Human pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) after repeated doses taken 2 h apart

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Abstract

Background 3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is one of the most abused recreational drugs. Its usual pattern of misuse includes repeated doses taken over a short time period that could influence MDMA pharmacology and toxicity.

Objective This study aims to evaluate the pharmacokinetics and pharmacologically induced effects of two MDMA consecutive doses separated by 2 h.

Methods A randomized, double-blind, crossover, and placebo-controlled trial included ten male volunteers participating in two experimental sessions. MDMA was administered as a single 100-mg dose or as a repeated dose (50 mg followed by 100 mg, administered at 2 h apart). Outcome variables included pharmacokinetics, physiological, subjective, and psychomotor effects.

Results Following the repeated doses, plasma concentrations of MDMA were higher than those expected by simple dose accumulation (+16.2 % AUC; +12.8 % C_{max}), but those of HMMA and HMA were significantly lower (−29.8 % AUC; −38.2 % C_{max}). After the second dose, physiological effects, psychomotor performance, and subjective effects were lower than expected especially for euphoria and stimulation. MDMA-induced increases in diastolic and systolic arterial pressure and body temperature were in the range of those expected following MDMA concentrations.

Conclusions MDMA pharmacokinetics and metabolic disposition following two doses separated by 2 h show that the contribution of the first dose to the MDMA-induced mechanism-based metabolic inhibition was already apparent. The concentrations of MDMA after the second dose were slightly higher than expected. The effects on blood pressure and temperature after the second administration were slightly higher than those following the first, but for heart rate and subjective variables these were lower than expected considering the MDMA concentrations achieved, suggesting a possible tolerance phenomenon.

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Abbreviations

ARCI	Addiction Research Center Inventory
AUC	Area under the curve
Peak	Peak effects
Time	Time course
C_{max}	Peak plasma concentration
DBP	Diastolic blood pressure

DSM-IV	Diagnosis and Statistical Criteria for Mental Disorders
DSST	Digit symbol substitution test
h	Hours
ECG	Electrocardiogram
HMA	4-Hydroxy-3-methoxyamphetamine
HMMA	4-Hydroxy-3-methoxymethamphetamine
HR	Heart rate
FPIA	Fluorescence polarization immunoassay
M50	50 mg of MDMA
M100	100 mg of MDMA
M50+100	A dose of 50 mg of MDMA followed by another of 100 mg at 2 h apart (repeated dose)
MDA	3,4-Methylenedioxyamphetamine
MDMA	3,4-Methylenedioxymethamphetamine
METH	Methamphetamine
ms	Milliseconds
PD	Pupil diameter
RT	Simple reaction time
SNS	Sympathetic nervous system
SBP	Systolic blood pressure
T	Temperature
T_{max}	Time to achieve peak plasma concentration
$t_{1/2}$	Elimination half-life
VAS	Visual Analog Scale

Introduction

The synthetic amphetamine analog (±)-3-4-methylenedioxy methamphetamine (MDMA, ecstasy) is a widely used psychoactive drug which exerts its effects by interacting with multiple neurotransmitter systems (e.g., release of serotonin, dopamine, and norepinephrine and re-uptake inhibition of these neurotransmitters). MDMA typically causes heightened feelings of well-being and euphoria (Camí et al. 2000). Ecstasy is associated with acute medical complications, long-term psychiatric disorders, and neuropsychological deficits (Greene et al. 2003; de Sola Llopis et al. 2008; Martín-Santos et al. 2010; Cuyàs et al. 2011).

Intensive ecstasy self-administration is often reported by experienced users. Most "binge" at least once, "stack" several pills together, and "boost" successive doses. The most common pattern of use consists in one half to five tablets taken 30 min to 2 h apart (Hammersley et al. 1999; Riley et al. 2001; Verheyden et al. 2003; Parrott 2005). The dose of MDMA per pill ranges from 0 to 245 mg, with the total dose consumed ranging up to 280 mg (Morefield et al. 2011). This practice presumably tries to minimize side effects or an ecstasy overdose and/or to prevent the "come-down": the experience of having a difficult transition from the peak effect back to baseline consciousness. This pattern of use could modify the MDMA pharmacological effects observed

after a single dose and incur toxicological consequences, aspects not examined experimentally in humans.

MDMA effects after repeated administrations have been investigated in several animal models where the dose and frequency of administration protocols applied had profound effects on the severity of acute (e.g., hyperthermia) and long-term (e.g., neurotoxicity) responses (O'Shea et al. 1998; Green et al. 2004a). In humans, several single-dose placebo-controlled studies have been reported (e.g., de la Torre et al. 2000a; Lester et al. 2000; Liechti et al. 2001), but only one MDMA repeated doses study taken 24 h apart. The results showed pharmacological tolerance to subjective effects, a possible sensitization phenomenon to some physiological effects and a MDMA metabolic autoinhibition (Farré et al. 2004), providing compelling reasons to evaluate pharmacological changes involving shorter intermittent MDMA dose regimens.

The present study was designed to determine the pharmacological effects and pharmacokinetics of two consecutive MDMA doses separated by 2 h, assuming that this time interval could be representative of MDMA recreational use.

