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Pharmacogenetics of Ecstasy: CYP1A2, CYP2C19, and CYP2B6 polymorphisms moderate pharmacokinetics of MDMA in healthy subjects

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Abstract

In vitro studies showed that CYP2C19, CYP2B6, and CYP1A2 contribute to the metabolism of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) to 3,4-methylenedioxyamphetamine (MDA). However, the role of genetic polymorphisms in CYP2C19, CYP2B6, and CYP1A2 in the metabolism of MDMA in humans is unknown. The effects of genetic variants in these CYP enzymes on the pharmacokinetics and pharmacodynamics of MDMA were characterized in 139 healthy subjects (69 male, 70 female) in a pooled analysis of eight double-blind, placebo-controlled studies. MDMA-MDA conversion was positively associated with genotypes known to convey higher CYP2C19 or CYP2B6 activities. Additionally, CYP2C19 poor metabolizers showed greater cardiovascular responses to MDMA compared with other CYP2C19 genotypes. Furthermore, the maximum concentration of MDA was higher in tobacco smokers that harbored the inducible CYP1A2 rs762551 A/A genotype compared with the non-inducible C-allele carriers. The findings indicate that CYP2C19, CYP2B6, and CYP1A2 contribute to the metabolism of MDMA to MDA in humans. Additionally, genetic polymorphisms in CYP2C19 may moderate the cardiovascular toxicity of MDMA.

Keywords: 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine, pharmacokinetics, CYP1A2, CYP2C19, CYP2B6

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) produces feelings of well-being, enhanced emotional empathy, and prosociality (Hysek et al., 2014a) and is used recreationally and as an adjunct to psychotherapy (Oehen et al., 2013). The recreational use of ecstasy has been associated with potentially severe toxicity, including agitation, hypertension, and hyperthermia (Halpern et al., 2011; Liechti, 2014; Liechti et al., 2005). Individually increased vulnerability to the clinical toxicity of MDMA may result from alterations in drug-metabolizing enzymes, such as cytochrome P450 monooxygenases (CYPs), that are involved in the metabolism of MDMA (de la Torre et al., 2012; Rietjens et al., 2012). Specifically, MDMA is O-demethylenated primarily by CYP2D6 to 3,4-dihydroxymethamphetamine (HHMA), which is then O-methylated to 4-hydroxy-3-methoxymethamphetamine (HMMA) by catechol-O-methyltransferase (COMT) (de la Torre et al., 2012; Kreth et al., 2000; Meyer et al., 2008; Segura et al., 2005), the main inactive metabolite of MDMA in humans (Kolbrich et al., 2008; Schindler et al., 2014). Additionally, MDMA is *N*-demethylated by CYP1A2, CYP2B6, CYP3A4, and CYP2C19 (Kreth et al., 2000; Meyer et al., 2008) to the minor active metabolite 3,4-methylenedioxyamphetamine (MDA) (de la Torre et al., 2004; Hysek et al., 2012c; Kolbrich et al., 2008).

Only limited controlled data are available on the pharmacogenetics/toxicogenetics of MDMA (Pardo-Lozano et al., 2012; Rietjens et al., 2012). The pharmacokinetics (PK) of a drug is in part determined by genetic variants in drug-metabolizing enzymes. Genetic polymorphisms in CYP2D6 have been shown to influence MDMA metabolism in humans (de la Torre et al., 2005; de la Torre et al., 2012; Hysek et al., 2013; Hysek et al., 2014b; O'Mathuna et al., 2008; Schmid et al., 2016; Yang et al., 2006) but the role of other CYPs is unknown. In vitro studies indicate that CYP2D6 is responsible for most of the clearance of MDMA, but CYP1A2, CYP2B6, and CYP2C19 also contribute to the N-demethylation of MDMA to MDA, and their role may become more important in cases of overdose (Meyer et al., 2008) or in

CYP2D6 poor metabolizers (PMs; de la Torre et al., 2012). However, the effects of polymorphisms in these CYPs on the metabolism of MDMA in humans have not yet been investigated. Therefore, the aim of the present study was to explore whether genetic variants in the CYP2C19, CYP2B6, and CYP1A2 genes alter the conversion of MDMA to MDA in humans. No common CYP1A2 loss-of-function polymorphisms have been identified to date. However, CYP1A2 is inducible by tobacco smoking in subjects with the common single-nucleotide polymorphism (SNP) rs762551 A/A genotype compared with the C/A and C/C genotypes (Sachse et al., 1999). Therefore, we tested whether MDA formation is greater in tobacco smokers who carry the A/A genotype to assess the contribution of CYP1A2 to the metabolism of MDMA for the first time in humans. Finally, we tested whether CYP2C19, CYP2B6 or CYP1A2 genotype influenced the pharmacodynamics of MDMA.

