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Population Pharmacokinetics of 3,4-Methylenedioxymethamphetamine and Main Metabolites in Rats

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The pharmacokinetics of the recreational drug 3,4-methylenedioxymethamphetamine (MDMA) and its mains metabolites have never been modeled together. We therefore designed a model with which to analyze the pharmacokinetics of MDMA, 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxymethamphetamine (HMMA), and 4-hydroxy-3-methoxyamphetamine (HMA) and to test the effect of covariates like gender and body weight on the pharmacokinetics. Rats (18 males and 18 females) were given 1 mg/kg MDMA iv, and the concentrations of MDMA, MDA, and HMMA were measured by high-performance liquid chromatography-mass spectrometry. Another 30 rats (15 males) were given 1 mg/kg MDA, and MDA and HMA were measured. A population pharmacokinetic model was developed to describe the changes in MDMA, HMMA, MDA, and HMA concentrations over time and to estimate interanimal variability. The influence of gender was tested using a likelihood ratio test. Estimated exposures of males and females to MDMA and its metabolites were compared using the Wilcoxon non-parametric test. An integrated six-compartment model adequately described the data. MDMA (two compartments) was transformed irreversible to HMMA (one compartment) and MDA (two compartments), which then produced HMA (one compartment). All rate constants were first order. Females given MDMA had significantly smaller MDMA distribution volumes than males, and they converted less MDMA to MDA than did males. Our MDMA, MDA, HMA, and HMMA model is suitable for examining the relationship between drug concentrations and its pharmacological/toxicological effects. Male rats were exposed to significantly more MDA and HMA than were females, which could explain why males are more sensitive to MDMA toxic effects than females.

Key Words: **MDMA; MDA; HMA; HMMA; population pharmacokinetics; SD rats.**

3,4-Methylenedioxymethamphetamine (MDMA, commonly called ecstasy) is a synthetic compound that has become a popular recreational drug among young people due to its entactogen (euphoria, friendliness, closeness, and empathy) properties (Nichols, 1986). MDMA can produce severe adverse reactions in humans, such as tachycardia, life-threatening hyperthermia, high blood pressure, and neurotoxicity; numerous deaths have been associated with the short-term use of this drug (Green *et al*., 2003; McKenna, 2002). Although the parent compound is probably responsible for some of these adverse events, as it stimulates the acute release of serotonin (5-hydroxytryptamine [5-HT]) and dopamine, it is unlikely to be responsible for the neurotoxic effects. Thus, MDMA must be metabolized to produce the toxic effect (Bai *et al*., 1999; Hiramatsu *et al*., 1990; Lim and Foltz, 1991b; Miller and O'Callaghan, 1995; Schmidt and Taylor, 1988; Zhao *et al*., 1992).

The pharmacokinetics of MDMA is very complex; at least 14 *in vivo* metabolites have been identified (Banks *et al*., 2007; Cho *et al*., 1990; de la Torre and Farre, 2004; Mechan *et al*., 2006). MDMA metabolism includes three pathways (Fig. 1). One is its N-demethylation to 3,4-methylenedioxyamphetamine (MDA), involving cytochrome P450 (CYP) 1A2 and 2D1 in rats and CYP2B6, 1A2, and 2D6 in humans (de la Torre and Farre, 2004). The second is demethylenation to 3,4-dihydroxymethamphetamine (HHMA), and the third is ring hydroxylation to 2-hydroxy-4,5-(methylenedioxy)methamphetamine (6-OH-MDMA) (Lim and Foltz, 1988, 1991a,b; Tucker *et al*., 1994). MDMA is further *O*-demethylenated to HHMA and MDA to 3,4-dihydroxyamphetamine (HHA). These transformations involve CYP2D1 and 3A2 in rats and CYP2D6, 1A2, 2B6, and 3A4 in humans (de la Torre and Farre, 2004). Both HHMA and HHA are subsequently *O*-methylated by catechol-*O*-methyltransferase (COMT) mainly to 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA) (Fig. 1). These four metabolites, particularly HMMA and HMA, are excreted in the urine as glucuronide or sulfate conjugates.

Although the MDMA metabolic pathways have been well characterized in man and rat using *in vitro* metabolism studies, no pharmacokinetic model is presently available to describe the simultaneous changes in the concentrations of MDMA and its

