Non-linear pharmacokinetics of MDMA ('ecstasy') in humans

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Aims 3,4-Methylenedioxymethamphetamine (MDMA, commonly called ecstasy) is a synthetic compound increasingly popular as a recreational drug. Little is known about its pharmacology, including its metabolism and pharmacokinetics, in humans in controlled settings. A clinical trial was designed for the evaluation of MDMA pharmacological effects and pharmacokinetics in healthy volunteers.

Methods A total of 14 subjects were included. In the pilot phase six received MDMA at 50 ($n=2$), 100 ($n=2$), and 150 mg ($n=2$). In the second phase eight received MDMA at both 75 and 125 mg ($n=8$). Subjects were phenotyped for CYP2D6 activity and were classified as extensive metabolizers for substrates, such as MDMA, whose hepatic metabolism is regulated by this enzyme. Plasma and urine samples were collected throughout the study for the evaluation of MDMA pharmacokinetics. Body fluids were analysed for the determination of MDMA and its main metabolites 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxy-methamphetamine (HMMA) and 4-hydroxy-3-methoxy-amphetamine (HMA).

Results As the dose of MDMA administered was increased, volunteers showed rises in MDMA concentrations that did not follow the same proportionality which could be indicative of nonlinearity. In the full range of doses tested the constant recovery of HMMA in the urine combined with the increasing MDMA recovery seems to point towards a saturation or an inhibition of MDMA metabolism (the demethylenation step). These observations are further supported by the fact that urinary clearance was rather constant while nonrenal clearance was dose dependent.

Conclusions It has previously been postulated that individuals genetically deficient for the hepatic enzyme CYP2D6 (about 10% of the Caucasian people) were at risk of developing acute toxicity at moderate doses of MDMA because the drug would accumulate in the body instead of being metabolized and inactivated. The lack of linearity of MDMA pharmacokinetics (in a window of doses compatible with its recreational use) is a more general phenomenon as it concerns the whole population independent of their CYP2D6 genotype. It implies that relatively small increases in the dose of MDMA ingested are translated to disproportionate rises in MDMA plasma concentrations and hence subjects are more prone to develop acute toxicity.

Keywords: ecstasy, humans, MDMA, pharmacokinetics

Introduction

3,4-Methylenedioxymethamphetamine (MDMA, commonly called ecstasy) is a synthetic compound that has become increasingly popular as a recreational drug amongst young people due to its entactogen (euphoria, friendliness, closeness, empathy) properties [1]. Recent reports have drawn attention to toxicity and deaths associated with MDMA use [2]. Neurodegenerative effects on the central serotonergic system observed in animal models after MDMA exposure have been postulated as clinically relevant long-term forms of toxicity [3–5]. However, little is known about MDMA pharmacology in humans as only a clinical trial in healthy volunteers given low doses of MDMA (as compared with those normally ingested for recreational purposes), and

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Received 13 January 1999, accepted 26 October 1999.

three pharmacokinetic studies in a controlled setting that included four subjects have been published to date [6–9].

The metabolism of MDMA involves *N*-demethylation to 3,4-methylenedioxyamphetamine (MDA) (Figure 1). The parent compound and MDA are further *O*-demethylenated to 3,4-dihydroxymethamphetamine (HHMA) and 3,4-dihydroxyamphetamine (HHA), respectively. Both HHMA and HHA are subsequently *O*-methylated by catechol-*O*-methyltransferase (COMT) mainly to 4-hydroxy-3-methoxy-methamphetamine (HMMA) and 4-hydroxy-3-methoxy-amphetamine (HMA). These four metabolites, particularly HMMA and HMA, are known to be excreted in the urine as conjugated glucuronide or sulphate metabolites but data on quantitative recovery of the metabolites and the potential relationship with the administered dose are scarce [10]. CYP2D6 as for other oxidative metabolic reactions in the phenylalkylamines series, regulates the demethylenation of MDMA [11]. This enzyme shows a genetic polymorphism causing variable metabolism for cosegregating substrates.

During an extensive study on MDMA pharmacology in healthy volunteers [12, 13], an unexpected observation was that data evaluation of disposition suggested nonlinear pharmacokinetics of MDMA. This has been further investigated in the present study.