2. Experimental procedures

2. 1. Study design

This was a prospectively designed pooled analysis of eight double-blind, placebo-controlled, crossover studies in healthy subjects (Hysek et al., 2012a; Hysek et al., 2013; Hysek et al., 2012b; Hysek et al., 2011; Hysek et al., 2012c; Hysek et al., 2014b; Schmid et al., 2014; Schmid et al., 2015) including a total of 142 subjects. The prespecified primary endpoint of the pooled analysis was to assess the effects of polymorphisms in CYP enzymes on the PK of MDMA in all of the studies. In seven studies each including 16 subjects, a total of 112 subjects received MDMA at a dose of 125 mg, placebo, one of eight pretreatments plus MDMA, or the pretreatment alone (Hysek et al., 2012a; Hysek et al., 2013; Hysek et al., 2012b; Hysek et al., 2011; Hysek et al., 2012c; Hysek et al., 2014b; Schmid et al., 2015). In one study, 30 subjects received MDMA at a dose of 75 mg, placebo, or methylphenidate (Schmid et al., 2014). Washout periods between treatment periods were at least 7 days. Only data after the administration of MDMA alone without other treatments were included in this analysis and the washout was considered sufficiently long to exclude any effects of the other treatments on the

effects of MDMA alone. All of the studies were registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT01386177, NCT01465685, NCT01616407, and NCT01771874). All of the studies were approved by the local ethics committee and the Swiss Agency for Therapeutic Products (Swissmedic) and conducted in accordance with the Declaration of Helsinki. The administration of MDMA in healthy subjects was authorized by the Swiss Federal Office for Public Health (BAG), Bern, Switzerland. Informed consent was obtained from all participants included in the studies.

2.2. Subjects

A total of 142 healthy European/Caucasian subjects, aged 18-45 years, were recruited from the University of Basel campus and participated in the study. One genotyping sample was missing, one participant did not give consent for genotyping, and a full concentration-time profile could not be obtained in one participant, resulting in data from 139 participants (69 male, 70 female, mean age ± SD: 24.9 ± 4.1 years; range: 18-44 years) that were included in the analysis. A total of 110 subjects (54 male, 56 female) received 125 mg MDMA (mean ± SD: 1.9 ± 0.3 mg/kg), and 29 subjects (15 male, 14 female) received 75 mg MDMA (1.1 ± 0.1 mg/kg) (Hysek et al., 2012a; Hysek and Liechti, 2012; Hysek et al., 2012b; Hysek et al., 2012c). The exclusion criteria were a history of psychiatric disorders, physical illness, a lifetime history of using illicit drugs more than five times (with the exception of past cannabis use), illicit drug use within the last 2 months, illicit drug use during the study, determined by urine tests that were conducted before the test sessions, and the use of drugs that interact with CYP function. Tobacco smoking (> 10 cigarettes/day) was an exclusion criterion, but light tobacco smokers (6-10 cigarettes/day) and very light tobacco smokers (1-5 cigarettes/day) were included in the study. The detailed exclusion criteria were reported elsewhere (Hysek et al., 2012a; Hysek and Liechti, 2012; Hysek et al., 2012b; Hysek et al., 2012c).

2.3. Study drug

(±)MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was administered orally in a single dose of 125 or 75 mg. Similar doses are found in ecstasy pills (Brunt et al., 2012) and have been used in clinical studies (Oehen et al., 2013). The dose range was 0.8-2.7 mg/kg (mean = 1.7 mg/kg).

2.4. Blood sampling and drug analysis

Blood samples were collected in lithium heparin tubes 0, 0.33, 0.67, 1, 1.5, 2, 3, 4, and 6 h after administration of MDMA or placebo and immediately centrifuged. Plasma was stored at -20°C until analysis. Plasma concentrations of MDMA, MDA, and HMMA were determined as previously described (Hysek et al., 2013; Hysek et al., 2012c). HMMA concentrations were determined after enzymatic deglucuronidation in 76 subjects. The lower limit of quantification concentrations were 1 ng/ml for all analytes (Hysek et al., 2012c).

2.5. Pharmacodynamic measures

Blood pressure, heart rate, and body temperature were assessed repeatedly before and 0, 0.33, 0.67, 1, 1.5, 2, 3, 4, 5, and 6 h after MDMA or placebo administration as previously described (Hysek and Liechti, 2012; Hysek et al., 2011). The rate pressure product (RPP), a measure of the overall cardiostimulant effects, was calculated as systolic blood pressure × heart rate. Core (tympanic) temperature was assessed using a GENIUS^{TM} 2 ear thermometer (Tyco Healthcare Group LP, Watertown, NY, USA). Subjective effects were measured using Visual Analog Scales (VAS) (Hysek et al., 2012a; Hysek and Liechti, 2012; Hysek et al., 2012b; Hysek et al., 2012c).