Materials and methods

Subjects

Ten healthy male volunteers were included in the definitive study (mean age of 24.9 years, range 22–35 years; mean weight 70.7 kg, range 57.5–81.6 kg; mean height 177.7 cm, range 171–190 cm). Two additional subjects were included in a pilot study (evaluating the effects of 50 plus 75 mg at 2 h later; data not shown). The subjects were recruited from the surrounding community by "word of mouth".

The subjects were recreational users of ecstasy with a lifetime use between five and 50 times, without any serious adverse reaction, and with no history of abuse or drug dependence according to DSM-IV for other substances except nicotine (in smokers). They all had previous experience with cannabis (100 %), cocaine (83.3 %), hallucinogens (LSD) (75 %), other amphetamines (25 %), and other psychotropic substances (e.g., ketamine, GHB) (50 %). All but two were smokers (less than 20 cigarettes per day). The subjects drank an average of 9.4 units of alcohol per week (range 1–25 [1 unit=8 g ethanol]).

Before their inclusion, the volunteers were submitted to a general medical examination, a psychiatric interview (DSM-IV), routine laboratory tests, urinalysis, and 12-lead electrocardiogram (ECG) to exclude any medical or psychopathological condition. The subjects were phenotyped for CYP2D6 activity using dextromethorphan as drug probe (de la Torre et al. 2005). Only phenotypic extensive metabolizers were included.

Protocol was approved by the local Research Ethics Committee (CEIC-Parc de Salut Mar, Barcelona, Spain)

and authorized by the Spanish Ministry of Health (AGEMED, Madrid, Spain). The subjects signed a written informed consent before participation. The study was conducted in accordance with the Declaration of Helsinki, and volunteers were economically compensated for their participation in the study.

Drugs

(R,S)-MDMA was supplied by the Spanish Ministry of Health and prepared by the Pharmacy Department (Hospital del Mar, Barcelona, Spain) as identical opaque gelatin capsules. Each treatment consisted of capsules that contained either placebo (placebo capsules) or MDMA (50 mg, active capsules). The two experimental sessions in the study were randomized. In one session, the subjects received placebo (two placebo capsules) and 2 h later 100 mg of MDMA (M100 condition, two active capsules). In the other session, the subjects received 50 mg of MDMA (one placebo and one active capsule) and 2 h later 100 mg of MDMA (M50+100 condition, two active capsules).

Study design

A double-blind, randomized, crossover, and controlled study with placebo design was used. Sessions were conducted once per week, with a 2-week washout period between them to minimize the influence of any carry-over effect.

Experimental sessions

The subjects were admitted to the clinical research facilities at 08:00 h after an overnight fast. Upon arrival, the subjects were interrogated about any drug consumption or any event that could affect their participation in the study. Volunteers were requested to refrain from using any psychoactive drugs a minimum of 3 days prior to the study and throughout it and from using caffeinated products or alcohol for 48 h prior to experimental sessions. A urine sample was collected for drug testing (opiates, cocaine metabolite, amphetamines, and cannabinoids) (FPIA, Abbott Laboratories, Chicago, IL, USA). An indwelling intravenous catheter was inserted into a subcutaneous vein in the forearm of the non-dominant arm, and the subjects remained seated in a calm and comfortable laboratory environment. A physician and a nurse were present during the entire session.

At the beginning of each experimental session, vital signs, body temperature, and pupil diameter were taken. The participants completed baseline questionnaires and performed psychomotor performance tests. At 09:00 h, they received the drug (placebo or MDMA 50 mg) in a fasting state with 200 mL of water. Two hours later, at 11:00 h, they received the second administration (MDMA 100 mg).

At 13:00 h, a first light meal was provided. At 15:00 h, they were allowed to engage in leisure activities. At 18:00 h, smoking was permitted and the study ended at 21:00 h. Adverse effects were assessed during the session and the day after.

Before beginning the experimental session, the volunteers completed a training session to familiarize themselves with the testing procedures and questionnaires and to reach a steady performance in the psychomotor tasks.

Physiological measures

Blood pressure, heart rate, oral temperature, and pupil diameter were recorded at ~15 min and immediately before oral drug administration (time ~5 min, baseline) and at 20 and 40 min and at 1, 1.5, 2, 2.3, 2.6, 3, 3.5, 4, 5, 6, 8, 10, 12, and 26 h after the first drug administration (Dinamap™ 8100-T vital signs monitor, Critikon, Tampa, FL, USA). Pupil diameter was recorded with a Haab pupil gauge. For safety reasons, ECG was continuously monitored during all of the sessions (Dinamap™ Plus vital signs monitor, Critikon, Tampa, FL, USA).

Psychomotor performance measures

Digit symbol substitution test, simple reaction time (RT), Pauli test (PC/Vienna System, Schufried, Austria), and Maddox wing device were used for psychomotor performance measures. The Maddox wing device measures the balance of extraocular muscles and quantifies exophoria as an indicator of extraocular muscle relaxation. This battery has been previously used in the evaluation of psychostimulants and MDMA effects (Mas et al. 1999; Camí et al. 2000; Hernández-López et al. 2002; Farré et al. 2004; 2007). Psychomotor performance measures were taken at ~45 min (baseline) and at 1, 1.5, 2, 3, 3.5, 4, 5, 6, 8, 10, 12, and 26 h after the first drug administration.