Methods

Patients and study design

The recreational use of MDMA on at least five occasions was required in subjects willing to participate in the study. Male subjects were recruited by 'word of mouth' and eligible subjects were interviewed to exclude concomitant underlying conditions. The presence of a major psychiatric disorder, assessed by means of the structured clinical interview for DSM-IV, was an exclusion criterion. Each subject underwent a physical examination, routine blood and urine laboratory testing and 12-lead electrocardiogram (ECG). A total of 14 volunteers were included, six in the pilot phase and eight in the final study. The mean age was 26.5 years (range 21–31 years), mean weight of 74.4 kg (range 66–83 kg), and mean height of 178 cm (range 169 to 186 cm). All but two subjects were current smokers. Their average consumption of alcohol was 2 units/day (1 unit = 8 g ethanol), and all of them had previous experience with cannabis, cocaine, and

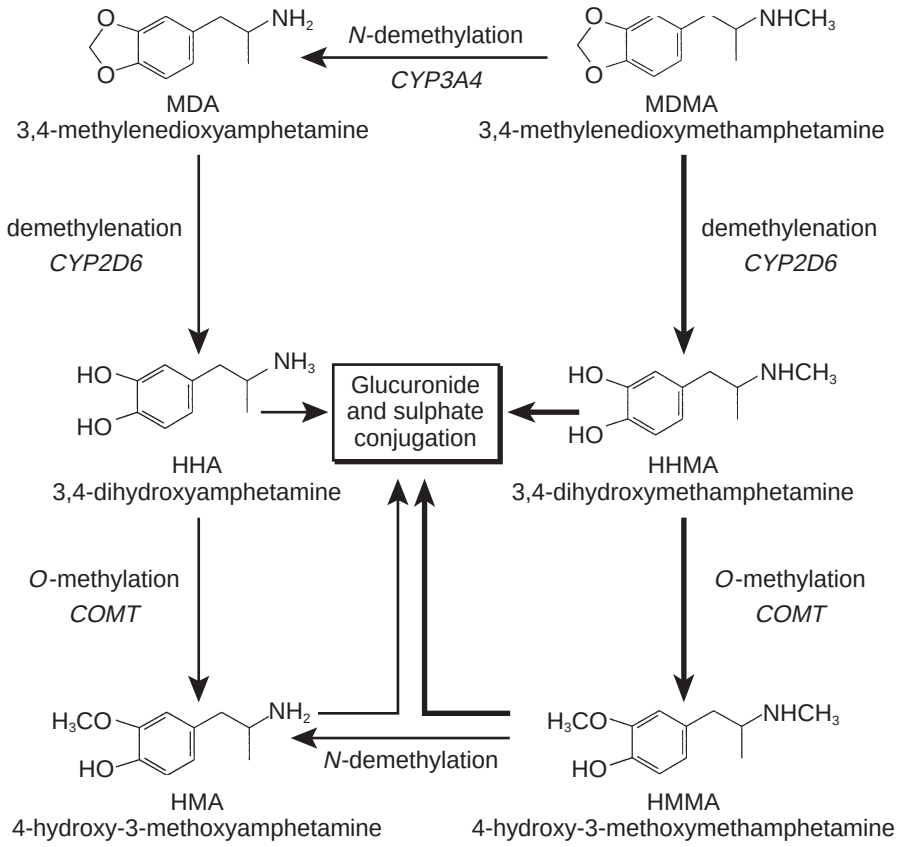

Figure 1 Metabolism of 3,4-methylenedioxymethamphetamine.

methamphetamine consumption. None had a history of abuse or drug dependence according to DSM-IV criteria (except for nicotine dependence), nor any medical or psychiatric adverse reaction after MDMA consumption.

All volunteers gave their written informed consent and were economically compensated for inconveniences caused by their participation in the study. The present study was performed in accordance with the Declaration of Helsinki and after completing the following steps: (1) peer review and approval of the clinical trial protocol by the institutional ethical committee (CEIC-IMAS); (2) peer review, authorization, and grant provision by public authorities for funding medical research in Spain (Fondo de Investigaciones Sanitarias, FIS); and (3) peer review and authorization by the General Directorate of Pharmacy and Health Products (DGFPS no. 95/297) of the Spanish Ministry of Health that procured also the MDMA (racemate) to be administered in the trial.