2.6. Genotyping

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hombrechtikon, Switzerland) and automated QIAcube system. Genotyping was performed using commercial TaqMan SNP genotyping assays (LuBio Science, Lucerne,

Switzerland). CYP2C19 TaqMan drug metabolism genotyping assays were used to determine the most common loss-of-function SNPs rs4244285 (CYP2C19*2, c.681G>A, assay: C_25986767_70) and rs28399504 (CYP2C19*4, c.1A>G, assay: C_30634136_10) and gain-of-function SNP rs12248560 (CYP2C19*17, c.806C>T, assay: C_469857_10)(Hicks et al., 2013). Predicted CYP2C19 PMs included CYP2C19*2/*2, intermediate metabolizers (IMs) included CYP2C19*1/*2 and CYP2C19*2/*17, extensive metabolizers (EMs) included CYP2C19*1/*1, and ultra-rapid metabolizers (UMs) included both CYP2C19*17/*17 and CYP2C19*1/*17 (Hicks et al., 2013). For CYP1A2, the CYP1A2 TaqMan drug metabolism genotyping assay (C_8881221_40) was used to determine the common SNP rs762551, the sole variant of the CYP1A2*1F haplotype. For CYP2B6, we determined the reduced-activity SNP rs3745274 (516G>T, CYP2B6*6 or CYP2B6*9, assay: C_7817765_60).

2.7. Pharmacokinetic analyses

Peak plasma concentrations (C_{max}) were obtained directly from the observed data. The area under the concentration-time curve (AUC) from 0 to 6 h after dosing (AUC_6) was calculated using the linear trapezoidal method. Plasma concentrations were determined up to 6 h after MDMA administration because the aim of the study was to assess potential changes in MDMA plasma levels while relevant pharmacodynamics effects or MDMA are present (Hysek et al., 2011; Hysek et al., 2012c).

2.8. Statistical analyses

The statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK, USA). Group differences were analyzed using one-way analysis of variance (ANOVA), with genotype as between-subjects group factors, followed by the Tukey *post hoc* test. Smoking status was included as factor with CYP1A2 genotype. To account for differences in body weight and dosing, plasma levels were dose-normalized to the mean dose per body weight (1.7 mg/kg), and the mg/kg dose of MDMA was included as a covariate in the analysis

of the pharmacodynamic effects. Sensitivity analyses were also conducted using only the 125 mg MDMA dose to exclude confounding by dose level. Additionally, CYP2D6 activity was determined using the dextromethorphan/dextrorphan ratio (Schmid et al., 2016) and the analyses were replicated in 111 subjects phenotyped as CYP2D6 EMs and after exclusion of 19 IMs and 9 PMs to exclude confounding by CYP2D6 activity.

3. Results

3.1. Effects of CYP2C19

Effects of the CYP2C19 genotype on the pharmacokinetics of MDMA are shown in Figure 1 and supplementary Table S1. MDMA plasma levels increased more rapidly in the two CYP2C19 PMs (Figure 1a) but this effect was not significant. The CYP2C19 genotype significantly influenced the AUC_6 of MDA ($F_{3,135}$ = 3.54, P < 0.05, Figure 1b) and the MDMA/MDA AUC_6 ratio ($F_{3,135}$ = 5.55, P < 0.01, Figure 1d), but not HMMA concentrations (Figure 1c). CYP2C19 genotype altered the E_{max} of the RPP ($F_{3,134}$ = 2.92, P < 0.05) with higher RPP values in CYP2C19 PMs compared with IMs and UMs (both P <0.05, Figure 2). CYP2C19 genotype had no effects on body temperature or any of the subjective effects of MDMA.

3.2. Effects of CYP2B6

Effects of the CYP2B6 rs3745274 SNP (G/G vs. G/T vs. T/T) on the pharmacokinetics of MDMA are shown in Figure 3 and supplementary Table S2. The CYP2B6 genotype significantly altered the MDMA C_{max} ($F_{2,136}$ = 3.72, P < 0.05, Figure 3a), with a higher concentration in subjects within the T/T compared to the G/G genotype (P < 0.05). The CYP2B6 genotype significantly influenced the MDMA/MDA AUC_6 ratio ($F_{2,136}$ = 3.67, P < 0.05, Figure 3d) with higher ratios in the T/T vs. G/T or G/G group (both P < 0.05), but had no

significant effects on plasma levels of MDA (Figure 3b) or HMMA (Figure 3c). CYP2B6 genotype did not alter the autonomic or subjective effects of MDMA.

