1A2 activity in humans.[23]

The main objective of the present study was to assess CYP1A2 activity after CYP2D6 had been previously inhibited by MDMA. The evaluation of CYP1A2 activity is of relevance in order to conciliate pre-clinical observations suggesting that the activity of cytochrome P450 isozymes would also be lowered by MDMA exposure, and the clinical observation showing that it is metabolically cleared in less than 48 h. Therefore, a relevant discrepancy exists between *in vitro* studies foreseeing a full inhibition of MDMA metabolic disposition in which the drug inhibits its own metabolism by inhibiting P450 CYPs, and the clinical observation that the drug is metabolically cleared from the body.

In order to study this matter, a clinical trial was designed in which caffeine pharmacokinetics, used as a probe drug for CYP1A2 activity, was evaluated in subjects, of both genders, previously administered with MDMA. Clinical studies have already reported that in less than 2 h after MDMA administration, CYP2D6 activity is almost completely inhibited. Therefore, caffeine was given 4 h after MDMA intake, making sure that CYP2D6 had been previously inhibited. The present clinical trial was developed under the same experimental conditions as those applied in a previous one examining CYP2D6 autoinhibition and CYP3A4 activity.[18]

Methods

Study participants: Twenty one healthy Caucasian subjects (12 males and 9 females), participated in a cross-over clinical trial. All were CYP2D6 extensive metabolizers and recruited by word of mouth. All but 5 subjects were current smokers (3 males and 2 females). Eligibility required the self-reported recreational use of MDMA on at least ten occasions including twice in the previous year. Subjects were interviewed by a psychiatrist (Psychiatric Research Interview for Substance and Mental Disorders for DSM-IV, PRISM-IV) to exclude those with a history of or currently suffering from major psychiatric disorders (schizophrenia, psychosis, and major affective disorders).[24] All subjects participated in a previously published study where the activity of CYP2D6 and CYP3A4 was assessed after administration of MDMA.[18] All of them were phenotyped for CYP2D6 activity, using dextromethorphan; the extensive metabolizer (EM) phenotype was required for participation in this study.[25] The participants had a mean (SD) age of 26.3 (3.7) years, mean weight of 63.4 (10.4) kg, and a mean height of 174.7 (9.8) cm. None met criteria of abuse or drug dependence (except for nicotine) and all had previous experience with other psychostimulants, cannabis or hallucinogens. Subjects were informed about the study, they provided written informed consent before inclusion, and they were compensated for their participation. The study was conducted in accordance with the Declaration of Helsinki (2000), approved by the local Institutional Review Board (CEIC-IMAS), and authorized by the Spanish Medicines Agency (AEM n. 04-0013) and registered at ClinicalTrials.gov (NCT01447472). Subjects were requested to refrain from consuming drugs of abuse two weeks before and throughout the duration of the study. Regular ingestion of medication in the month preceding the study was an exclusion criterion although single doses of symptomatic medication (*i.e.*, paracetamol) were accepted up to the week preceding the trial. Females were not taking oral contraceptive steroids (OCS) and all of them participated during the follicular menstrual period. At each session, and before drug administration, urine samples were collected for drug testing (opiates, cocaine, amphetamines, and cannabis) by a rapid test device (Instant-View®, Alpha Scientific Designs, Inc., Poway, CA, USA) and for pregnancy testing if required. A positive screen test was considered an exclusion criterion.

Study design and drugs: Subjects followed a xanthine-free diet ingestion 48 h prior to beginning the session. Each session began at 07:30 h following an overnight fast. Drug administration commenced at 08:30 h. In session 1, 100 mg of caffeine was given at 0 h. There was a wash-out period of at least 3 days between sessions. In session 2, a 1.5 mg/kg MDMA dose (minimum 75 mg, maximum 100 mg) was given at 0 h followed by a 100 mg dose of caffeine 4 h later. The dose of MDMA was chosen in order to be within the range of the amounts reported in a single recreational dose of ecstasy.[15] All drugs were administered orally. A light meal was provided 6 h after initial drug administration. Blood samples were obtained through an indwelling catheter inserted into a subcutaneous vein in the forearm of the non-dominant arm. In session 1, blood samples (8 mL) were taken at 0, 0.5, 1, 2, 4, 6, 8, and 24 h after caffeine administration; in session 2, samples were collected at 4, 4.5, 5, 6, 8, 10, 12, and 28 h after MDMA administration. After centrifugation at 4°C, four 1 mL aliquots of plasma were stored at −20°C until analysis. Cardiovascular effects were recorded during the sessions for safety reasons (continuous ECG, blood pressure, heart rate, temperature) but are not presented in this manuscript.