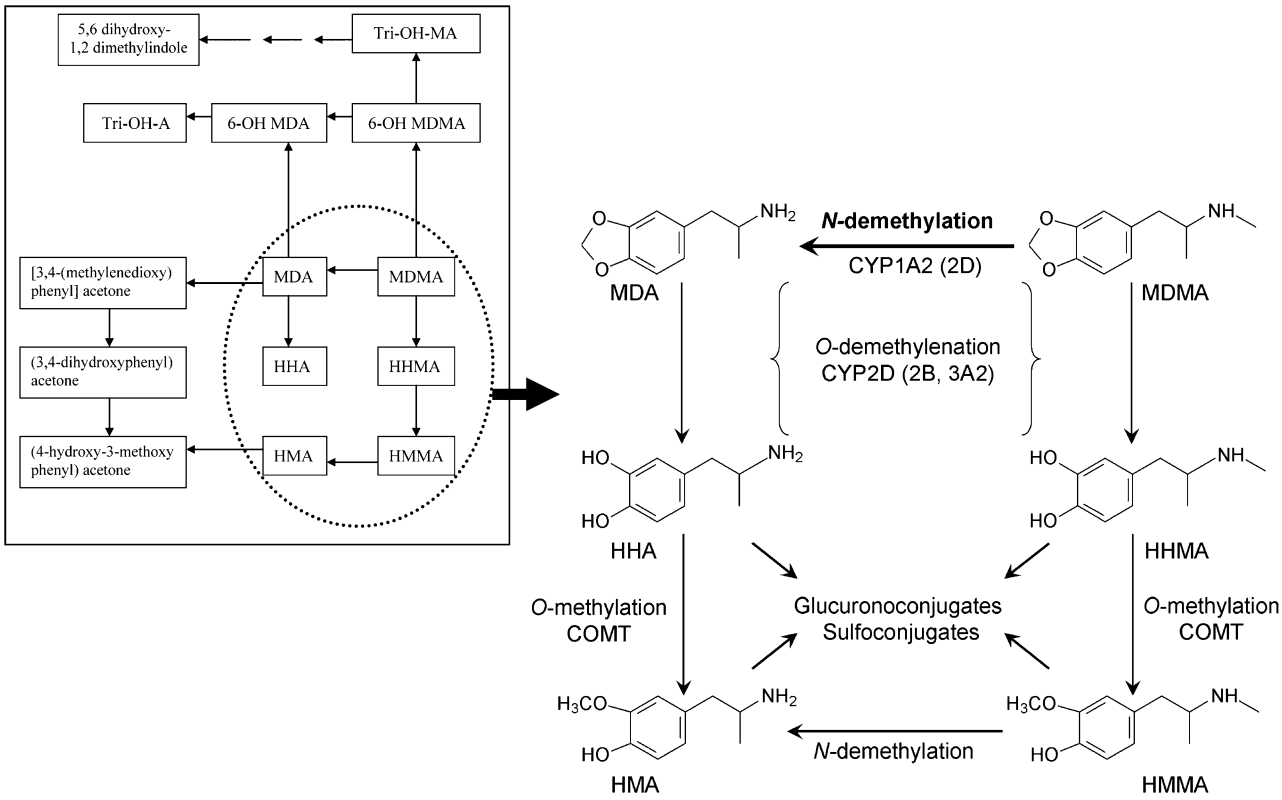

FIG. 1. Upper left: Postulated pathways of MDMA metabolism in rats, adapted from Green *et al*. (2003). MDMA and the 14 identified *in vivo* metabolites. Bottom right: only structures considered in this paper are shown. MDMA; MDA; HHMA; HHA; HMA, 4-hydroxy-3-methoxyamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine. CYP for cytochrome P450; COMT for catechol-*O*-methyltransferase; 6-OH-MDMA for 2-hydroxy-4,5-(methylenedioxy)metham; 6-OH-MDA for 2-hydroxy-4,5-(methylenedioxy)amphetamine; Tri-OH-MA for 2,4,5-trihydroxymethamphetamine; Tri-OH-A for 2,4,5-trihydroxyamphetamine. The major metabolic pathway is in bold. Minor enzymes are shown in brackets.

three main circulating metabolites MDA, HMMA, and HMA over time. Such a pharmacokinetic model is needed in order to establish the relationships between the pharmacokinetics and the pharmacodynamics (PD) of the drug. Indeed, PD analysis of a drug has limited utility unless it can be related to the concentration over time and total exposure profile of the drug in the recipient. When considering the dose-response relationship in the organism, pharmacokinetics (PK) offers, in the place of dose, a concentration profile, which provides a more accurate estimate of the amount of drug that is available to enter the tissues and, thus, interacts with the host at the level of the receptor. The resultant concentration or exposure profile for a given drug administered at a given dose provides a more suitable surrogate to explain interindividual variability in response than does dose alone.

This preclinical study provides a detailed analysis of the pharmacokinetics of MDMA and its metabolites in the rat blood plasma and evaluates the influence of covariates like gender and body weight on these pharmacokinetic parameters. The key tool used to analyze the fate of iv injected MDMA and MDA in male and female Sprague-Dawley (SD) rats was a population pharmacokinetic model. Conclusions were that an integrated six-compartment model adequately described the pharmacokinetics of MDMA and its metabolites MDA, HMMA, and HMA. HHMA and HHA, nearly undetectable in blood samples due to rapid methylation to HMA and HMMA, were not modeled. Females given MDMA had a significantly smaller MDMA distribution volume than males, and they converted less MDMA to MDA than did males. Male rats were thus exposed to significantly more metabolites MDA and HMA than were females.

MATERIALS AND METHODS

Experiments

Chemicals and Drugs

(+) and (−) HMA, (+) and (−) HMMA, (+) and (−) MDA, and (+) and (−) MDMA (99% pure) were synthesized as hydrochloride salts in the laboratory of Prof H. Galons (INSERM U648, Université Paris Descartes, Paris, France). d5-MDMA and d5-MDA (1 mg/ml in methanol) were obtained from Cerilliant (LGC Promochem, Molsheim, France). 3-Hydroxy,4-methoxyphenylethylamine (HMP) and xylazine hydrochloride were obtained from Sigma (Saint-Quentin Fallavier, France). Sodium pentobarbital and ketamine were purchased from Centravet (Nancy, France). All other chemicals used were of analytical grade, and the solvents used were of chromatographic grade.

Animals

Thirty-three male and 33 female SD rats (Janvier, Le Genest-St-Isles, France) weighing 310.1 ± 2.0 g (females) and 467.3 ± 4.2 g (males) (means ± SEM)

were housed in groups of four in an air-conditioned room (22 ± 1°C) with 12/12-h light/dark cycles (lights on 8:00 A.M.–8:00 P.M.) and acclimated to the laboratory for at least 1 week before experiments. Standard rat chow and water were available *ad libitum*. Animal experiments were carried out in compliance with the European Community Council (86/609/EEC) and French laws, with the standard ethical guidelines and under control of the faculty ethical committee.

Jugular Veins Catheterization

Catheters were inserted into the jugular veins of each rat (Thrivikraman *et al*., 2002). Briefly, the rat was placed in a chamber gassed with 5% isoflurane (AErrane; Bayer S.A., Lessines, Belgium) in oxygen (1 l/min) for 4 min and then quickly given an ip injection of ketamine/xylazine (80 and 10 mg/ml, 1 ml/kg). The depth of anesthesia was confirmed by the absence of a response to repeated toe pinching, and the dorsal and ventral cervical areas of the rat were shaved. Incisions were made dorsally and over the jugular vein. A catheter (Silastic: i.d. 0.51 mm; o.d. 0.94 mm; Dow Corning, Seneffe, Belgium), filled with sterile heparinized saline (20 U/ml), was tunneled under the skin from the back of the neck area to the jugular vein. The right and left veins were isolated and their distal ends tied. One catheter was inserted into the right vein and advanced into the atrium for blood sampling, and the other was used for drug injection. The neck wounds were then sutured, and 100 U of heparinized saline/kg body weight was injected iv.