3.3. Interacting effects of CYP1A2 and smoking

Smoking status interacted with CYP1A2 genotype (inducible rs762551 A/A vs. non-inducible A/C and C/C) to affect MDA C_{max} and AUC_6 values ($F_{5,133}$ = 5.56, P < 0.001 and 4.04, P < 0.01, respectively; Table S3 and Figure 4). Smoking status altered MDA formation only in subjects with the inducible rs762551 A/A genotype, with higher MDA formation in light tobacco smokers (6-10 cigarettes/day) compared with nonsmokers and very light smokers (1-5 cigarettes/day, both P < 0.001; Figure 4). No effect of smoking status on MDA levels was found in subjects with the rs762551 A/C and C/C genotypes. There were no effects of CYP1A2 genotype or smoking or interaction on the plasma concentrations of MDMA or HMMA (Table S3) or on the pharmacodynamic autonomic and subjective effects of MDMA.

3.4. Effect of dose and dose normalization

As expected, peak plasma concentrations of MDMA were greater after the 125 mg dose vs the 75 mg dose (mean ± SD: 230 ± 46 vs. 125 ± 29 ng/ml; $F_{1,137}$ = 140.20, P < 0.001). Consistently, the 125 mg dose produced greater subjective peak drug effects (80 ± 23 vs. 57 ± 30%; $F_{1,137}$ = 21.5, P < 0.001) and cardiovascular stimulant peak responses (RPP = 14728 ± 3278 vs. 12067 ± 3159 mmHg × bpm; $F_{1,137}$ = 15.3, P < 0.001). After dose normalization, the subjective and cardiovascular effects of MDMA did not differ between the dose groups. However, dose-normalized C_{max} values of MDMA were near-significantly greater at the 125 mg compared with the 75 mg dose ($F_{1,137}$ = 4.08, P = 0.05) indicating a trend towards nonlinear pharmacokinetics at the doses used in this study.

4. Discussion

The present study described the pharmacogenetics of CYP1A2, CYP2C19 and CYP2B6 in the disposition of MDMA in healthy human subjects. We documented a role for CYP2C19 and CYP2B6 in the conversion of MDMA to MDA in humans, confirming in vitro metabolism studies (Kreth et al., 2000; Meyer et al., 2008). The MDMA/MDA AUC6 ratio was greater in subjects with low CYP2C19 or low CYP2B6 function, consistent with a contributing role for both CYP2C19 and CYP2B6 in the N-demethylation of MDMA to MDA in humans and confirming in vitro studies (Kreth et al., 2000; Meyer et al., 2008). Additionally, subjects with genetically determined low CYP2C19 function showed a more rapid and greater cardiovascular response to MDMA, although only two subjects with CYP2C19 PM genotype were included in the present study. In contrast to the CYP2C19 genotype, the CYP2B6 genotype altered MDMA concentrations later in time 3-4 hours after drug administration. This finding may indicate that CYP2B6 becomes more important when CYP2D6 function decreases over time due to auto-inhibition by MDMA (de la Torre et al., 2012; O'Mathuna et al., 2008; Schmid et al., 2016; Yang et al., 2006). MDA is pharmacologically active *in vitro* (Hysek et al., 2012c; Rickli et al., 2015) and in rats (Schindler et al., 2014). One might therefore predict that alterations in the conversion of MDMA to MDA should not have a relevant effect on the pharmacodynamics of MDMA. However, the present study showed greater cardiostimulant effects of MDMA in subjects with slower MDMA to MDA conversion suggesting that MDMA contributes more to the cardiostimulant effects of MDMA than MDA.

Similar to CYP2C19 and CYP2B6, CYP1A2 contributes to the N-demethylation of MDMA to MDA *in vitro* (Meyer et al., 2008). CYP1A2 can be induced by tobacco smoking (Sachse et al., 1999). CYP1A2 activity increased with the number of cigarettes smoked per day (Dobrinas et al., 2011) and normalized with a half-life of 39 hours when smoking is stopped (Faber and Fuhr, 2004). Additionally, CYP1A2 function is greater in smokers with the inducible SNP rs762551 A/A genotype compared with smokers with the non-inducible A/C and C/C genotypes (Sachse et al., 1999). We found higher MDA levels in tobacco smokers with the inducible vs. non-inducible genotypes and compared with nonsmokers. This finding indicates

that CYP1A2 contributes to the N-demethylation of MDMA to MDA in humans. However, CYP1A2 genetics did not alter the response to MDMA.

Overall, polymorphism in CYP1A2, CYP2C19, and CYP2B6 influenced the metabolism of MDMA but none of the polymorphism altered the subjective response to MDMA.

The present study has several limitations. Although it is a relatively large study, it included only two subjects with the 2C19 PM genotype. While consistent with the higher concentrations of MDMA, the greater cardiostimulant response to MDMA in these two subjects may represent a chance finding. Similarly, there were only 4 light smokers with the inducible CYP1A2 genotype and this interaction of CYP1A2 and smoking in the metabolism of MDMA needs to be confirmed in a larger study. We also included only smokers (<10 cigarettes/day) and it is likely that heavy smokers would show greater CYP1A2 induction (Dobrinas et al., 2011).