Analytical methods: All chemical reagents were of the highest grade available. Caffeine (137X), paraxanthine (17X), and the internal standard, diphylline, were purchased from Sigma-Aldrich, Spain. Caffeine and paraxanthine plasma concentrations were determined using a 1090 II high performance liquid chromatography coupled to a 1,100 UV detector at 274 nm (Agilent, Palo Alto, CA, USA) following a previously published methodology.[26] Briefly, caffeine and paraxanthine were extracted from 500 µL of

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plasma using a liquid/liquid extraction procedure; 4 mL of ethyl acetate and 200 µL of a saturated potassium sulfate solution were added to 500 µL of plasma. The mixture was vortexed for 30 s. Samples were agitated for 20 min and then centrifuged at room temperature for 5 min. The organic layer was separated and evaporated to dryness at 40°C using a gentle stream of nitrogen (Turbovap®, Zymark®, Kopkinton, MA, USA). The residue was reconstituted in 100 µL mobile phase, and 25 µL was injected into the HPLC system. The mobile phase used was acetonitrile: acetic acid (0.05%) (99:1, v/v) applied as an isocratic gradient with a flow rate of 1 mL/min for a run of 15 min. The HPLC column used was an Ultrasphere ODS (4.6 cm × 7.5 cm × 3 µL) (Beckman Coulter™, Palo Alto, CA, USA). The inter-assay precision and accuracy was 10.0% and 7.8% for caffeine, and 4.2% and 5.9% for paraxanthine, with limits of detection of 3.5 µg/L and 12 µg/L for caffeine and paraxanthine, respectively. Plasma MDMA and metabolites were measured by GC/MS.[27]

Data analysis: Values of the peak plasma concentration (C_{max}), time to reach C_{max} (t_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration-time curve from 0 to 8 h (AUC_{0-8h}) and from zero to infinity (AUC_{0-inf}) were derived directly from the plasma concentration-time profiles of 137X and 17X. Plasma clearance (CL_P) was calculated as the ratio of the total administered dose and AUC_{0-inf}. AUC values (AUC [0,t]) were determined from 0–8 h (session 1 data) and 4–12 h (session 2 data) using the trapezoidal rule. Elimination rate constants (Ke) were estimated by log-linear regression of terminal data points. AUC_{0-8h} was extrapolated to infinity (AUC_{0-inf}) by adding the last quantifiable concentration divided by Ke. Volume of distribution (Vd) was estimated as the ratio between AUC_{0-inf} and Ke.

To study the effect of MDMA intake and gender on each of the response variables of interest, repeated measure ANOVA models were used which included MDMA and gender, their interaction, and smoking as factors. Whenever the MDMA-gender interaction could be discarded, it was removed from the model and the two factors of interest were studied separately. If, in contrast, the interaction was statistically significant, the MDMA effect was studied separately for males and females and vice versa. In all cases, the mean effects were estimated and the 95% confidence intervals were calculated within the framework of the corresponding ANOVA model.

Statistical significance was set at 0.1 in the case of the MDMA-gender interactions to protect against Type-II error, and at 0.05 in the case of MDMA and gender effects to protect against Type-I error. The statistical software package R (The R Foundation for Statistical Computing), version 2.14.1, was used for the analyses.

AUC_{0-8h} and C_{max} ratios 17X/137X were calculated to assess the activity of CYP1A2 in male and female subjects before and after MDMA intake. In order to evaluate whether the change in CYP2D6 activity (inhibition) was in some way related to a change in CYP1A2 activity, we collected the data from a previous trial regarding the percentage of inhibition of CYP2D6 of the subjects in both genders.[18] The rate of CYP2D6 inhibition (urinary molar concentrations ratio dextromethorphan/dextrorphan in an 8 h collection period prior to MDMA intake and 4 h after) and the increase in CYP1A2 activity (C_{max} 17X/137X ratio prior and 4 h after MDMA intake) were evaluated. The Spearman correlation was calculated to study this association.