Dosing and Blood Sampling

Because of the difficulties in handling rats after injection of (+) and (−) MDMA, we checked the effect of anesthesia (ketamine/xylazine 80/10 mg/kg ip and 1% isoflurane 0.4 l/min) on the metabolism of MDMA 45 min before its administration. Experiments comparing treated and untreated animals revealed no significant difference in the concentrations of MDMA and its metabolites. Subsequently, all rats were anesthetized with ketamine/xylazine, followed by 1% isoflurane (0.4 l/min), before injecting MDMA or MDA. The depth of anesthesia was assessed periodically by a negative toe-pinch response.

(+) and (−) MDMA or (+) and (−) MDA (1 mg/ml) were dissolved in sterile 0.9% saline and injected iv via the catheter in the right jugular vein. Blood samples were taken at 5, 10, 20, 30, 60, and 120 min after drug injection for the first animal and at 180, 240, 300, 360, and 420 min for the second. Three hundred and fifty microliters of blood was withdrawn into a heparinized syringe; the same volume of sterile heparinized saline (25 U/ml, 37°C) was then injected. The blood samples were centrifuged at 10,000 × g for 10 min at 4°C; 200-µl plasma samples were withdrawn and mixed with 5 µl 1 N HCl to minimize the breakdown of MDMA and its metabolites. Samples were immediately vortexed and stored at −30°C. The rats were killed at the end of the experiment by iv injection of 300 mg sodium pentobarbital. Each set of blood samples was taken from separate rats to avoid excessive blood loss (limit of 10% of the total blood volume in a single bleed), according to the ethical guidelines on blood collection in rodents (McGill University Animal Care Committee, 2005). Plasma concentrations of MDMA, MDA, and HMMA were collected in 36 rats (18 males and 18 females) given iv MDMA, and plasma concentrations of MDA and HMA were collected in another 30 rats (15 males and 15 females) given iv MDA.

Determination of MDMA and Its Metabolites

MDMA and its metabolite concentrations were determined by high-performance liquid chromatography with online mass spectrometry, as previously described (Fonsart *et al*., 2009).

The intra-assay precision (relative standard error [RSE]) of the method was 1–4.3%, and the accuracy (percent deviation from the nominal concentration [DEV]) was 0.9–3.7% ($n = 6$). The interassay precision (RSE) of the method was 1.5–12.6%, and the accuracy (DEV) was 0.2–7% ($n = 6$). These values are within the acceptable limits previously specified (Shah *et al*., 2000).

Population Pharmacokinetic Analysis

Data were analyzed using the nonlinear mixed effect modeling software program NONMEM (version VI, level 1.0) with the DIGITAL FORTRAN compiler (NONMEM). The first-order conditional estimation with interaction method was used. The pharmacokinetics of MDMA and its metabolites were studied sequentially. Rats were given 1 mg/kg MDA or MDMA, so a linear body weight scale was used for distribution volumes. The MDMA, MDA, HMA, and HMMA concentrations were measured in µg/l and transformed to µmol/l (dividing by the corresponding molecular weight) to estimate the real amounts transformed. Goodness of fit was evaluated by visual inspection. Observed concentrations against population predictions, weighted residuals against time, and weighted residuals against predictions were plotted in order to evaluate structural model. Observations should be homogeneously distributed around the identity line for the first graph and on both sides of the abscise axis for the others. Similar graphs using individual predictive *post hoc* estimation were displayed. Individual-weighted residuals against individual-predicted concentrations, evaluating residual error model, should be homogeneously distributed on both sides on the x-axis, with same amplitude for low and high concentrations.

The diagnostic graphs were prepared using RfN (S. Urien, RFN-831-20070911, https://sourceforge.net/project/showfiles.php?group_id=29501&package_id=140129&release_id=538680) with the R program (R).

Model I: MDMA-MDA-HMMA Pharmacokinetic Model Building

The first experiments examined the plasma concentrations of MDMA, MDA, and HMMA in rats (18 males and 18 females) given iv MDMA. A two-compartment model with first-order elimination best described the MDMA data. Two additional compartments were used for the MDA and HMMA concentrations. The distribution volumes of MDA (V_4) and HMMA (V_3) could not be identified because no urinary concentrations were available for these metabolites. The parameters of this model were therefore the MDMA distribution volume (V_1), the MDMA central-to-peripheral compartment rate constant (k_{12}), the MDMA peripheral-to-central compartment rate constant (k_{21}), the MDMA-to-MDA transformation (k_{14}/V_4), the MDA elimination rate constant (k_{40}), the MDMA-to-HMMA transformation (k_{13}/V_3), and the HMMA elimination rate constant (k_{30}) (Fig. 2). A first-order and nonlinear transfers were then tested for MDMA-to-MDA and for the MDMA-to-HMMA transformations.

Model II: MDA-HMA Pharmacokinetic Model Building

We next analyzed the concentrations of MDA and HMA in the blood plasma of rats (15 males and 15 females) given iv MDA and led to model II. A two-compartment model with first-order elimination best described the MDA data. An additional compartment was used for the HMA concentrations. The HMA distribution volumes (V_6) could not be identified. The model parameters were the MDA distribution volume (V_4), the MDA elimination rate constant (k_{40}), the MDA central-to-peripheral compartment rate constant (k_{45}), the MDA peripheral-to-central compartment rate constant (k_{54}), the MDA-to-HMA transformation (k_{46}/V_6), and the HMA elimination rate constant (k_{60}). First-order and nonlinear transfers were then tested for MDA direct elimination.