Subjective effects

Mood and subjective effects were evaluated using a series of Visual Analog Scales (VAS) and the Addiction Research Centre Inventory (ARCI). Measures from both questionnaires were taken at ~45 min (baseline), at 20 and 40 min, and at 1, 1.5, 2, 2.3, 2.6, 3, 3.5, 4, 5, 6, 8, 10, 12, and 26 h after first drug administration.

VAS is a 100-mm line associated with adjectives and labeled at either end with opposites such as "not at all" and "extremely". The participants had to place a vertical mark along the continuum at a location appropriate to their current mood state at a total of 21 VAS. They were asked to rate effects of "stimulated", "high", "any effect", "good effect", "bad effect", "liking", "drowsiness", "changes in

distances", "changes in colors", "changes in shapes", "changes in lights", "hallucinogens—seeing of lights or spots", "changes in hearing", "hallucinations—hearing sounds or voices", "dizziness", "hallucinations—seeing animals, things, insects or people", "confusion", "fear", "depression or sadness", "different, changes or unreal body feeling", and "different or unreal surroundings".

The short form of ARCI was used. It consisted of 49 true or false statements containing five major subscales: MBG (a measure of euphoria), PCAG (a measure of sedation), LSD (a measure of dysphoria and somatic symptoms), and two empirically derived amphetamine-sensitive scales (a measure of stimulant-like effects), the ARCI-BG (stimulation) and ARCI-A (amphetamine-like) scales (Lamas et al. 1994).

Pharmacokinetics

Blood samples for the determination of MDMA and metabolite (HMMA, HMA, and MDA) plasma concentrations were collected during each experimental session at ~5 min (baseline), at 20 and 40 min, and at 1, 1.5, 2, 2.3, 2.6, 3, 3.5, 4, 5, 6, 8, 10, 12, 26, 30, and 50 h after first drug administration. Plasma concentrations were measured by gas chromatography coupled to mass spectrometry (Pizarro et al. 2002).

Statistical analysis

Values from physiological and subjective effects and psychomotor performance were transformed to differences from baseline measurements obtained before the first MDMA administration at 09:00 h. For each variable, two experimental parameters were obtained: the peak effect from 2 to 6 h after the 100-mg MDMA administration of both condition treatments (peak or E_{max}, maximum absolute change from baseline values) and the area under the curve from 2 to 6 h ($AUC_{0–6\ h}$) of effects calculated by the trapezoidal rule. These pharmacodynamic parameters (peak effects, AUC) were analyzed by a one-way repeated-measures analysis of variance (ANOVA) with drug conditions (M100 or M150) as factors. Furthermore, a detailed evaluation of the time course of effects was conducted using repeated-measures two-way ANOVA with treatment condition and time (from 2 to 6 h) as factors. When treatment condition or treatment condition×time was statistically significant, a Student t-test post-hoc comparison was performed at each time point (M100 vs. M50+150). Differences associated with $p < 0.05$ were considered to be statistically significant.

Pharmacokinetic parameters were calculated using WinNonlin software (Pharsight Corporation, Palo Alto, CA, USA). Compartmental analysis was performed in order

to set up the extent of the 50-mg dosage contribution when 50 and 100 mg after 2 h were administered. The estimated parameters $AUC_{0–\infty}$ and $AUC_{2–\infty}$ after 50 mg of MDMA administration were obtained as follows:

$$AUC_{0–\infty} = \frac{D}{V_c \times k_e}$$

and

$$AUC_{2–\infty} = AUC_{0–\infty} - AUC_{0–2}$$

where D is the dose (50 mg), V_c is the apparent volume of distribution, k_e is the first-order elimination constant in a one-compartmental open model, $AUC_{0–\infty}$ is the area under the curve from 0 h to infinity, $AUC_{2–\infty}$ is from 2 h to infinity, and $AUC_{0–2}$ is from 0 to 2 h.

Non-compartmental analysis was performed in order to obtain the following pharmacokinetic parameters: maximum concentration in the concentration–time profile (C_{max}), time after dosing required for the maximum concentration (T_{max}), slope of the terminal phase of the pharmacokinetic profile(λ_z), half-life ($t_{1/2}$), area under the curve from time point 0 to time point t ($AUC_{0–t}$), area under the curve from time point 0 to infinity ($AUC_{0–\infty}$), apparent volume of distribution (V_z), and plasmatic clearance. The ratios $AUC_{2–inf(50)}/AUC_{0–\infty(50+100)}$ and $AUC_{0–\infty(pbo+100)}/AUC_{0–\infty(50+100)}$ were calculated to obtain the percent of contribution of 50 and 100 mg in the total $AUC_{0–\infty}$ in the second MDMA administration.