A pharmacokinetic model was constructed to describe the time course of plasma concentrations of caffeine and paraxanthine in plasma. The model incorporates the time course of plasma disposition of caffeine and includes the metabolic formation and elimination of paraxanthine from plasma. The differential equations for the model were as follows:

Equation 1 for caffeine concentrations:

$$\frac{dC_{137X}}{dt} = \text{Dose} \times \text{Ka} - C_{137X} \times [(\text{Ke}_{137X} + \text{Kf}_{17X})]$$

Equation 2 for paraxanthine concentrations:

$$\frac{dC_{17X}}{dt} = \text{Kf17X} \times \text{C17X} - \text{Ke17X} \times \text{C17X}$$

C_{137X} and C_{17X} are the concentrations of caffeine and paraxanthine in plasma (ng/mL), Ka the absorption constant (h^{-1}), and Ke_{137X} (h^{-1}) the elimination constant. The constants Kf_{17X} (h^{-1}) and Ke_{17X} (h^{-1}) correspond to the formation and elimination rates of paraxanthine, respectively.

The pharmacokinetic software SAAM II (SAAM Institute Inc., Seattle, WA, USA) was used for nonlinear least square analysis to fit the parameters Ke_{137X}, Kf_{17X}, Ke_{17X}, Vd_{137X}, Vd_{17X}, CLp_{137X} (caffeine plasmatic clearance; mL/h) and Cl_{17X} (paraxanthine plasmatic clearance; mL/h) to the set of plasma concentrations of caffeine and paraxanthine using equations 1 and 2 and fixing the Ka values.

Results

Table 1 summarizes the effect of MDMA on caffeine and paraxanthine pharmacokinetics, in males and females, taken separately in both experimental sessions (session 1 *vs.* session 2). Mean plasma concentrations over time of 137X and 17X before and after a 1.5 mg/kg dose of MDMA for both genders are shown in **Figure 2**. **Table 2** summarizes the results of the ANOVA model looking at MDMA and gender effects on caffeine pharmacokinetics, controlling by smoking status of subjects. Results show that there were significant gender differences at basal condition, as reflected in C_{max}, AUC_{0-8h} T_{max}, CL, Vd and AUC_{0-inf} for caffeine parameters. These gender differences are also observed after adjusting results for body weight (data not shown). Preliminary data on gender differences at baseline led us to construct another

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Table 1. Caffeine (137X) and paraxanthine (17X) pharmacokinetics before (session 1) and after (session 2) a 1.5-mg/kg dose of MDMA ($n = 21$; 12 males and 9 females)

	Males				Females			
	137X		17X		137X		17X	
	Session 1	Session 2	Session 1	Session 2	Session 1	Session 2	Session 1	Session 2
C_{max} (μg/L)	2,154.5 ± 475.4	1,895.3 ± 534.3	726.3 ± 89.8	753.1 ± 121.4	3,645.5 ± 781.8	3,077.7 ± 699.6	814.4 ± 161.8	1,025.1 ± 260.4
T_{max} (h)	0.8 (0.5–1)	0.8 (0.5–1)	4.0 (0.5–8)	6.0 (2–8)	1.0 (0.5–2)	1.0 (0.5–4)	6.0 (2–8)	6.0 (2–8)
Ke (h^{-1})	0.27 ± 0.06	0.24 ± 0.08	NA	NA	0.23 ± 0.11	0.21 ± 0.14	NA	NA
AUC_{0-8h} (μg·h/L)	7,267.0 ± 2,223.7	7,629.4 ± 2,737.1	4,696.4 ± 452.3	4,647.8 ± 726.6	15,197.9 ± 5,145.3	14,645.9 ± 6,079.8	4,880.3 ± 1,130.3	5,508.3 ± 1,034.7
$t_{1/2}$ (h)	2.7 ± 0.8	3.3 ± 1.3	10.6 ± 6.1	11.2 ± 8.4	4.2 ± 3.1	5.0 ± 3.6	15.8 ± 17.4	13.5 ± 9.4
CL_P (L/h)	14.7 ± 3.9	14.2 ± 4.9	NA	NA	7.1 ± 3.2	7.8 ± 4.3	NA	NA
Vd (L)	54.94 ± 9.3	61.99 ± 15.9	NA	NA	34.41 ± 11.6	40.77 ± 9.9	NA	NA
$AUC_{0\text{-}Inf}$ (μg·h/L)	7,347.7 ± 2,336.3	8,043.6 ± 3,285.4	NA	NA	17,054.9 ± 7,608.9	16,767.4 ± 8,954.2	NA	NA
$17X/137X_{cmax}$	0.35 ± 0.07	0.42 ± 0.08	NA	NA	0.25 ± 0.1	0.35 ± 0.13	NA	NA
$17X/137X_{AUC}$	0.69 ± 0.19	0.68 ± 0.23	NA	NA	0.38 ± 0.19	0.46 ± 0.24	NA	NA