Model III: Integrated Model

All the data could be analyzed together, which allowed us to model the two compartments for MDA, to dissociate the MDMA-to-MDA transformation (k_{14}) from the MDA distribution volume (V_4) and to estimate the effect of gender on each of these parameters.

The random effects were estimated thanks to residual variabilities for each compound and intersubject variabilities on pharmacokinetic parameters when we could estimate it.

Residual variabilities link observed ($C_{i,j}$) to predicted concentrations ($f(t_{i,j},\theta_i)$) thanks to $C_{i,j} = f(t_{i,j},\theta_i) + \varepsilon_{i,j}$, θ_i is the vector of the logarithm of all the PK parameter of patient i, at sampling $t_{i,j}$. We assumed that $\varepsilon_{i,j}$ the errors for patient i at sampling j were independent and normally distributed with a null mean and an heteroscedastic variance $\sigma^2_{i,j}$. The variance can be modeled using different error models (e.g., mixte, multiplicative, and additive error models).

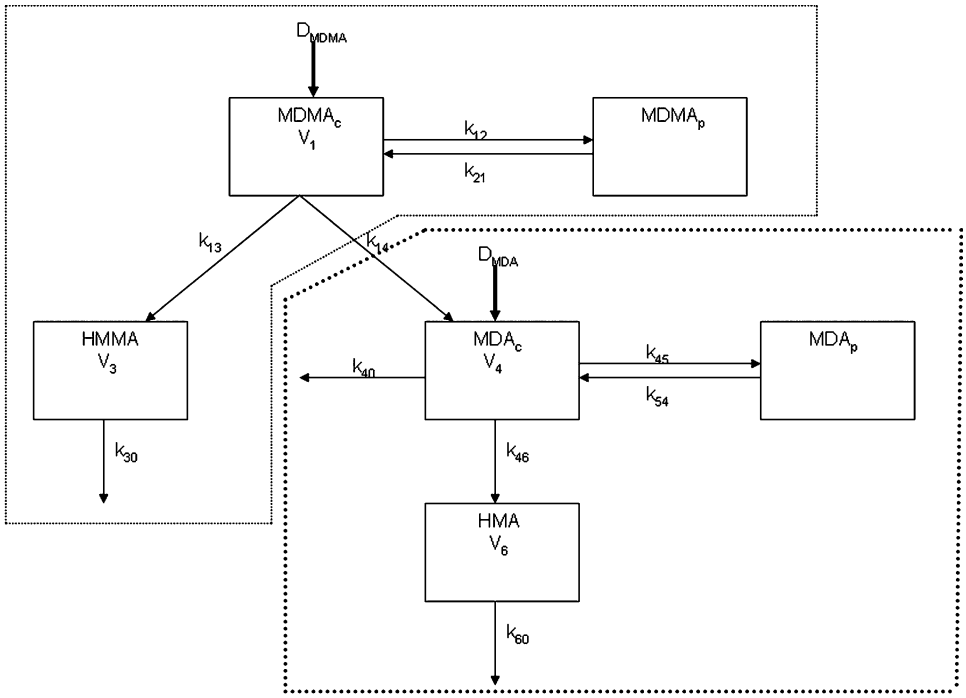

FIG. 2. Pharmacokinetic compartmental model for MDMA, MDA, HMA, and HMMA plasma concentrations in rats given an iv bolus dose of MDMA or a MDA (D_{MDMA} or D_{MDA}) (model III). MDMA (in compartments 1 and 2) is irreversibly transformed to HMMA (in compartment 3) and MDA (in compartments 4 and 5). MDA produces HMA (in compartment 6). The distribution volumes are V_1 for MDMA, V_3 for HMMA, V_4 for MDA, and V_6 for HMA. The elimination rate constants are k_{30} for HMMA, k_{40} for MDA, and k_{60} for HMA. Central-to-peripheral compartment rate constants are k_{12} for MDMA and k_{45} for MDA. Peripheral-to-central compartment rate constants are k_{21} for MDMA and k_{54} for MDA. The transformation rate constants are k_{13} from MDMA to HMMA, k_{14} from MDMA to MDA, and k_{46} from MDA to HMA. The thin dashed box corresponds to model I and the bold dash box to model II.

An exponential model was used for intersubject variability (ISV) of a pharmacokinetic parameter; e.g., the volume of distribution for a patient i: $V_i = \theta_V \times e^{\eta_{V_i}}$, where θ_V is the typical distribution volume and η_{V_i} the ISV expressed with an exponential model. η_{V_i} is assumed to be normally distributed with zero mean and a variance ω_V^2.

For each model, the objective function value (OFV) was computed as -2 times the log of the likelihood. Two nested models (e.g., a model with ISV on clearance and volume and model with only ISV on clearance or one vs. two compartments) could thus be compared by calculating the difference between their OFVs referred to as log-likelihood ratio; the log-likelihood ratio is assumed to be chi-squared distributed. When two models were not nested (e.g., a model with linear elimination and a model with nonlinear elimination), they were compared using the difference in Akaïke information criterias (AIC) computed as the difference between their OFV plus the difference between the numbers of the parameters estimated.

When the best structural and statistical model was found (lowest OFV or AIC, best visual, diagnostic graphs, and parameters correctly estimated), covariates were tested to improve the modeling. The effect of gender (SEX) and body weight (BW) was tested according to the following equation: using V, e.g., $V_i = \theta_V \times \left(\frac{BW_i}{\text{median (BW)}}\right)^{\beta} \times e^{\eta_{V_i}}$ or $V_i = \theta_V \times \beta^{SEX_i} \times e^{\eta_{V_i}}$, where β is the estimated influential factor for the body weight and sex (sex coded as 0 or 1). A covariate was retained if

- its effect was biologically plausible,
- it produced a minimum reduction of 6.63 U (log–likelihood ratio follows a $\chi^2_{1ddl,\alpha=0.01}$) in the OFV between the model without the covariate and the model with the covariate (nested models),
- it produced a reduction in the variance ω^2 of the ISV η_i of thepharmacokinetic parameter where the covariate was added.