In a preliminary phase six healthy volunteers received 50, 100 and 150 mg of MDMA (two subjects for each dose) to determine the subsequent doses of MDMA to be used in the definitive trial. The main criteria for the selection of doses included tolerance to the dose given and sensitivity to a battery of tests regarding subjective effects and psychomotor performance. It appeared that 50 mg was a quite low dose and that volunteers given 150 mg experienced several side-effects (cardiovascular, subjective effects) that prevented the use of this dose, so that 75 and 125 mg were the doses finally selected. In the second phase, eight subjects participated in a double-blind, randomised, crossover and controlled clinical study. Treatment conditions (MDMA 75 mg, MDMA 125 mg, (±)-amphetamine 40 mg and placebo) were randomly assigned using a balanced 4×4 latin-square design. Subjects were phenotyped for CYP2D6 activity using dextrometorphan as a probe drug [14] and the dextrometorphan/dextrorphan urinary metabolic ratio allowed all the subjects to be classified as extensive metabolizers. Psychomotor performance tasks, subjective effects and physiological variables (heart rate, blood pressure, oral temperature, pupillary diameter and ECG continuous monitoring) were measured.

An indwelling catheter was inserted in a peripheral vein and a 0.9% sodium chloride solution was infused at a rate of 20 ml h^{-1}. Blood was collected at baseline and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 h after MDMA administration. Urine samples were collected in two periods (0–8 h and 8–24 h) for the preliminary phase and at three different periods (0–4 h, 4–8 h and 8–24 h) in the second phase (doses of 75 and 125 mg). Urine was stored at -20° C until analysis. Blank urine samples were collected before drug administration to verify the absence of MDMA metabolites.

Analytical methods

Urine and plasma samples were analysed following a previously reported method based on solid-liquid extraction and gas chromatography separation with nitrogen specific detection. MDMA, MDA, HMMA and HMA, were measured in plasma and urine samples either directly or after hydrolysis of glucuronide conjugates [15].

Data analysis

Area under the plasma MDMA and metabolites concentration *vs* time curve (AUC(0,24)) was determined by the linear trapezoidal rule. AUC were extrapolated to infinity (AUC$(0,\infty)$) by adding the last quantifiable concentration divided by the elimination constant (λ_z). Calculations were performed using PKCALC software [16].

Results

An initial assessment of MDMA and MDA concentrations in plasma samples of the definitive study suggested a nonproportional dose-dependent kinetics of MDMA and its metabolite MDA (Table 1). A paired Student's *t*-test of normalized AUC$(0,\infty)$ (AUC/dose) obtained for MDMA 75 and 125 mg ($n=8$) showed statistically significant differences ($P<0.03$). When combining these results with those obtained in the pilot studies, it was observed that whilst the range of doses evaluated had a factor of 3 (50–150 mg), the area under the curve (AUC(0,24 h)) for MDMA showed a variation higher than 10 times. A non parametric comparison (Kruskal–Wallis) of normalized data to a 100 mg MDMA dose of AUC$(0,\infty)$ of the five doses assayed (50 $n=2$, 75 $n=8$, 100 $n=2$, 125 $n=8$ and 150 $n=2$) showed marginally significant differences amongst doses ($P<0.1$). A bioequivalence test comparing normalized AUC$(0,\infty)$ (AUC/dose) for 75 and 125 mg (same eight subjects for each dose level) showed that the 90% confidence interval of the AUC within subjects ratio was -0.3–89.3%. As standard bioequivalence tolerance intervals are set-up at $\pm 20\%$, one can consider that the 125 mg MDMA dose was not bioequivalent showing a higher bioavailability. MDMA plasma clearance has been evaluated and divided into the renal and the nonrenal components of this pharmacokinetic parameter (see Table 1). It is apparent that while urinary clearance was rather constant (an ANOVA analysis for all doses tested was non significant), nonrenal clearance was dose dependent (ANOVA analysis, $P<0.03$). These observations are of particular interest for those subjects (the same eight subjects) that received both doses of 75 and 125 mg MDMA. Non-renal clearance is reduced by one-half for the 125 mg dose, strongly

Table 1 Pharmacokinetic parameters for MDMA and MDA.