AUC_{0-8h}, area under the plasma concentration-time curve from 0 to 8 h; $AUC_{0\text{-}Inf}$, AUC from zero to infinity; CL_P, plasmatic clearance; C_{max}, maximum plasma concentration; Ke, elimination rate constant; NA, not applicable; $t_{1/2}$, elimination half life; t_{max}, time to reach C_{max}; Vd, volume of distribution.

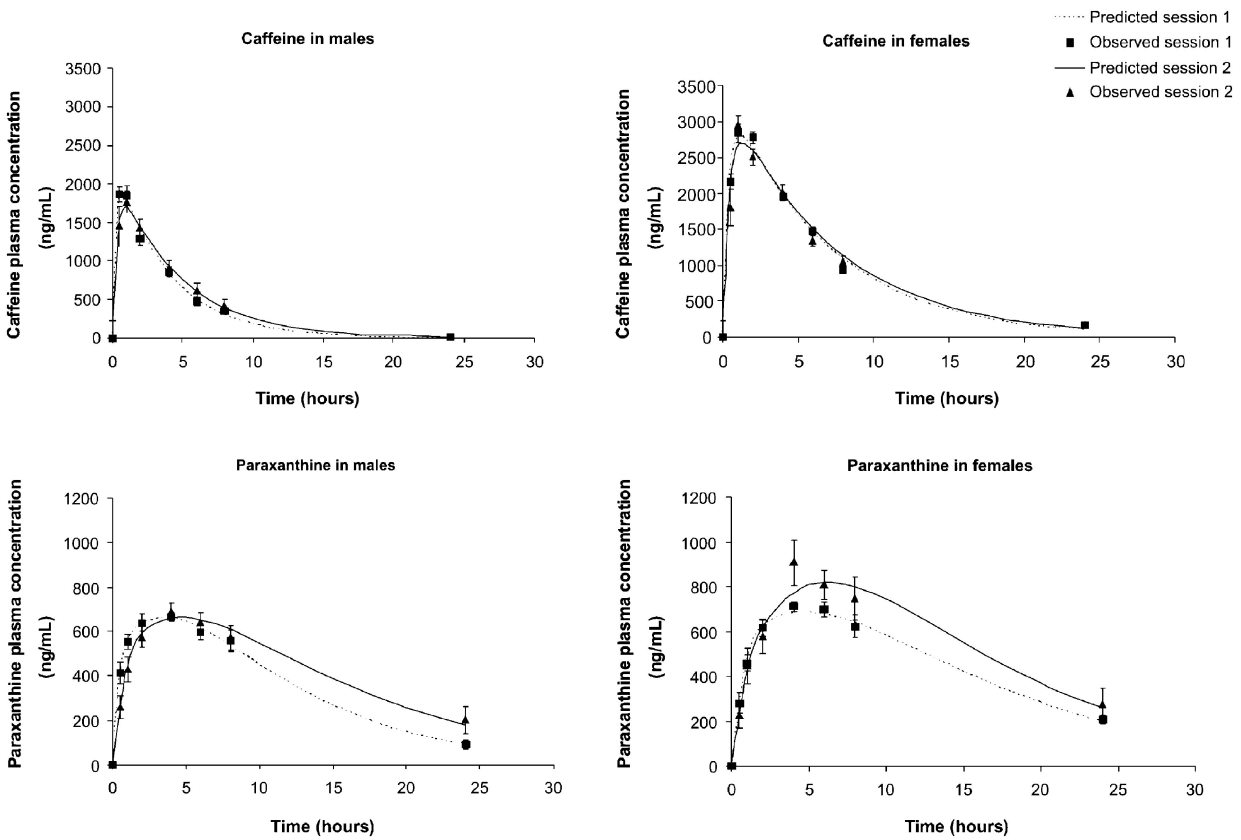

Fig. 2. Mean (± standard error) plasma concentrations of caffeine and paraxanthine in men ($n = 12$) and in women ($n = 9$) before (session 1) and after (session 2) a 1.5 mg/kg dose of 3,4-methylenedioxymethamphetamine (MDMA; ecstasy)

ANOVA model that would consider the possible interaction between the effects of MDMA and gender.