An intermediate model with all significant covariates was obtained. A backward elimination phase was finally performed by deleting each covariate from the intermediate model, to obtain the final model, using also a likelihood ratio test.

Evaluation and Validation

Bootstrap evaluation. From the original data set of 66 rats, 1000 bootstrap sets of 66 rats were drawn with replacement (resampling). The precision and robustness of the final population model were assessed using a nonparametric bootstrap with 1000 replicates (without stratification by rat group) (Parke *et al.*, 1999).

Visual predictive check. Concentration profiles were simulated and compared with the observed data using a visual predictive check to validate the model. The vectors of pharmacokinetic parameters from 1000 animals were simulated using the final model. Each vector parameter was plotted in a log-normal distribution with a variance corresponding to the ISV previously estimated. A simulated residual error was added to each simulated concentration. The simulations were performed using NONMEM. The 5th, 50th, and 95th percentiles of the simulated concentrations at each time were then overlaid on the observed concentration data using the R program and the relationship inspected visually.

Gender Differences in Exposure to MDMA, MDA, HMMA, and HMA

The areas under the curves (AUCs) for MDMA, MDA, and HMMA were calculated by integrating blood concentration over time (from 0 to infinity). Estimated individual pharmacokinetic parameters of each rat given MDMA were used for the calculation. The AUC for HMA was simulated using the model, as if the rat in the second experiment had been given MDMA. The influence of sex was tested using a Wilcoxon nonparametric test.

RESULTS

Population Pharmacokinetics

Model I: MDMA-MDA-HMMA Pharmacokinetic Model Building

A two-compartment model better described the MDMA data than a one-compartment model (statistically, the OFV was decreased by 44.2 U, and visually, the diagnostic graphs were improved). Two additional compartments were used for the MDA and HMMA concentrations. The direct elimination of MDMA (k_{10}) could not be estimated. Michaelis-Menten kinetics did not improve the model for the MDMA-to-MDA transformation, the MDMA-to-HMMA transformation, or MDA elimination (no decrease in AIC and visually no improvement of diagnostic graphics). Hence, only first-order rate constants were used in the model. The exponential model was used for ISV. The final model and the visual predictive check enabled us to estimate the ISV for only V_1, k_{14}, k_{13}, k_{30}, and k_{40}. Residual variabilities were best described by proportional error models. Female rats had significantly smaller V_1, k_{14}, and k_{40} than males; backward elimination of gender on these parameters led to increases in OFV of 14.61, 45.04, and 19.37 U and increases in the corresponding ISV from 24 to 34%, 16 to 43%, and 11 to 18%.

Model II: MDA-HMA Pharmacokinetic Model Building

A two-compartment model best described the MDA data than a one-compartment model (statistically, the OFV was decreased by 137 U, and visually, the diagnostic graphs were improved), and an additional compartment was used for HMA concentrations. Linear elimination was used as nonlinear MDA elimination did not improve the OFV or the fit. An exponential model was used for ISV. The final model and the visual predictive check enabled us to estimate the ISV for only V_4 and k_{40}. Residual variabilities were best described by proportional error models. Female rats had significantly smaller k_{40} than males, removing it resulted in a 38.87 increase in OFV and a k_{40} ISV increase from 13 to 58%.

Model III: Integrated Model

Analytical solutions of the differential equations describing this model were used to estimate V_1, k_{12}, k_{21}, k_{14}, k_{40}, k_{13}/V_3, k_{30}, V_4, k_{40}, k_{45}, k_{54}, k_{46}/V_6, and k_{60} as nonlinear transfer did not improve the model (AIC did not decrease and diagnostic graphs were not improved). Two values were estimated to describe k_{40} because the elimination of MDA was significantly different in rats given MDMA or MDA. The influences of covariates found in the two previous steps were then added to the integrated model. Estimates of the MDA distribution volume with two compartments and body weight scaling indicated that gender significantly influenced MDA formation (k_{14}) but not MDA elimination (k_{40}). Finally, female given MDMA had significantly smaller V_1 and k_{14} than male rats; backward elimination led to increases of 11.77 and 73.98 U in OFV and increases in the corresponding ISVs from 17 to 24% and from 10 to 12%. The k_{13} and k_{14} ISVs were perfectly correlated. Table 1 summarizes the final population pharmacokinetic estimates. Figure 3 shows the observed and predicted MDMA and MDA concentrations plotted against time for male and female rats given MDMA or MDA. Final model performance was also assessed by comparing population- and individual-predicted plasma concentrations of MDA, MDA, HMMA, and HMA with observed concentrations, and population weighted residuals with predicted concentrations and with time (not shown).

Evaluation and Validation

Bootstrap evaluation. The bootstrap means and coefficients of variation of parameter estimates obtained from the 1000 bootstrap runs were close to the estimates obtained with the original data set (Table 1).

Visual predictive check. The average predictions matched the observed concentration time courses, and the variability was reasonably estimated (Fig. 4). Thus, the numbers (percentage) of observed points outside the 90% prediction interval for rats given MDMA were 9/132 (7%) for MDMA, 10/132 (8%) for MDA, and 20/132 (15%) for HMMA. They were 11/110 (10%) for MDA and 10/110 (9%) for HMA in rats given MDA. None of these percentages was significantly different from the expected 10%.