Differences in metabolic behavior were assessed by calculating the linear trapezoidal rule $AUC_{0–t}$ of MDMA, MDA, HMMA, and HMA. The sum of metabolic areas HMMA + HMA and HMMA + HMA + MDA and the metabolic ratios HMMA + HMA + MDA/MDMA, HMMA + HMA/MDA + MDMA, and MDA/MDMA were compared in order to evaluate the capacity of the O- and N-demethylation of MDMA through cytochrome CYP2D6 and cytochrome CYP3A4.

The paired Student's t-test for the pharmacokinetic parameters and the metabolic ratio results and the Wilcoxon test for T_{max} were used for statistical analysis. Differences associated with $p < 0.05$ were considered to be statistically significant.

Results

Global results

The pharmacological effects observed after a single dose M100 versus repeated doses M50+100 of MDMA on physiological measures, psychomotor performance, and subjective effects are presented in Table 1 and in Fig. 1. The pharmacokinetic parameters are presented in Tables 2 and 3 and in Fig. 2.

Table 1 Physiological parameters, psychomotor performance, and subjective effect results (*n*=10, mean, standard deviation) after a single dose [M100] vs. a repeated MDMA dose [M50+100] administration

Variable		M100		M50+100		M100 vs. M50+100
		Mean	SD	Mean	SD	*p*
Physiological						
SBP	Peak	29.50	9.69	33.30	4.13	N.S.
	AUC	49.35	24.28	72.96	22.12	0.029
	T-C				2.3 h; 3.5 h	<0.001
DBP	Peak	11.85	13.83	18.90	12.33	N.S.
	AUC	18.81	21.09	46.82	27.82	0.015
	T-C				2.3; 3.5 h; 5 h; 6 h	<0.001
HR	Peak	33.50	11.87	33.05	13.25	N.S.
	AUC	57.75	30.11	69.98	40.02	N.S.
	T-C				2.3 h; 5 h	0.004
T	Peak	0.20	0.52	0.56	0.44	N.S
	AUC	0.50	0.86	1.61	1.30	N.S
	T-C				2.3 h; 2.6 h; 3 h	<0.001
PD	Peak	3.39	0.58	3.74	0.87	N.S.
	AUC	9.42	1.83	11.92	3.33	0.037
	T-C				2.3 h; 2.6 h	<0.001
Psychomotor						
RT total	Peak	34.70	24.86	54.20	56.80	N.S.
	AUC	65.07	63.09	83.55	110.52	N.S.
	T-C				3 h	0.009
Subjective						
VAS—changes in colors	Peak	20.60	24.62	7.50	9.69	N.S.
	AUC	18.90	22.73	7.70	12.13	0.042
	T-C				none	0.044
ARCI-MBG	Peak	8.40	2.91	8.60	4.14	N.S.
	AUC	16.81	10.31	19.39	12.39	N.S.
	T-C				2.3 h	0.008
ARCI-LSD	Peak	3.50	2.64	4.90	0.99	N.S.
	AUC	5.23	4.79	8.99	4.25	0.007
	T-C				2.3 h	<0.001
ARCI-A	Peak	5.60	1.90	5.30	2.50	N.S.
	AUC	10.89	5.20	12.26	8.99	N.S.
	T-C				2.3 h	0.017

AUC area under the curve, 2–6 h, *Peak* peak effects from 2 to 6 h, *T-C* time course from 2 to 6 h indicating time points when conditions were statistically significant, *M100* single dose of 100 mg (±)-3-4-methylenedioxymethamphetamine, *M50+100* repeated dose of 50 mg and 2 h later 100 mg (±)-3-4-methylenedioxymethamphetamine, *SD* standard deviation, *p* statistical significance level obtained with ANOVA, *N.S.* not significant

MDMA single dose versus repeated doses administered 2 h apart

Physiological measures

The magnitude of MDMA-induced effects on DBP, SBP, and HR and their duration were related to the dose administered (Fig. 1; Table 1). MDMA induced hypertensive effects and sinus tachycardia that peaked within 1 h and returned to baseline after 3–5 h from drug ingestion. The average increases in peak effects for M100 and M50+100 were 29.5 and 33.3 mmHg for SBP, 11.9 and 18.9 mmHg for DBP, and 33.5 and 33.1 for HR, respectively. Increases in blood pressure were statistically significant when comparing M100 and M50+100 AUC and at some points of the time course after the second MDMA administration (SBP at 2.3 and 3.5 h; DBP at 2.3, 3.5, 5, and 6 h). The diagnostic criteria for hypertension (SBP/DBP>140/90) were met in the ten subjects (100 %). Increases in HR were statistically significant at 2.3 and 5 h of the time course.

Oral temperature increased at 1 and 2 h after MDMA repeated administration. Increases in oral temperature were statistically significant at some time points of the time course (at 2.3, 2.6, and 3 h).