Although impairments in CYP2C19 or CYP2B6 alone may have only small effects on MDMA pharmacokinetics and its effects, the presence of multiple enzymes with impaired function such as combinations of 2D6 PM with CYP2C19 PM and CYP2B6 T/T may result in more pronounced consequences. The present study did not include subjects with such rare combinations that may predispose to MDMA toxicity.

Plasma for pharmacokinetic analyses was sampled only up to 6 hours. However, this time covers the actual pharmacodynamic effects of MDMA which are shorter than its presence in plasma due to acute tolerance. Finally, we tested only doses of MDMA up to 125 mg which is in the upper range of recreational doses (Brunt et al., 2012) and identical to the dose used in clinical studies (Mithoefer et al., 2010; Oehen et al., 2013) but may not represent all cases of overdosing.

In conclusion, the results indicate that CYP1A2, CYP2C19, and CYP2B6 contribute to the conversion of MDMA to MDA in humans. Additionally, genetic polymorphisms in CYP2C19 may play a role in the clinical toxicity of MDMA.

Conflict of interest

The authors do not have any conflicts of interest to declare for this work.

Contributors

YS, PV, and MEL designed the study. MEL obtained funding. YS, PV, and KP performed the research. YS, PV, KP, HMS, and MEL analyzed the data. PV, YS, and MEL wrote the manuscript. All the authors reviewed and approved the manuscript.

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Figure Legends

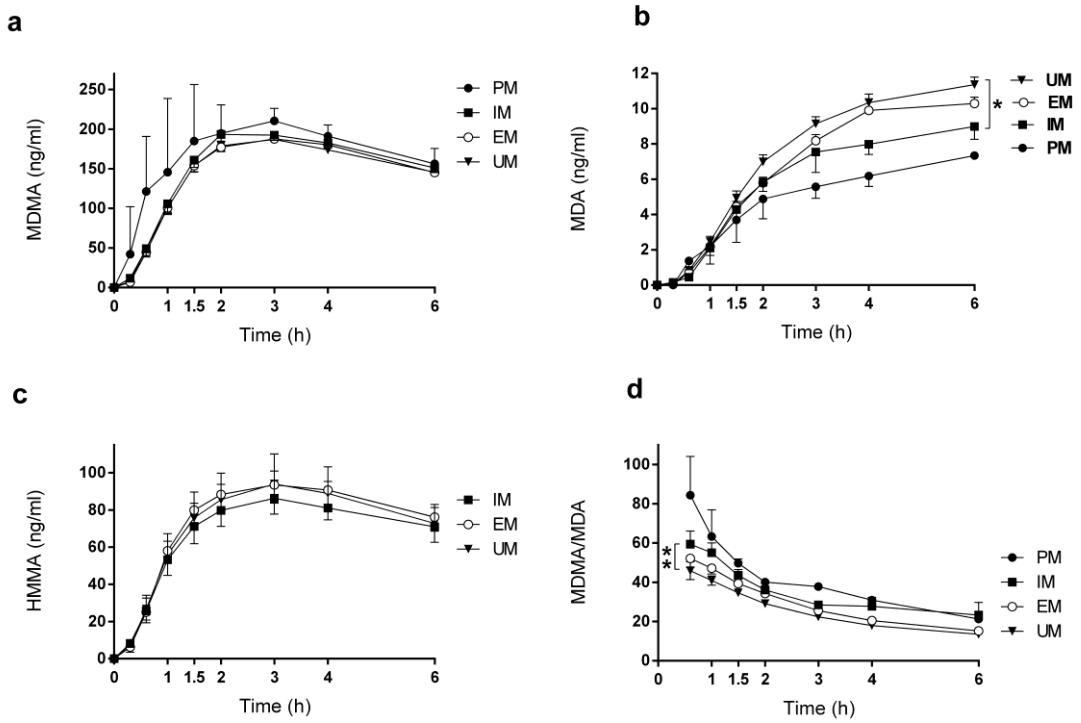

Figure 1. Effect of the CYP2C19 polymorphism on the plasma concentrations of MDMA **(a)**, MDA **(b)**, HMMA **(c)** and the MDMA/MDA ratio **(d)**. The MDMA/MDA ratio **(d)** decreased with increasing CYP2C19 function, while MDA levels **(b)** increased with decreasing CYP2C19 function, indicating influence on the *N*-demthylation of MDMA to MDA. The data are expressed as mean ± SEM in 2 PMs, 24 IMs, 66 EMs and 47 Ums (except for HMMA). *P < 0.05 for the AUC_6 of MDA in UM vs. IM based on a significant main effect over all genotypes ($F_{3,135}$ = 3.54, P < 0.05). **P < 0.01 for the MDMA/MDA AUC_6 ratio in UM vs. IM based on a significant main effect over all genotypes ($F_{3,135}$ = 5.55, P < 0.01).