Influence of Gender on Exposure to MDMA, MDA, HMMA, and HMA

The exposures of rats, given MDMA, to MDMA, HMMA, MDA, and HMA are shown in Figure 5. Female rats were exposed to significantly higher MDMA (mean: 2.35, range: 1.58–2.89) than were male rats (mean: 1.43, range: 1.02–1.90), $p < 10^{-5}$. The exposures of male and female rats to HMMA were not significantly different (males: 0.56, 0.31–1.03; females: 0.60, 0.34–1.32, $p = 0.48$). Male rats given MDMA were exposed to significantly more MDA (0.65, 0.56–0.85) and HMA (0.15, 0.15–0.17) than were female rats (0.41, 0.36–0.55, $p < 10^{-6}$ and 0.09, 0.08–0.10, $p < 10^{-5}$).

DISCUSSION

This study provides the pharmacokinetic modeling of MDMA-MDA-HMMA-HMA together in male and female SD rats, following a subtoxic exposure to MDMA and MDA. Both MDMA and MDA were given at 1 mg/kg iv because preliminary experiments showed that these doses had few deleterious toxic effects, allowing us to perform an extensive pharmacokinetic study of MDMA and the main metabolites. Two distinct experiments were necessary to identify the main pharmacokinetic parameters of this model. In one, rats were

TABLE 1
Population Pharmacokinetic Parameters of MDMA and Its Metabolites from the Final Model for SD Rats

Parameter	Final model, mean (RSE%)	Bootstrap × mean (RSE%)	Parameter	Final model, mean (RSE%)	Bootstrap × mean (RSE%)
Structural model			Statistical model		
V_1 (l/kg)	0.00394 (9)	0.00384 (13)	σ_{MDMA} (%)	14 (21)	13 (14)
k_{12} (h^{-1})	1.78 (18)	2 (23)	σ_{MDA} (%)	16 (23)	15 (14)
k_{21} (h^{-1})	1.2 (19)	1.33 (33)	σ_{HMMA} (%)	22 (26)	21 (16)
k_{13}/V_3 (kg/l/h)	126 (10)	143 (19)	σ_{HMA} (%)	36 (21)	32 (12)
k_{30} (h^{-1})	1.31 (10)	1.31 (14)	ω_{V_1} (%)	17 (51)	18 (48)
k_{14} (h^{-1})	0.685 (8)	1.01 (35)	$\omega_{k_{13}}$ (%)	29 (38)	47 (45)
k_{40} (h^{-1})	1.91 (13)	1.69 (18)	$\omega_{k_{30}}$ (%)	38 (29)	38 (22)
V_4 (l/kg)	0.00238 (11)	0.00231 (14)	$\omega_{k_{40}}$ (%)	15 (56)	25 (50)
k_{45} (h^{-1})	1.71 (33)	2.16 (37)	ω_{V_4} (%)	10 (54)	18 (43)
k_{54} (h^{-1})	1.64 (22)	1.91 (24)	k as $\eta_{k_{14}} = e^{\eta_{k_{13}} \times k}$ with correlation $\omega_{k_{13}} - \omega_{k_{14}} = 1$	0.33 (27)	1.18 (72)
k_{46}/V_6 (kg/l/h)	132 (14)	150 (15)			
k_{60} (h^{-1})	1.46 (15)	1.56 (13)			
V_1, θ_{SEX}	0.715 (9)	0.736 (14)			
k_{14}, θ_{SEX}	0.501 (8)	0.372 (32)			
k_{40} (h^{-1}) (MDA adm.)	0.545 (19)	0.531 (19)			
k_{40}, θ_{SEX} (MDA administration)	0.278 (25)	0.266 (30)			

Note. RSE%, relative standard error (standard error of estimate/estimate × 100); V_1 for MDMA and V_4 for MDA volume of distribution, k_{30} for HMMA, k_{40} for MDA and k_{60} for HMA elimination rate constants, k_{12} for MDMA and k_{45} for MDA central-to-peripheral compartment rate constants, and k_{21} for MDMA and k_{54} for MDA peripheral-to-central compartment rate constants. k_{13}/V_3 for MDMA to HMMA, k_{46}/V_6 from MDA-to-HMA and k_{14} from MDMA-to-MDA transformation rate constants, θ_{SEX} for the estimated influential factor for sex on a pharmacokinetic parameter, σ residual variability estimates (coefficient of variation [CV] of residual variability, %), and ω, interindividual variability estimates (CV of ISV, %).

given MDMA iv and the concentrations of MDMA, MDA, and HMMA were measured. In the other, rats were given MDA iv and the MDA and HMA concentrations were measured. Analysis of the two data sets allowed to build a two-compartment model for MDA and identify the MDA distribution volume and the MDMA-to-MDA transformation rate constant.

We used a population pharmacokinetic model to take into account the provenance of the samples. Two rats were needed