MDMA dose	C_{max} *(μg l^{-1})*	t_{max} *(h)*	*AUC (0,24 h) (μg l^{-1} h)*	*Normalized AUC*[1]$_e$	$t_{1/2}$ *(h)*	CL_T *(l h^{-1})*	CL_R *(l h^{-1})*	$CL_{non\text{-}R}$[4] *(l h^{-1})*
Dose 50 mg								
MDMA ($n=2$)	19.8–82.8	2–3	100.1–813.9	2.0–16.3	2.7–5.1	807.4–61.4	73.3–4.9	734.1–56.5
MDA ($n=1$[2])	5.1	6	51.1	1.0	5.6			
Dose 75 mg								
MDMA ($n=8$)								
Mean (s.d.)[3]	130.9 (38.6)	1.8 (0.4)	1331.5 (646.0)	17.8 (8.6)	7.7 (3.2)	86.9 (74.4)	12.8 (5.6)	74.0 (71.1)
MDA ($n=8$)								
Mean (s.d.)	7.8 (2.5)	5.1 (2.6)	122.3 (66.7)	1.6 (0.9)	16.1 (18.3)			
Dose 100 mg								
MDMA ($n=2$)	209.7–189.9	2–3	2256.6–1447.8	22.6–14.5	5.8–5.8	45.4–83.6	20.4–12.3	24.9–71.3
MDA ($n=2$)	22.4–14.2	6–4	345.4–61.5	3.4–0.6	6.3–6.4			
Dose 125 mg								
MDMA ($n=8$)								
Mean (s.d.)	236.4 (58.0)	2.4 (1.0)	2623.7 (572.9)	21.0 (4.6)	8.6 (3.2)	51.1 (14.1)	13.0 (5.4)	38.1 (13.3)
MDA ($n=8$)								
Mean (s.d.)	13.7 (1.6)	7.1 (2.8)	215.2 (68.5)	1.7 (0.5)	27.7 (26.0)			
Dose 150 mg								
MDMA ($n=2$)	441.9–486.9	1.5–2	5132.8–5232.0	34.2–34.9	6.9–7.2	29.2–26.3	5.2–11.3	24.0–15.0
MDA ($n=2$)	34.2–31.4	4–10	590.0–373.9	3.9–2.5	37.3–23.2			

[1]AUC (0,24 h) divided by dose administered.
[2]The MDA concentrations of the second volunteer were under the quantification limit.
[3]Mean ± s.d. (when $n=2$, individual values are reported)
[4]$CL_{non\text{-}R}$ refers to nonrenal clearance.

suggesting an impairment in the MDMA hepatic clearance.

Urine samples for the 14 participating subjects were also analysed for MDMA, MDA, HHMA and HMA. Urinary recoveries are shown in Table 2. Some other minor MDMA metabolites already described were detected, but were not relevant quantitatively. MDA and HMA appeared to be very minor metabolites of MDMA. Independent of the dose administered, about 50% of the dose was recovered in 24 h. However, it was noted that while the HMMA (mainly excreted as conjugated with glucuronic acid) total recovery was almost constant (around 100 μmol) for the whole range of doses studied the recovery of MDMA increased in a nonproportional dose–response pattern. Although there was a factor of 3 in the range of doses administered, recovery of MDMA was affected by a factor of nearly 20. Urinary pH and creatinine excretion rate were monitored in each urine collection period in order to exclude any bias induced by changes in the normal renal excretion of these compounds. No significant changes were observed along the study. Urinary pH (always lower than 6.8) varied in less than 0.3 units and maximal differences in total creatinine clearance were always lower than 0.25 l h^{-1}.

Plasma samples from five volunteers, one for each dose level, where re-analysed specifically for HMMA levels after enzymatic hydrolysis with -glucuronidase, as HMMA was absent in plasma in the nonconjugated fraction. Results are presented in Figures 2 and 3. Whilst HMMA was the major product in plasma after the administration of 50, 75 and 100 mg, the situation was the opposite when higher doses were given, with MDMA being predominant. This observation was based on AUCs and C_{max} at the five doses tested. A cardiovascular variable,

Table 2 Urinary excretion of MDMA and its main metabolites.