As seen in **Table 2**, there was a gender effect for all caffeine pharmacokinetic parameters except Ke, thus justifying the interaction model that reflects differences already seen at baseline condition. MDMA intake significantly changed the caffeine pharmacokinetic parameters: C_{max} and Vd. There was no interaction between gender and

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Table 2. Mean differences, 95% confidence intervals and the p-values obtained from ANOVA models with repeated measures including smoking: In the case of a significant Gender * MDMA interaction (p < 0.1), values are shown for both categories of the other variable

		MDMA[a]	Gender[b]	Interaction (p-value) MDMA × Gender
137X	C_{max} (μg/L)	−314.317; [−542.6, −86.0]; **p < 0.01	1250.928; [719.6, 1782.2]; ***p < 0.001	0.572
	T_{max} (h)	0.214; [−0.090; 0.518]; p = 0.518	0.638; [0.214; 1.062]; **p < 0.01	0.308
	Ke (h^{-1})	−0.025; [−0.060; 0.010]; p = 0.152	−0.036; [−0.122; 0.051]; p = 0.397	0.563
	AUC_{0-8h} (μg·h/L)	−29.471; [−1135.6; 1076.6]; p = 0.956	7,531.060; [3,889.1; 11,173.0]; ***p < 0.001	0.404
	$t_{1/2}$ (h)	0.681; [−0.278; 1.640]; p = 0.153	1.626; [−0.280; 3.532]; p = 0.090	0.864
	CL_P (L/h)	0.026; [−1.108; 1.160]; p = 0.962	−7.009; [−10.812; −3.207]; ***p < 0.001	0.267
	Vd (L)	6.728; [0.183; 13.272]; *p < 0.05	−20.713; [−29.701; −11.726]; ***p < 0.001	0.909
	$AUC_{0\text{-}Inf}$ (μg·h/L)	275.302; [−1195.587; 1746.192]; p = 0.699	9,312.479; [4,207.453; 14,417.504]; ***p < 0.001	0.502
17X	C_{max} (μg/L)	Males: 26.3; [−50.5; 103.0]; p = 0.482 Females: 210.7; [122.0; 299.3]; ***p < 0.001	Session 1: 86.3; [−66.5; 239.2]; p = 0.251 Session 2: 270.7; [117.9; 423.6]; **p < 0.01	0.004
	T_{max} (h)	0.643; [−0.560; 1.845]; p = 0.276	0.935; [−0.699; 2.568]; p = 0.245	0.538
	Ke (h^{-1})	0.013; [−0.019; 0.044]; p = 0.402	0.013; [−0.038; 0.063]; p = 0.610	0.262
	AUC_{0-8h} (μg·h/L)	Males: −48.682; [−403.924; 306.559]; p = 0.777 Females: 627.971; [217.774; 1038.169]; **p < 0.01	Session 1: 169.900; [−598.065; 937.865]; p = 0.648 Session 2: 846.553; [78.589; 1614.518]; *p < 0.05	0.017
	$t_{1/2}$ (h)	−0.631; [−6.371; 5.109]; p = 0.820	3.952; [−3.492; 11.396]; p = 0.279	0.606
17X/137X	$17X/137X_{cmax}$	0.083; [0.050; 0.116]; ***p < 0.001	0.085; [−0.177; 0.007]; p = 0.067	0.324
	$17X/137X_{AUC}$	Males: 0.015; [−0.091; 0.062]; p = 0.694 Females: 0.082; [−0.006; 1.171]; p = 0.067	Session 1: −0.316; [−0.517; −0.115]; **p < 0.01 Session 2: −0.219; [−0.421; −0.018]; *p < 0.05	0.098

AUC_{0-8}, area under the plasma concentration-time curve from 0 to 8 h; $AUC_{0\text{-}Inf}$, AUC from zero to infinity; CL_P, plasmatic clearance; C_{max}, maximum plasma concentration; Ke, elimination rate constant; $t_{1/2}$, elimination half life; t_{max}, time to reach C_{max}; Vd, volume of distribution.