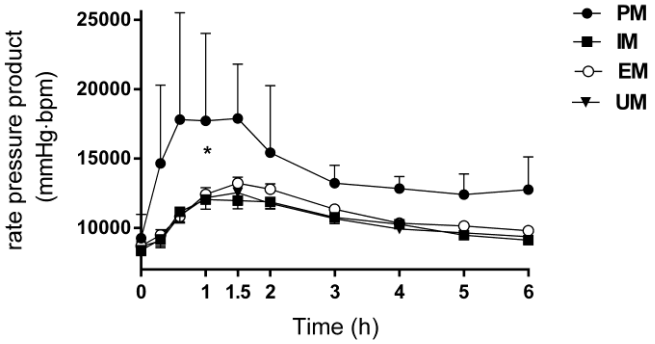

Figure 2. Effect of CYP2C19 polymorphism on cardiovascular stimulation. The heart rate systolic blood pressure product increased more in the two CYP2C19 PMs compared to IMs or UMs (*for both $P < 0.05$ based on a significant main effect of genotype on E_{max} values: $F_{3,134} = 2.92$, $P < 0.05$). The data are expressed as the mean ± SEM in 2 PMs, 24 IMs, 66 EMs and 47 UMs.

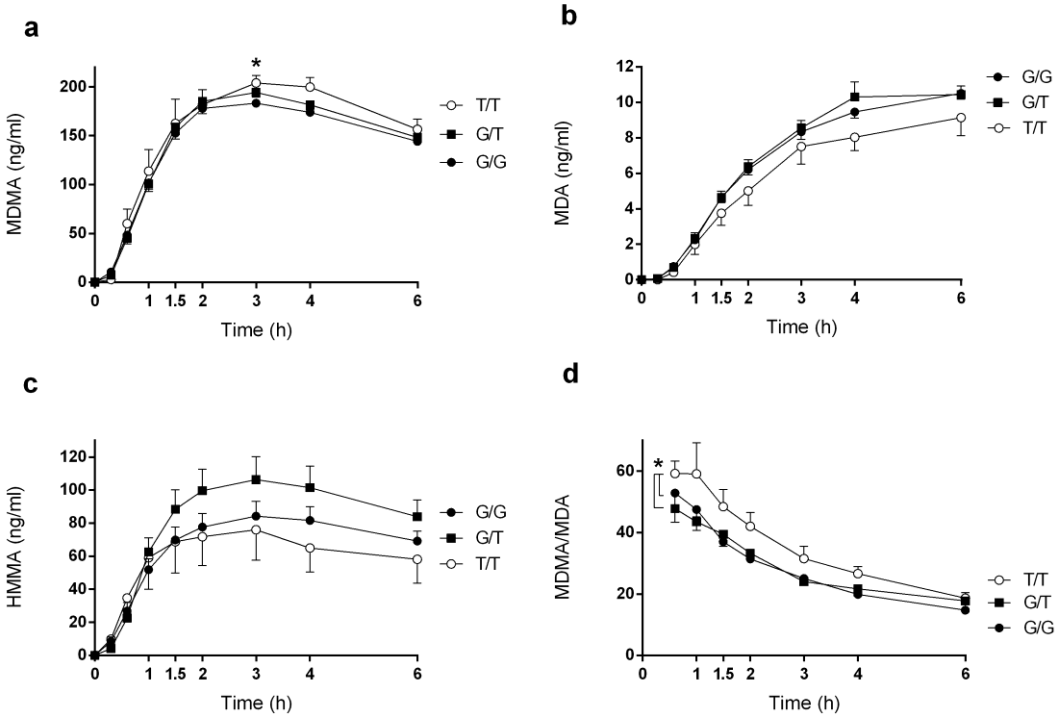

Figure 3. Effect of CYP2B6 genotype on the plasma concentrations of MDMA **(a)**, MDA **(b)**, HMMA **(c)** and the MDMA/MDA ratio **(d)**. Maximal MDMA concentrations were higher in the reduced-function CYP2B6 rs3745274 T/T genotype group compared with the normal functioning G/G and G/T genotype groups **(a)** (*for both $P < 0.05$). The CYP2B6 genotype also significantly influenced the MDMA/MDA ratio **(d)** with higher ratios in the T/T *vs*. G/T groups and T/T *vs*. G/G group (*for both $P < 0.05$), but had no significant effects on plasma levels of MDA **(b)** or HMMA **(c)**. The data are expressed as mean ± SEM in 78 G/G, 51 G/T and 10 T/T for **(a)**, **(b)**, and **(d)** and in 42 G/G, 29 G/T and 5 T/T for **(c)**.