Dose		*Urinary recovery (μmol)*				
mg (μmol)	n	*MDMA*	*MDA*	*HMMA*	*HMA*	*Dose excreted (%)*
50 (259)	2	20.7–40.9	1.4–1.0	152.0–89.2	4.7–4.2	69.1–38.3
75 (388)	8	71.2 ± 13.7[1]	3.5 ± 0.9	128.3 ± 21.8	5.4 ± 0.4	53.7 ± 11.4
100 (518)	2	232.6 − 74.7	1.4 − 5.6	59.8 − 124.0	2.9 − 6.8	57.3 − 40.7
125 (647)	8	169.6 ± 69.5	6.4 ± 2.7	148.3 ± 102.8	6.2 ± 3.7	51.0 ± 16.2
150 (776)	2	160.3 − 333.3	2.6 − 4.7	122.2 − 82.4	4.1 − 3.7	37.3–54.7

[1]Mean ± s.d. (when $n=2$, individual values are reported)

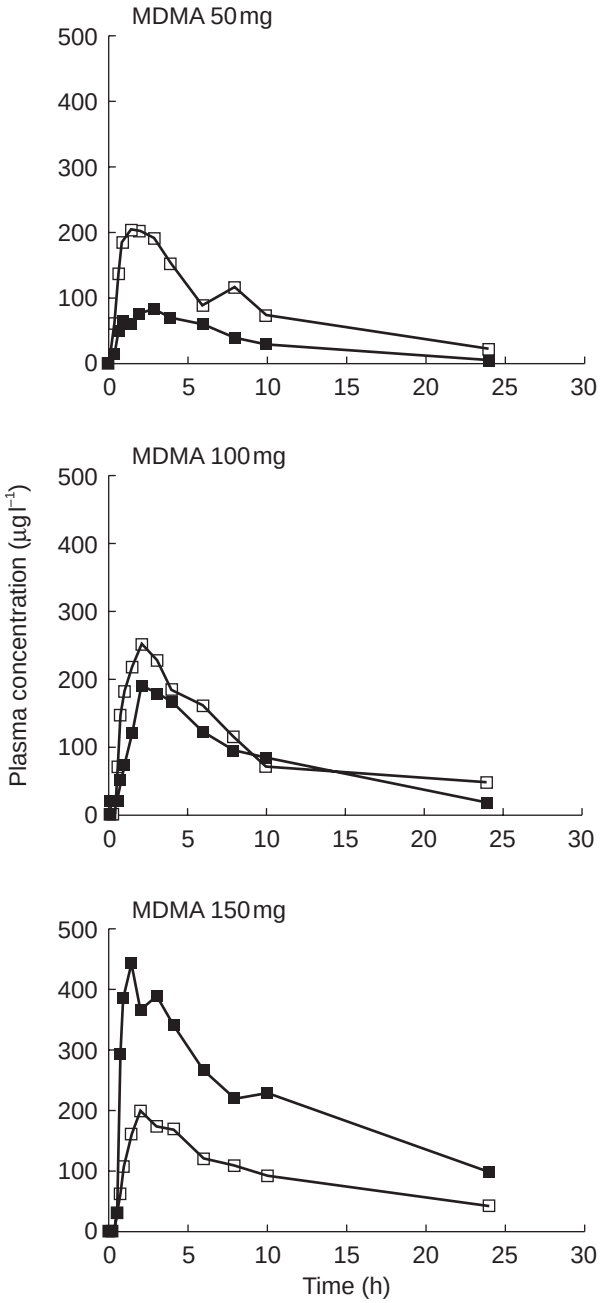

Figure 2 MDMA (■) and HMMA (□) plasma concentration *vs* time curve in three subjects administered 50 mg, 100 mg and 150 mg (one subject per dose).

such as diastolic blood pressure (DBP), supposed to be sensitive to MDMA plasma concentrations, was plotted against MDMA C_{max} for each dose evaluated. DBP showed a similar behaviour to the plasma concentrations of MDMA (Figure 3), with clear nonlinearity at the highest dose level. Taking into account the cardiovascular effects experienced by the volunteers at the dose of 150 mg, it was considered not ethically feasible to administer higher doses.