[a]A positive value indicates higher values in session 2 (MDMA).

[b]A positive value indicates higher values among females.

MDMA. In the case of paraxanthine parameters (C_{max} and AUC_{0-8h}) there was a drug x gender interaction: the MDMA effect was only observed in females.

More importantly, the C_{max} ratio 17X/137X showed that there was an MDMA effect in all subjects regarding CYP1A2 activity ($p < 0.001$), the gender effect was marginally significant ($p = 0.067$), and, therefore, no interaction was observed ($p = 0.324$). Consequently, this ratio reflects the fact that MDMA actually changes the activity of CYP1A2. Taking each gender separately, CYP1A2 activity increased 40% in females (0.25 ± 0.10 *vs.* 0.35 ± 0.13) and 20% in males (0.35 ± 0.07 *vs.* 0.42 ± 0.08) in both sessions. Results for the 13X/137X AUC_{0-8h} ratio showed an interaction ($p = 0.098$) and differences before and after MDMA administration which were gender related, the drug effect being more marked in women.

The pharmacokinetic model constructed reported a good adjustment between observed and predicted data points (see **Fig. 2**) and also provided some predicted parameters such as the formation rate constant of paraxanthine (Kf17X), and the volume of distribution of caffeine (Vd137X) and paraxanthine (Vd17X). The formation of paraxanthine was similar in both genders. Females showed a slight increase in its value—0.25 ± 0.12 h^{-1} *vs.* 0.26 ± 0.17 h^{-1} (an increase of approximately 6% of activity) in both sessions—which also occurred in males: 0.28 ± 0.10 h^{-1} *vs.* 0.31 ± 0.16 h^{-1} (an increase of 9%) in both sessions. Vd137X values were similar after MDMA administration (44.8 ± 9.4 L *vs.* 42.8 ± 11.1 L) in males and in females (25.1 ± 6.1 L *vs.* 25.0 ± 6.1 L). In contrast, Vd17X values increased after MDMA administration—12.5 ± 5.4 L *vs.* 14.7 ± 10.1 L (an increase of approximately 17%) in males in both sessions—and similarly increased in females: 13.1 ± 8.3 L *vs.* 14.9 ± 19.1 L (an increase of approximately 14%) in both sessions. Taking into account that subjects were the same in both studies, we collected the data from a previous trial to see if changes in CYP1A2 activity were related with CYP2D6 activity after MDMA intake. A significant correlation between the rate of CYP2D6 inhibition and the increase in CYP1A2 was observed ($r = 0.57$, $p = 0.007$).

The administration of a single dose of 1.5 mg/kg MDMA produced the typical effects described for this substance in an experimental laboratory setting (increases in blood pressure and heart rate, mydriasis, feelings of well-being and euphoria). No adverse effects were noted after MDMA administration. No major differences were observed in MDMA doses or plasma concentrations (in both cases, corrected by weight) between genders (data not shown).

Discussion

The main finding of this study was a modest but significant increase in CYP1A2 activity after MDMA administration in humans which was more marked in females. This observation would, in principle, contradict preclinical data that predicted the inhibition of this isozyme.[10,28)]

MDMA and caffeine displayed differences in drug concentration of approximately one order of magnitude; caffeine being higher 4 h after MDMA administration. Therefore, no

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major changes due to a competitive mechanism on caffeine metabolic disposition after MDMA administration were expected and, in any case, this interaction should have resulted in an increased caffeine metabolism. The present work shows an increase of one of the CYP 450 isozymes *in vivo* when another one is inhibited by MDMA. It seems, therefore, that a compensatory mechanism exists between the CYP 450 isozymes in order to metabolically clear the drug.

A potential explanation for the results observed, and one of the underlying hypotheses that were considered when planning this study, was that MDMA is structurally related to a group of chemicals known as methylenedioxyphenyl (benzodioxole) (MDP) synergist pesticides. MDP compounds function as both inhibitors and inducers of cytochrome P450 isoforms, the two processes proceeding, *in vivo*, at different rates. Inhibition is mediated through the formation of metabolite inhibitory complexes while induction involves both aryl hydrocarbon (Ah)-dependent and Ah-independent mechanisms.[29,30] MDMA and synergistic pesticides display similar metabolic behavior by inducing a mechanism-based inhibition of their main metabolic pathway (related to the methylenedioxy grouping) and apparently increasing the activity of one isozyme, CYP1A2, regulated by the aromatic hydrocarbon receptor (AhR) and induced through AhR-mediated transactivation following ligand binding and nuclear translocation.[31]

There have been several studies concerning natural and chemical compounds (*e.g.* rosiglitazone, modafinil) which reported biphasic activities such as inhibitors and inducers on human liver cytochrome p450 isoenzymes.[32,33] Nevertheless, *in vitro* studies are not always predictive of effects *in vivo*, and the concentrations used of these compounds in order to get an effect sometimes are very high and, therefore, unlikely to occur *in vivo*.