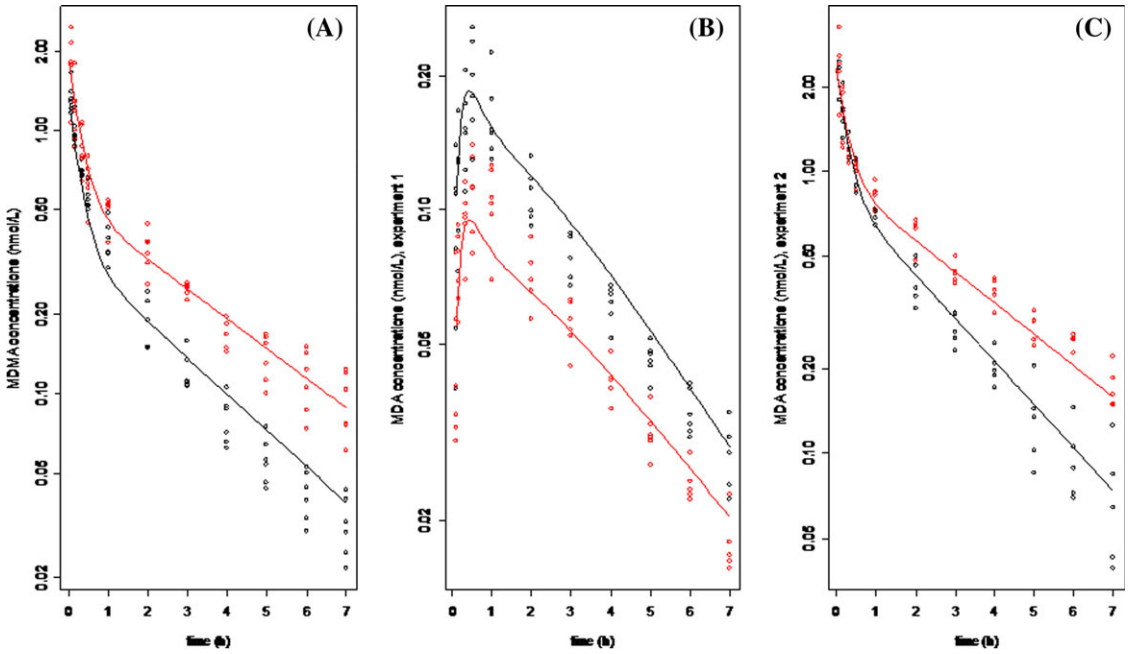

FIG. 3. Observed (points) and predicted (lines) plasma concentrations of MDMA (A) and MDA after iv bolus injections of MDMA (B) or MDA (C) plotted against time for females (red) and males (black).

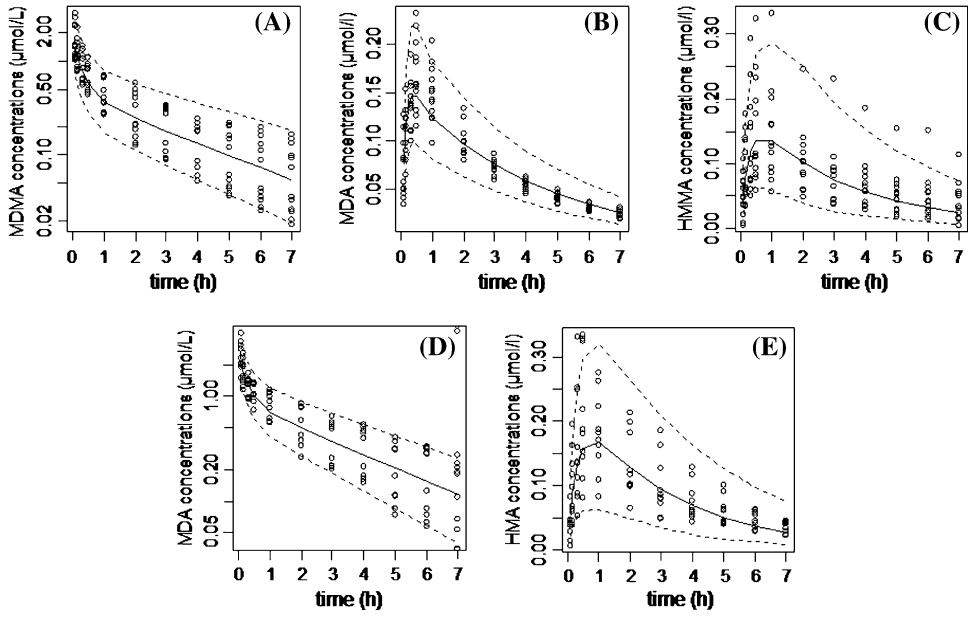

FIG. 4. Evaluation of the final model. Comparison between the 5th (lower dashed line), 50th (full line), and 95th (upper dashed line) percentiles obtained from 1000 simulations and the observed data (o) for MDMA concentrations (A), MDA concentrations after injection of MDMA (B) and MDA (D), and HMMA concentrations (C) and HMA concentrations (E).

to obtain one complete set of blood samples from 5 min to 7 h after iv injection. It was also used to estimate interindividual variability and to examine this with respect to body weight and gender.

The model reproduced the pathways of MDMA metabolism shown on the right in Figure 1. MDMA (described by two compartments) formed HMMA (described by an additional compartment linked to MDMA central compartment) and MDA (described by two compartments). MDA further formed HMA (described by an additional compartment linked to MDA central compartment). HHMA and HHA were nearly undetectable in blood samples because they are rapidly methylated to HMA and HMMA. These intermediates were therefore not modeled. The transformation of HMMA to HMA could not be estimated as the HMMA pharmacokinetic parameters came only from experiment 2 and those for HMA only from experiment 1. MDMA, MDA, HMMA, and HMA could be detected in urine samples (unpublished data). We therefore looked at direct elimination from MDMA central, MDA central, and HMA and HMMA compartments. However, the

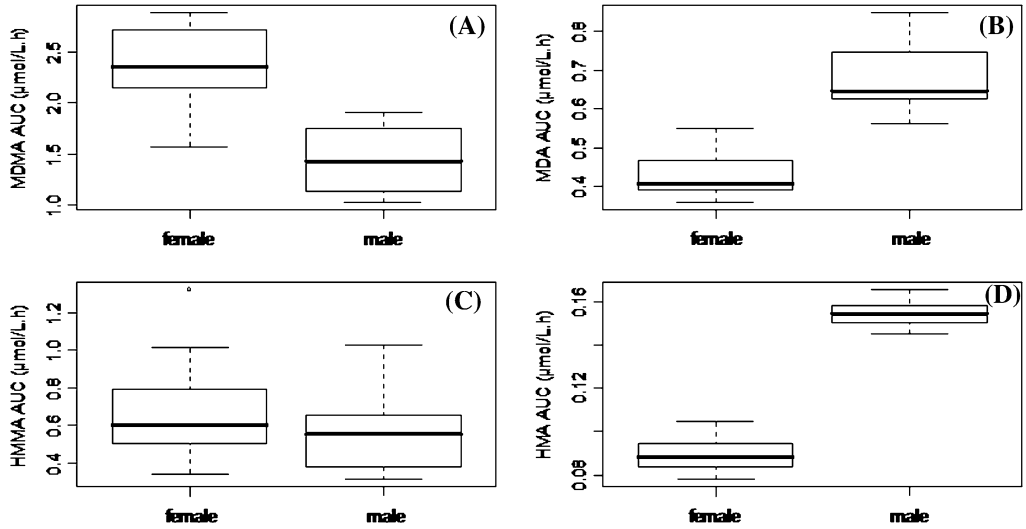

FIG. 5. Box plots of exposures of male and female rats to MDMA (A), MDA (B), HMMA (C), and HMA (D), after a 1 mg/kg iv dose of MDMA. Exposure was calculated from experiment 1 for MDMA, MDA, and HMMA and simulated for HMA.

direct elimination of MDMA could not be estimated in our model.