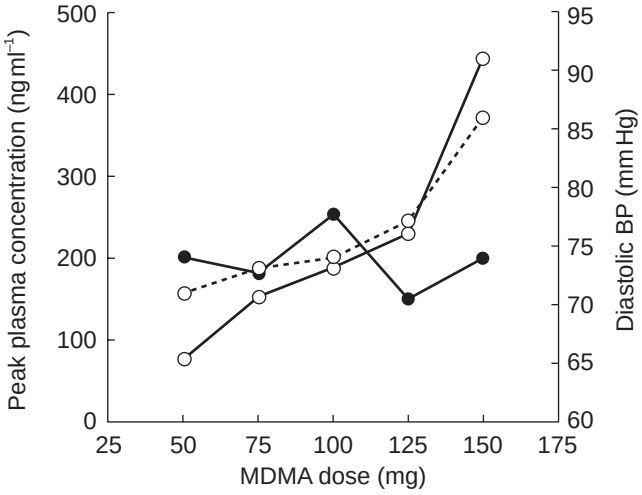

Figure 3 Peak plasma concentrations for MDMA (○) and HMMA (●) (C_{max}) and diastolic blood pressure values (DBP at the time of C_{max} , –o–) as a function of the dose administered in five subjects, one for each experimental point.

Discussion

The main finding of the present study is that as the MDMA dose is increased, the rise in MDMA concentrations does not follow the same proportionality, which could be indicative of nonlinearity. MDMA pharmacokinetic studies are scarce in the literature with data from only three volunteers (one from the study of Verebey and associates [7] and two from the study of Helmlin and coworkers [8]). In one of these volunteers [8] who received a dose of 135 mg, the C_{max} was 330 $\mu g\ l^{-1}$. This result is consistent with our findings of a C_{max} of 236.4 $\mu g\ l^{-1}$ for the dose of 125 mg dose and a C_{max} of 464.5 $\mu g\ l^{-1}$ for dose of 150 mg. The constant recovery of HMMA in the urine combined with the increasing MDMA accumulation seems to point towards an inhibition of MDMA demethylenation.

The possibility of a saturation of MDMA metabolism cannot be discarded as the simplest explanation of the observed phenomenon. Alternatively, a more complex explanation could involve an interaction of MDMA metabolites on some of its metabolic pathways. *In vitro* studies with cell cultures expressing human CYP2D6 suggested that the demethylenation of MDMA to 3,4-dihydroxymethamphetamine (HHMA) was regulated by this enzyme [11, 17]. Nevertheless, HHMA is nearly undetectable in biological fluids as it is quickly methylated to HMMA most probably by the COMT enzyme. *In vitro* data also suggest that MDMA, in addition to being a substrate, acts as an inhibitor of CYP2D6 activity. Mechanisms involved in such inhibitory activity most probably include not only a competitive interaction between MDMA and CYP2D6 probe substrates but also the formation of a metabolic complex with CYP2D6

[18]. In fact, when MDMA is preincubated with human microsomes before performing inhibition studies, there is an increase in the inhibitory capacity of MDMA (up to 95% of control activity at 1.5 μM MDMA final concentration). MDMA concentrations at which these observations were made are compatible with concentrations in body fluids *in vivo*. On the other hand, structural requirements of phenylisopropylamines for becoming substrates/inhibitors of CYP2D6 are quite well defined [17, 19]. In the methylendioxyamphetamines series, it is apparent that the maintenance of methoxy groups in position 3 and 4 confers to molecules an increased affinity for CYP2D6 as compared with their respective *O*-demethylated product (i.e. HMMA *vs* HHMA). It is conceivable that the nonlinearity in the MDMA pharmacokinetics is associated to an inhibition of its *O*-demethylenation with both MDMA and HMMA playing an important role.

Thus, our results seem to support nonlinear kinetics of MDMA in the range of doses usually taken by recreational abusers. However, due to the fact that the observations were made retrospectively and that the purpose of the clinical studies was not specifically orientated to the detection of the phenomenon described, one cannot exclude that observations may be biased by the fact that the same inviduals were not tested at each dose level. However, this observation is clinically relevant as some acute toxic effects reported, not only in overdose cases but after the ingestion of recreational doses, should be interpreted in the light of the dose-dependent kinetics of MDMA.

This study was supported by grants FIS 97/1198, CIRIT (1997SGR00077), ISCIII 97/4344 and Plan Nacional sobre Drogas, Madrid. We thank E. Menoyo and S. Poudevida for assistance in clinical studies, N. Pizarro for chemical analysis and M. Pulido for editing the manuscript and editorial assistance.

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