Gender differences were observed in the modification of CYP1A2 phenotype. The exclusion of oral contraceptives was a particularly restrictive criterion for study eligibility and reduced the number of female subjects who enrolled in the study. The benefit of this approach, however, was that potential drug interactions with oral contraceptives were excluded and, as a result, pharmacokinetic changes could be attributed to gender differences. In fact, genders already varied at baseline (caffeine alone, session 1) in the metabolic disposition of caffeine used as probe drug and such differences persisted after MDMA administration (session 2). Females displayed a slower caffeine metabolism which was translated into an apparent increased effect on CYP1A2 activity once MDMA inhibited CYP2D6 (40% increase in females *vs.* 20% in males, based on the C_{max} 17X/137X ratio). Gender differences at baseline are in agreement with some previously published work.[10,34,35] There is, however, controversy as other authors have described no gender differences in CYP1A2 activity.[36,37]

Previous work has reported that CYP1A2 activity is induced by smoking.[38,39] In this study, 16 subjects were smokers and only 5 were non-smokers. The ANOVA analysis had already controlled the smoking status of subjects. Ideally, a more balanced distribution between smokers and non-smokers would be desirable. Nevertheless, it should be taken into account that in populations of MDMA users smoking rates are even higher (>90%) than for the participants included in the study (16/21, 76%). In addition, the second most common drug misused by MDMA users is cannabis, which is consumed in the form of hashish resin mixed with tobacco. Therefore, it is quite difficult to enlarge MDMA populations with non-smokers.

A potential limitation of this study is that CYP1A2 genetic polymorphisms have not been investigated; inducibility of CYP1A2 varies among individuals due to genetic factors.[31]

A pharmacokinetic model was built to analyze the data which provided some predicted parameters such as the formation (Kf17X) rate constant and the volume of distribution (Vd137X) of caffeine and paraxanthine. This provided some additional information which allowed for a better assessment of caffeine metabolism in the study. Globally, an increase in 17X formation (reflected in higher Vd17X and Kf17X values) was observed in session 2, which is in agreement with experimental data.

The present work holds that CYP2D6 and CYP1A2 metabolic activities are not independent from each other, which helps to give a deeper insight into drug–drug interactions *in vivo*. This observation has some clinical implications: (i) after MDMA CYP2D6 is fully inhibited, the intake of drugs whose metabolism is regulated by this isozyme should be avoided (ii) after MDMA there is a temporary increase in CYP1A2 activity that compensates for the decreased CYP2D6 activity. This helps to explain why MDMA is still metabolically cleared even after repeated doses, (iii) an enhanced clearance of medications whose metabolism is regulated by CYP1A2 is expected.

In summary, the results presented here show an increased metabolic disposition of caffeine after MDMA intake. This observation is more relevant in females than in males. We are unable to comment on enzyme induction as this aspect was not examined, and only changes in CYP1A2 phenotype were evaluated. The increase in CYP1A2 activity may further contribute to MDMA metabolic disposition once CYP2D6 has been irreversibly inhibited by a mechanism-based process induced by this drug. A good correlation between the CYP2D6 and CYP1A2 activities indicated that the more CYP2D6 is inhibited, the more CYP1A2 activity is enhanced.

The overall picture from a clinical perspective is that subjects consuming MDMA, because of the interaction of this substance with several isozymes of cytochrome P450 (CYP2D6, CYP3A4, CYP1A2), display alterations in their ability to clear metabolically therapeutic drugs. This is not limited to CYP2D6, although this enzymatic activity is the one most severely altered. Further studies should evaluate the interaction of the polymorphic CYP2C19 isozyme with

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MDMA, bearing in mind that the clearance of a number of drugs is regulated by this enzyme.

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