The pharmacokinetics of MDMA has been shown to be nonlinear in rats. Chu *et al*. (1996) found that increases in brain and plasma MDMA concentration were greater than the proportional increases in dose. They have also been found to be nonlinear in monkeys (Mueller *et al*., 2008) and humans (de la Torre *et al*., 2000; Kolbrich *et al*., 2008), indicating that nonlinear MDMA pharmacokinetics occurs in many species. However, the transformations of MDMA to MDA and MDMA to HMMA were not significantly nonlinear in our model, and this may be because we used only one low dose of MDMA (1 mg/kg), while others have used more (from 5 to 40 mg/kg) (Chu *et al*., 1996). Thus, toxicological effects of higher MDMA doses cannot be predicted with our model.

We found that significantly more MDMA was converted to MDA in male rats than in females. Sex had a more significant effect on this biotransformation than body weight had. This is in accordance with Chu *et al*. (1996), who measured significantly lower concentrations of MDA in female SD rats than in males. The isoenzymes CYP1A2 and CYP2D1 are involved in the conversion of MDMA to MDA in rats. We used rats of the same age, which should have equivalent enzyme activities, but body weight was significantly lower in females than in males, which could have introduced a bias. Our results suggest that these enzymes are more active in males than in females. These results are supported by other studies. Fonsart *et al*. (2008) reported a twofold higher CYP1A2 activity in male rats than in females, leading to differences in the N-demethylation metabolic pathway of MDMA. There may also be gender-specific differences in the concentrations of CYP2D enzymes in both humans and mice (Lofgren *et al*., 2004; Meibohm *et al*., 2002). The CYP2D pathway was studied in SD and Dark Agouti (DA) rats; DA rats are deficient in CYP2D isozymes. Chu *et al*. (1996) found that DA rats given MDMA had brain MDA concentrations that were more similar to those of female SD rats than male SD rats.

Although a body weight linear scaling was applied, the MDMA distribution volume in females was smaller than that in male. Thus, the 30% smaller MDMA distribution volume and the 50% smaller MDMA-to-MDA transformation in females than in males can account for the significantly greater exposure of females to MDMA and of males to MDA. As gender had no effect on the conversion of MDA to HMA, the increased MDA in males led to more HMA in males than in females.

It has been suggested that MDMA is unlikely to be responsible for the observed neurotoxic effects and that systemic metabolism is required for the development of toxicity (Bai *et al*., 1999; Hiramatsu *et al*., 1990; Lim and Foltz, 1991b; Miller and O'Callaghan, 1995; Schmidt and Taylor, 1988; Zhao *et al*., 1992). Injecting MDMA directly into the brain does not produce serotoninergic neurotoxicity. Several pathways seem to be involved in MDMA metabolism in the rat, resulting in the formation of 14 metabolites *in vivo* (Lim and Foltz, 1988, 1991a,b) (Fig. 1). Green *et al*. described three main MDMA transformation: hydroxylation to 6-OH MDMA, N-demethylation to MDA (leading to HHA and HMA), and demethylenation to HHMA (leading to HMMA). It is unclear which MDMA metabolites are responsible for the neurotoxic effects of MDMA. Two studies explored the neurotoxicological properties of the first pathway (hydroxylation) and found that intrastriatal administration of 6-OH-MDMA to SD rats had no effect on the brain concentrations of 5-HT or dopamine (Johnson *et al*., 1992; Zhao *et al*., 1992). Thus, this metabolite probably has no direct role in the neurotoxic effects of MDMA. Studies on the two other pathways showed that Glutathione and N-acetylcysteine conjugates of HHMA and HHA are present in the striatum of rats given MDMA and that these mediate the serotoninergic neurotoxicity (Jones *et al*., 2005).

We have used another approach to identify the metabolites that could contribute to MDMA-mediated neurotoxicity. Male mice and rats have been shown to be more sensitive to the toxic lethal effects of high doses of MDMA than are females (Cadet *et al*., 1994; Fonsart *et al*., 2008; Koenig *et al*., 2005; Miller and O'Callaghan, 1995) and that the ecstasy-related death rate is significantly higher in men than in women (Cregg and Tracey, 1993; Schifano *et al*., 2003). We therefore looked for the MDMA metabolites to which males were more exposed than females that could lead more often to neurotoxic effects. The hydroxylation pathway was not considered because this metabolite has been shown to be nontoxic (Johnson *et al*., 1992; Zhao *et al*., 1992). Only the two pathways previously implicated (HHA and HHMA) were modeled. Male rats given MDMA were exposed to significantly more MDA and HMA than were female rats ($p < 10^{-5}$). This is in agreement with a recent study (Mueller *et al*., 2009) that used correlations between pharmacokinetic parameters and lasting serotoninergic deficits to show that the conversion of MDMA to MDA is a more important determinant of brain 5-HT neurotoxicity than is the HHMA to HMMA pathway in the rat.

In conclusion, we have developed a useful compartmental pharmacokinetic model of MDMA and its metabolites in the rat that can serve as a platform for studies on the relationship between the pharmacological or toxicological effects of MDMA and plasma metabolite concentrations. Analysis of our data using this model in female and male rats suggests that the MDA-to-HMA pathway could be involved in the neurotoxic effects. We find that male rats are more exposed to these compounds than are females, which could lead to greater toxic lethal effects of high doses in males.

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