Maximal pupil diameter (PD) increase was observed at 2 h and remained at 6 h after MDMA second dose. The mean peak increase was 3.4 mm (M100) and 3.7 mm (M50+100), and statistically significant differences between

Fig. 1 Time course of pharmacological effects following two repeated doses of 50 and 100 mg MDMA over a period of 6 h ($n=10$, mean, standard error). Placebo and 100 mg MDMA (*unfilled diamond*, P+M100) or 50 +100 mg MDMA (*filled square*, M50+100)
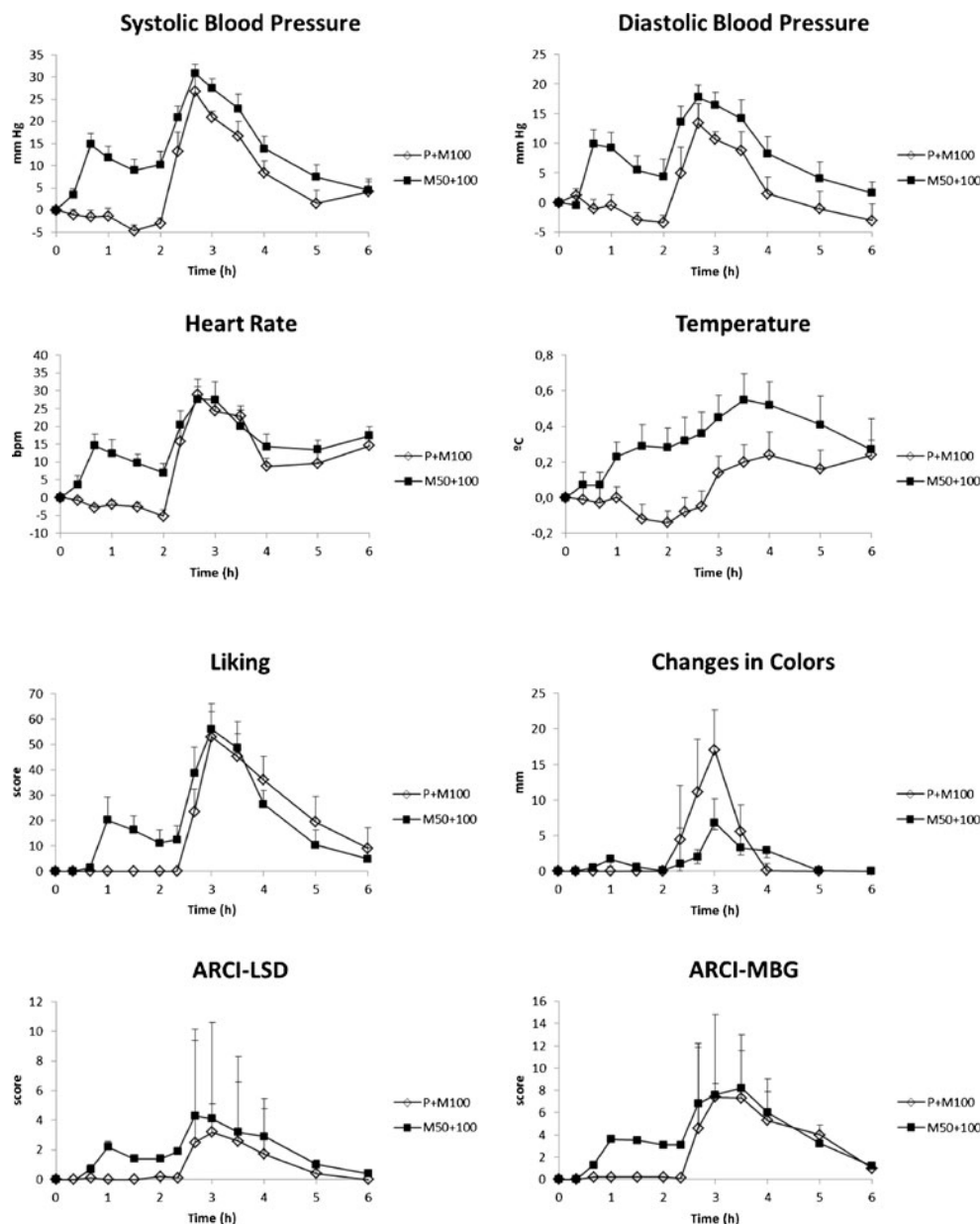
single and repeated doses were observed in the AUC and at some time points (2.3 and 2.6 h of the time course) after drug administration.

Psychomotor performance

No differences were observed in peak or AUC in any performance test (Table 1). Total RT increased significantly after repeated doses, with peak increases of 34.7 ms (M100) and 54.2 ms (M50+100) and differences at 3 h of time course.

Subjective effects

MDMA repeated doses produced an increase in the scores of most subjective effects in comparison to the single dose

(Fig. 1; Table 1). In general, the subjective effects reached their maximum between 1 and 2 h and returned to baseline at 4 h after drug repeated administration. This increase was statistically significant in the scores of some scales such as "changes in colors" (AUC), ARCI-MBG (euphoria) (at 2.3 h of time course), ARCI-LSD (dysphoria) (at 2.3 h of time course and AUC), and ARCI-A (amphetamine-like) (at 2.3 h) in comparison with single dose (M100). No statistically significant changes were observed in other VAS or the ARCI-PCAG (sedation) or ARCI-BG (stimulant) scores.