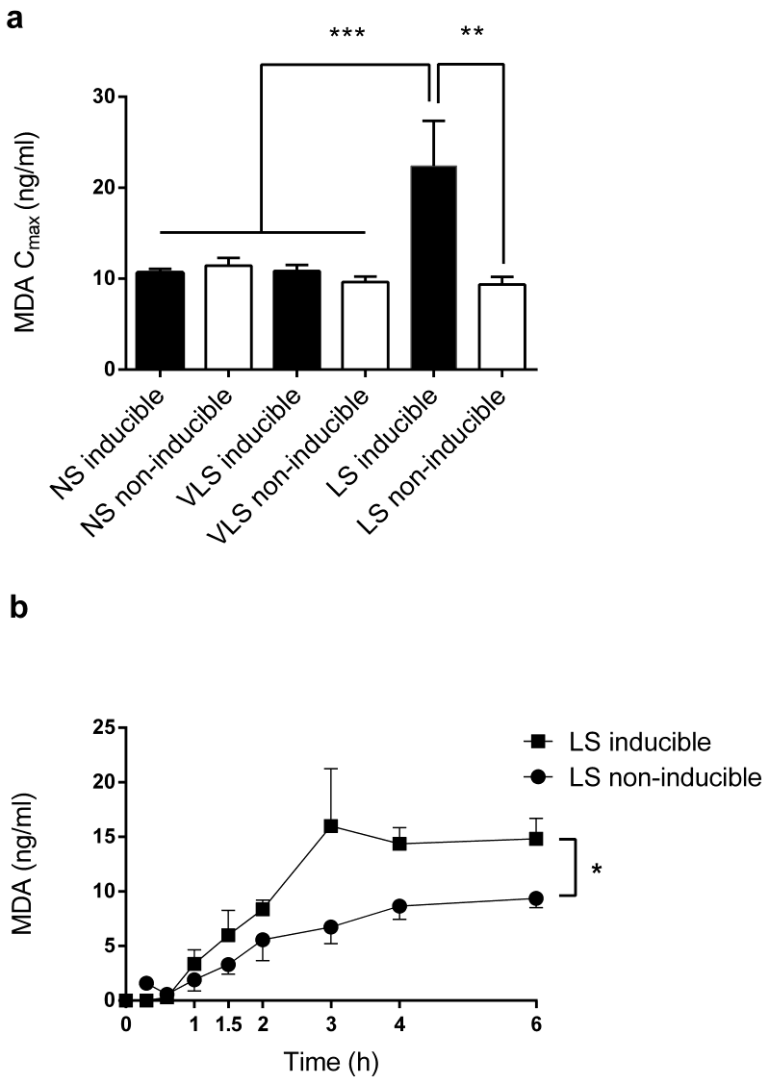

Figure 4. Effects of the CYP1A2 SNP rs762551 and smoking status on MDA plasma levels. Maximal plasma concentrations of MDA were higher in light smokers (LS; 6-10 cigarettes/day) with the inducible CYP1A2 rs762551 A/A genotype compared with nonsmokers (NS), very light smokers (VLS; 1-5 cigarettes/day), and smokers with the non-inducible CYP1A2 rs762551 A/C and C/C genotypes **(a and b)**. The data are expressed as the mean ± SEM in 57 inducible NS, 50 non-inducible NS, 9 inducible VLS, 16 non-inducible VLS, 4 inducible LS, 3 non-inducible LS. **P < 0.01, ***P < 0.001.

Supplemental Material

Table S1. Effect of CYP2C19 activity on the pharmacokinetics of MDMA (n=139)

CYP2C19 genotype	PM	IM	EM	UM	$F_{3,135}$ =	P =
subjects (n, total n=139)	2	24	66	47		
subjects with lower dose (n, n=29)	0	6	17	6		
Alleles (n)	*2/*2(2)	*1/*2(20) *2/*17(4)	*1/*1(66)	*1/*17(37) *17/*17(10)		
Women (n, %)	0 (0)	13 (54)	33 (50)	24 (51)		
Women with lower dose (n, %)	0 (0)	4 (67)	7 (41)	3 (50)		
MDMA C_{max} (ng/ml)	229±7	211±5.8	210±4.6	204±4.7	0.61	0.61
MDMA AUC_6 (ng·h/ml)	1007±61	903±28	878±20	866±22	0.80	0.50
MDA C_{max}	7.3±0	10.2±1.1	11.1±0.7	11.8±0.6	1.00	0.38
MDA AUC_6	29.3±0.4	36.2±2.7	40.8±1.5	45.7±2.1*	3.54	<0.05
MDMA AUC_6/MDA AUC_6	34.3±1.6	27.1±1.6	23.3±0.9	20.6±1**	5.55	<0.01
HMMA C_{max}		93.1±15	99.6±10	99.4±16	[b]0.059	0.94
HMMA AUC_6		457±70	447±46	409±71	[b]0.16	0.85