No hallucinations, psychotic episodes, or serious adverse reactions were observed among subjects during the study (including hyperthermia, moderate/severe hypertension, or tachycardia). None of the participants required specific

Table 2 Pharmacokinetic results ($n=10$, mean, standard deviation) after a single [M100] vs. a repeated MDMA dose [M50+100] administration

Variable	M100		M50+100	
	Mean	SD	Mean	SD
Pharmacokinetic parameters				
λ_z, h^{-1}	0.07	0.02	0.07	0.01
$t_{1/2}$, h	10.41	3.55	10.48	2.12
t_{max}, h	3.75		3.25	
C_{max}, µg/L	202.92	30.30	311.16***	66.48
CL (L/h)	34.60	13.66	33.42	10.58
V_z, L	480.82	116.88	489.00	103.00
$AUC_{(0-2)}$, gh/L	–	–	101.86	25.15
$AUC_{(2-50)}$, µgh/L	2,890.73	605.59	4,426.60	945.11
$AUC_{(0-t)}$, µgh/L	2,949.74	637.30	446.88*	958.90
$AUC_{(0-\infty)}$, µgh/L	3,162.91	818.19	4,781.13**	1,062.34
$AUC_{(\%Extrap)}$, %	14.42	9.11	12.81	5.00
$AUC_{(0-\infty)\ 50*}$, µgh/L	–	–	1,414.28	324.34
$AUC_{(0-\infty)\ 50*}/AUC_{(0-\infty)\ M50+100}$, %	–	–	0.30	0.01
$AUC_{(0-\infty)\ M100}/AUC_{(0-\infty)\ M50+100}$, %	–	–	0.67	0.13

λ_z first order elimination constant, $t_{1/2}$ half-life, C_{max} maximum plasma concentration, CL plasmatic clearance, V_z apparent volume of distribution, $AUC_{(0-2)}$ area under the curve from 0 to 2 h, $AUC_{(2-50)}$ area under the curve from 2 to 50 h, $AUC_{(0-t)}$ area under the curve from 0 to the last quantified concentration at time t, $AUC_{(0-\infty)}$ area under the curve from 0 to infinity, $AUC_{(\%Extrap)}$ area under the curve extrapolated, $AUC_{(0-\infty)\ 50*}$ area under the curve from 0 to infinity corresponding to 50 mg dose, $AUC_{(0-\infty)\ 50*}/AUC_{(0-\infty)\ M50+100}$ contribution of 50 mg to the total MDMA exposure after M50+100 intake—shown as mean and SD values, $AUC_{(0-\infty)\ M100}/AUC_{(0-\infty)\ M50+100}$ contribution of 100 mg to the total MDMA exposure after the intake of M50+100 —shown as mean and SD values. T_{max} is shown as a median value. The pharmacokinetic parameters were obtained by non-compartmental analysis except for $AUC_{(0-\infty)\ 50*}$ where individual data were fitted to a monocompartmental open pharmacokinetic model

*$p<0.05$; **$p<0.005$; ***$p<0.001$

therapy or special care during the experimental sessions and all of them completed the study.

Pharmacokinetics

All subjects presented quantifiable concentrations of MDMA at 2 h after the administration of the first 50-mg dose (Fig. 2), with a mean C_{max} of 84.71 ng/mL and a mean concentration at 2 h (C_{min}) of 79.92 ng/mL. MDMA and MDA concentrations (C_{max} and AUC) after the second dose (M50+100) were higher than that observed after the 100-mg single dose (M100). No changes were observed in the elimination half-life, T_{max}, volume of distribution, and plasmatic clearance of

MDMA between both experimental conditions (see Table 2). The concentrations following the second dose were higher than expected if we consider the proportionality in the exposition, obtained by fitting the plasma MDMA concentrations by a one-compartmental open pharmacokinetic model (see Table 3) in terms of C_{max} (+12.8 %) and AUC (+16.2 %).

Following the second MDMA dose, HMMA plasma concentration decreased by 29.8 % (AUC) and 38.2 % (C_{max}) from that expected by simple dose proportionality. The plasma concentrations of HMA and MDA showed the same trend. A significant decrease in all metabolic ratios was found except for the metabolic ratio MDA/MDMA (see Table 3).

Discussion

This study provides, for the first time, valuable clinical information about the pharmacological effects of MDMA after two doses taken 2 h apart as practiced in recreational settings. Our results have implications on MDMA adverse effects because, after repeated doses, some effects (e.g., euphoria, liking, HR) are lower than expected following drug plasma concentrations and doses administered, while others may increase more as expected in a dose concentration-dependent manner (e.g., SBP, DBP, T, and pupil diameter).

Pharmacological effects

MDMA produced its prototypical effects (increased blood pressure, heart rate, temperature, and pupil diameter) (Mas et al. 1999; Lester et al. 2000; de la Torre et al. 2000b; Hernández-López et al. 2002; Farré et al. 2004; 2007) due to its sympathomimetic properties and interaction with serotonergic system (Liechti et al. 2000a; 2000b; Hysek et al. 2011). Impairments on psychomotor performance were higher after MDMA repeated doses, evidencing a dose-dependent influence. This is in agreement with reports of difficulties in coordination, concentration, impairment of performance, and divided attention (Camí et al. 2000; Kuypers et al. 2007). In relation to subjective effects, MDMA induce a state of well-being, euphoric-like feelings, stimulation plus mild changes in perceptions, and some degree of dysphoria without illusions or hallucinations (Camí et al. 2000; Liecthi and Vollenweider 2000; Tancer and Johanson 2001).

Pharmacokinetics

MDMA and MDA plasma concentrations observed after the second dose can be explained considering dose proportionality. Following the repeated MDMA administration (M50+100), C_{max} (311.16 ng/ml) was in the range of those observed by two 100 mg doses (232 ng/ml) taken 24 h apart after a single dose

Table 3 Exposition and metabolic rates of MDMA and metabolites (HMMA, HMA, and MDA) results (mean, standard deviation) after a single [M100] vs. a repeated MDMA dose [M50+100] administration ($n=10$)

Variable	M100 Mean	SD	M50+100 Mean	SD	p	M50+100 vs M100 Increment×times
Exposition (AUC$_{0-t}$)						
MDMA, µgh/L	2,949.74	637.30	4,460.88	958.90	0.00001	1.5
MDA, µgh/L	288.56	75.14	462.02	43.45	0.000004	1.6
HMMA, µgh/L	129.20	33.94	163.58	66.58	0.034	1.3
HMA, µgh/L	2,365.09	768.52	2,897.47	996.23	0.005	1.2
HMMA + MHA + MDA, µgh/L	2,782.85	777.19	3,523.07	1,017.87	0.001	1.3
HMMA + HMA, µgh/L	2,494.29	795.46	3,061.05	1,058.15	0.006	1.2
Metabolic ratios						
MDA/MDMA	0.10	0.02	0.1	0.02	N.S.	1
MDA + HMA + HMMA/MDMA	1.05	0.51	0.85	0.41	0.002	0.81
HMMA + HMA/MDA + MDMA	0.86	0.45	0.68	0.37	0.001	0.79
HMA + HMMA/MDMA	0.94	0.51	0.75	0.41	0.0021	0.79

AUC area under the curve (0–th), *M100* single dose of 100 mg (±)-3-4-methylenedioxymethamphetamine, *M50+ 100* repeated dose of 50 mg and at 2 h later 100 mg (±)-3-4-methylenedioxymethamphetamine, *MDA* 3,4-methylenedioxyamphetamine, *HMA* 4-hydroxy-3-methoxyamphetamine, *HMMA* 4-hydroxy-3-methoxymethamphetamine, *p* statistical significance level obtained with ANOVA, *N.S.* not significant

of 125 mg (229 ng/ml) and 150 mg (465 ng/ml) (de la Torre et al. 2000a; 2000b; Farré et al. 2004). The increase of MDA could be related to a higher availability of MDMA as substrate and to the mechanism-based autoinhibition of CYP2D6 that regulates the formation of 3,4-dihydroxyamphetamine from MDA. However, the drastic reduction in HMMA and metabolic ratio significant differences is explained by a MDMA-induced inhibition of CYP2D6 (de la Torre et al. 2000a; Farré et al. 2004; Yang et al. 2006; Yubero-Lahoz et al. 2011). This

point is pharmacologically relevant since the metabolic disposition of several relevant drugs (e.g., opiates, SSRIs, antiarrythmics) are regulated by the same enzyme. Our results show that a low dose of 50 mg is able to inhibit the metabolism of the next 100 mg dose at 2 h later. In comparison with the previous study with two 100 mg doses administered at 24-h interval (Farré et al. 2004), the increase in MDMA concentrations was higher than in this study, probably related to the dose and interval differences.

Fig. 2 Plasma concentration over time curves of MDMA and metabolites MDA, HMMA, and HMA following two repeated doses M50+100 of MDMA over a period of 6 h ($n=10$, mean, standard error). Placebo and 100 mg MDMA (*unfilled diamond*, P+M100) or 50 +100 mg MDMA (*filled square*, M50+100)
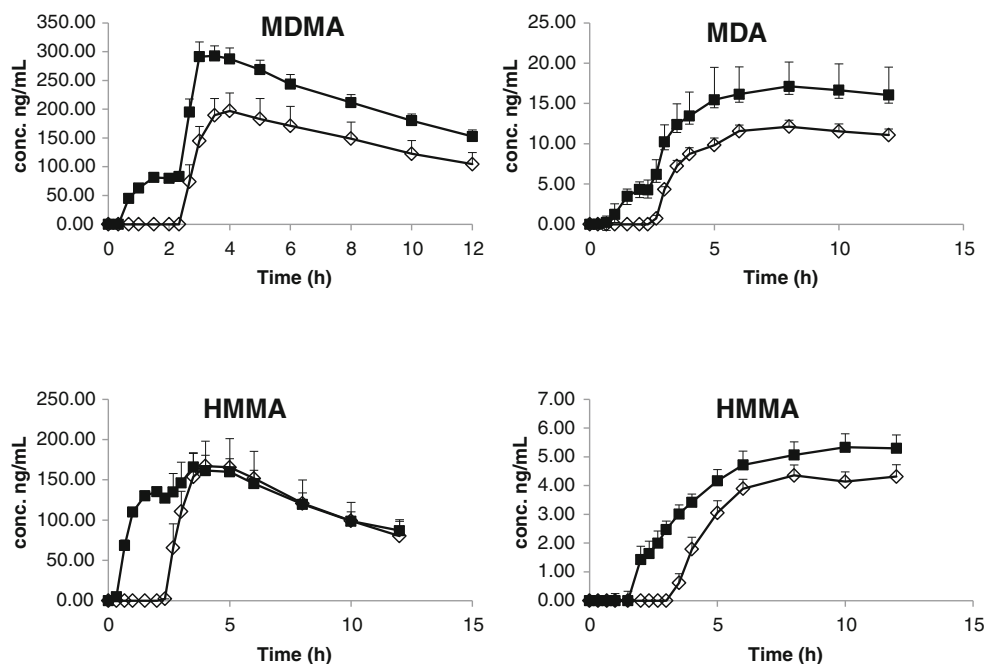
Pharmacological effects in relation to pharmacokinetics