*P<0.05, **P<0.01 compared with IMs. PM, poor metabolizer; IM, intermediate metabolizer; EM, extensive metabolizer; UM; ultra-rapid metabolizer; C_{max} maximum plasma concentration, AUC, area under the plasma concentration-time curve; AUC_6, AUC from time 0-6h. Values are means±SEM. [b]$F_{2,73}$ (N=76; 0 PMs, 16 IMs, 37 EMs and 23 UMs).

Table S2. Effect of CYP2B6 activity on the pharmacokinetics of MDMA (n=139)

CYP2B6 genotype (rs3745274)	homozygous G/G	heterozygous G/T	homozygous T/T	$F_{2,136}$ =	P =
subjects (n, total n=139)	78	51	10		
subjects with lower dose (n, n=29	17	11	1		
Women (n, %)	46 (59)	19 (37)	5 (50)		
Women with lower dose (n, %)	9 (53)	4 (36)	1 (100)		
MDMA C_{max} (ng/ml)	203±3.8*	212±4.9	232±8.5	3.72	<0.05
MDMA AUC_6 (ng·h/ml)	860±18	896±21	950±21	2.03	0.14
MDA C_{max}	10.8±0.4	12±0.9	9.3±1	1.71	0.18
MDA AUC_6	41.2±1.5	43.2±2	35.3±3.7	1.46	0.24
MDMA AUC_6/MDA AUC_6	22.9±0.9*	22.3±0.9*	29.4±2.8	3.67	<0.05
HMMA C_{max}	91.4±9	111.4±14.1	78.3±19.3	[b]1.06	0.35
HMMA AUC_6	404±41.2	499±63.4	359±88	[b]1.07	0.35

*P<0.05 compared with homozygous T/T. $C_{max,}$ maximum plasma concentration; AUC, area under the plasma concentration-time curve; $AUC_{6,}$ AUC from time 0-6h. Values are means±SEM. [b]$F_{2,73}$ (N=76; 42 homozygous G/G, 29 heterozygous G/T, 5 homozygous T/T).

Table S3. Effect of CYP1A2 activity on the pharmacokinetics of MDMA (n=139)

CYP1A2 genotype	A/A			A/C | C/C			$F_{5,133}$ =	P =
functionality	inducible			non-inducible				
Smoking status	nonsmokers	very light smokers (1-5 cigarettes/day)	light smokers (6-10 cigarettes/day)	nonsmokers	very light smokers (1-5 cigarettes/day)	light smokers (6-10 cigarettes/day)		
subjects (n, total n=139)	57	9	4	50	16	3		
subjects with lower dose (n, n=29)	11	0	1	12	3	2		
Women (n, %)	29 (51)	4 (44)	2 (50)	24 (48)	9 (56)	2 (67)		
Women with lower dose (n, %)	3 (27)	0 (0)	0 (0)	7 (58)	1 (33)	2 (100)		
MDMA C_{max} (ng/ml)	209±4.0	222±13	221±7.5	206±5.3	210±8.1	175±33	1.02	0.41
MDMA AUC_6 (ng·h/ml)	891±19	922±54	938±65	861±23	889±35	752±149	0.92	0.47
MDA C_{max}	10.7±0.4***	10.8±0.7***	22.4±5	11.4±0.8***	9.6±0.6***	9.4±0.8**	5.56	<0.001
MDA AUC_6	41.1±1.7***	42.4±3.2**	68.8±8.9	41.1±1.9***	37.6±2.7***	36±6.5*	4.04	<0.01
MDMA AUC_6/MDA AUC_6	23.4±0.9	22.7±2	14.5±2.3	22.9±1.1	25.9±2.6	22±5	1.47	0.20
HMMA C_{max}	98.9±11	78.4±23	58.4	106±15	76.3±12	123±51	[b]0.49	0.78
HMMA AUC_6	436±49	338±94	128	499±66	330±53	392±118	[b]0.81	0.55

*P<0.05, **P<0.01, ***P<0.001 compared with inducible low smokers. C_{max} maximum plasma concentration, AUC, area under the plasma concentration-time curve; $AUC_{6,}$ AUC from time 0-6h. Values are means±SEM. [b]$F_{5,70}$ (N=76; 30 inducible nonsmokers, 30 non-inducible nonsmokers, 4 inducible very light smokers, 9 non-inducible very light smokers, 1 inducible light smokers, 2 non-inducible light smokers).