After a single dose administration, earlier MDMA effects show a relationship with plasma MDMA concentrations (Mas et al. 1999; Camí et al. 2000; de la Torre et al. 2000b; Hernández-López et al. 2002; Farré et al. 2004; 2007). Taking into account that MDMA concentrations increased approximately 50 % after the second administration (C_{max}, 202.92 to 311.16 ng/mL), most of the pharmacological effects observed were in the range or lower than expected. This phenomenon could indicate some degree of adaptation or possible tolerance which seems especially obvious in pleasant effects such as euphoria and stimulation as has been described by many recreational users (Hammersley et al. 1999; Verheyden et al. 2003; Parrott 2005). In other laboratory studies (Hysek et al. 2011; Hysek et al. 2012), pharmacological effects lower than expected based on plasma exposure have been described. Tolerance is possibly linked to acute neurotransmitter depletion. In comparison with the previous study with two 100 mg doses administered at 24-h interval (Farré et al. 2004), the results were similar; the pharmacological effects after the second administration were higher than those following the first but lower than expected.

In contrast, the effects were in the range of those expected in (1) DBP (SBP/DBP mean increase ranged between 67 and 109 %) and (2) body temperature (sustained increase of 0.3–0.7 °C). We did not evidence hyperthermia, but an increase in oral temperature was observed earlier after the repeated MDMA administration. Our results in body temperature agree with previous studies of MDMA acute administration. To date, only marginal increases in body temperature have been observed in laboratory settings (Mas et al. 1999; de la Torre et al. 2000b; Hernández-López et al. 2002; Farré et al. 2004). Nevertheless, higher alterations have been reported in acute intoxications (Greene et al. 2003) and animal models (e.g., rhesus macaques models; Crean et al. 2006; Von Huben et al. 2007). Much of the concern is that MDMA use in certain environmental conditions (room temperature, poor ventilation, heightened physical activity, limited water consumption, and frequency of administration) could be more relevant than its simple use (O'Connor 1994; Greene et al. 2003; Freedman et al. 2005; Von Huben et al. 2007). Also, in several animal models, serotonergic neurotoxicity may affect the hyperthermic response of subsequent doses which occurs independently of increased muscular activity (Green et al. 2004b; Mills et al. 2004).

Few controlled studies in humans have provided evidence of the phenomenon of sensitization ("kindling-like" phenomena) in cardiovascular effects (Walsh et al. 2000; Kollins and Rush 2002; Cleary and Docherty 2003; Farré et al. 2004). This was not observed in the present study where the effects were in line with plasma concentrations or lower than expected.

Limitations of the study

The lower effect found in some variables may be due the tests used (e.g., many of the ARCI parameters are at the upper scale limit score being unable to detect increases), the ceiling effect achieved in some variables (e.g., PD or HR), and a sample size that may limit the statistical power to show differences. Also, laboratory setting is very different from recreational conditions; MDMA doses were administered at different times of day and recreational individuals might use MDMA in a different temporal pattern and/or dose levels (e.g., our volunteers did not experience "come down"). In relation to the doses administered, we selected two different doses for the study (50 and 100 mg) for safety reasons, but probably the administration of a single 150 mg or two equal doses (50 plus 50 mg; 100 plus 100 mg) could permit a more precise comparison. The study is also limited by the fact that we compared the effect of the second dose of MDMA at a time when the effect of the first low dose was still present, adding the exposure or dynamic changes of the second dose. An interval of 6–8 h between doses would have allowed the pharmacodynamic effects to return to baseline before the next administration of MDMA. However, this dose interval would not reflect recreational use patterns.

In conclusion, MDMA given at repeated doses 2 h apart in a laboratory setting autoinhibits its own metabolism. The pharmacological effects after the second administration in blood pressure and temperature were slightly higher than those following the first, but for heart rate and subjective effects these were lower than expected considering the MDMA concentrations achieved, suggesting acute pharmacological tolerance. In recreational settings, if individuals attempt to maintain a certain level of euphoria by repeated MDMA use, they may be at an increased risk for cardiovascular toxicity and increased body temperature.

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