Metabolism and Disposition of 3,4-Methylenedioxymethamphetamine ("Ecstasy") in Baboons after Oral Administration: Comparison with Humans Reveals Marked Differences

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ABSTRACT

The baboon is potentially an attractive animal for modeling 3,4-methylenedioxymethamphetamine (MDMA) effects in humans. Baboons self-administer MDMA, are susceptible to MDMA neurotoxicity, and are suitable for positron emission tomography, the method most often used to probe for MDMA neurotoxicity in humans. Because pharmacokinetic equivalence is a key feature of a good predictive animal model, we compared the pharmacokinetics of MDMA in baboons and humans. Baboons were trained to orally consume MDMA. Then, pharmacokinetic profiles of MDMA and its major metabolites were determined after various oral MDMA doses using the same analytical method recently used to perform similar studies in humans. Results indicate that MDMA pharmacokinetics after oral ingestion differ markedly between baboons and humans. Baboons had little or no MDMA in their plasma but

had high plasma concentrations of 3,4-dihydroxymethamphetamine (HHMA), pointing to much more extensive first-pass metabolism of MDMA in baboons than in humans. Other less prominent differences included less O-methylation of HHMA to 4-hydroxy-3-methoxymethamphetamine, greater N-demethylation of MDMA to 3,4-methylenedioxyamphetamine, and a shorter half-life of HHMA in the baboon. To our knowledge, this is the first study to characterize MDMA metabolism and disposition in the baboon. Differences in MDMA pharmacokinetics between baboons and humans suggest that the baboon may not be ideal for modeling human MDMA exposure. However, the unusually rapid conversion of MDMA to HHMA in the baboon may render this animal uniquely useful for clarifying the relative role of the parent compound (MDMA) versus metabolites (particularly HHMA) in the biological actions of MDMA.

Introduction

($\pm$)-3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy) is a psychoactive drug with significant abuse liability and neurotoxic potential (Kalant, 2001; Green et al., 2003; Capela et al., 2009). A recent national survey indicates that recreational MDMA use may be once again on the rise (Substance Abuse and Mental Health Services Administration, 2010). In an effort to better understand effects of MDMA in humans, various laboratory animal models have been used to study the behavioral pharmacology and neurotoxicology of

MDMA. Intravenous self-administration of MDMA (a measure of abuse liability) has been demonstrated in baboons (Lamb and Griffiths, 1987), rhesus macaques (Beardsley et al., 1986; Fantegrossi et al., 2002; Lile et al., 2005), and rats (Schenk et al., 2007). Likewise, the neurotoxic effects of MDMA have been reported in baboons (Scheffel et al., 1998), rhesus monkeys (Kleven et al., 1989), and rats (Commins et al., 1987; Schmidt, 1987).

Translating MDMA findings from laboratory animals to human MDMA users has proved challenging for a number of reasons. In most laboratory animal studies, MDMA has been administered parenterally (intravenously, intramuscularly, and subcutaneously) rather than orally, even though MDMA is nearly always ingested orally by people. In addition, as is usually the case, doses used in animal studies have generally been higher than those used by humans (Green et al., 2003).

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ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; HHMA, 3,4-dihydroxymethamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; $C_{\max}$, peak plasma concentration; $T_{\max}$, time of peak plasma concentration; AUC, area under the concentration-time curve; COMT, catechol O-methyltransferase.

Although interspecies dose-scaling principles dictate that small laboratory animals will require higher milligram per kilogram doses than humans to achieve the same pharmacological effect (Mordenti, 1986), dose-scaling methods have been the subject of debate (de la Torre and Farré, 2004; Fantegrossi, 2007). A third factor complicating generalization of preclinical MDMA findings to humans has been the difference in MDMA metabolism across species. In particular, MDMA is metabolized via two main pathways that are coexistent but operate at different rates in different species. In rodents (rats and mice) N-demethylation of MDMA predominates, whereas in primates (human and nonhuman) ring demethylation is more prevalent (Meyer et al., 2008) (Fig. 1).

To circumvent uncertainties related to dose and metabolism, there has been recent interest in relating MDMA effects to plasma drug (and metabolite) concentrations (which can readily be compared across species), rather than only to MDMA dose (Mechan et al., 2006; Mueller et al., 2009). With respect to neurotoxicity, studies taking this approach have shown that plasma MDMA concentrations that are associated with neurotoxicity in squirrel monkeys overlap those found in some recreational Ecstasy consumers (Samyn et al., 2002; Morefield et al., 2011) and are only 2 to 3 times higher than those produced by a single 100- to 150-mg dose of MDMA in humans (Mechan et al., 2006; Kolbrich et al., 2008b; Mueller et al., 2009a). Related studies have also shown that the metabolism and disposition of MDMA in squirrel monkeys closely parallel those in humans (Mueller et al., 2009a). Given these findings, there is concern that the margin of safety of MDMA in humans may be narrow (at least with respect to neurotoxicity).

To determine whether the safety margin of MDMA in humans is, in fact, narrow, it would be ideal if a large primate animal model could be developed, one that metabolized MDMA in a manner similar to that in humans and one that could be studied with the same methods used to study the

neurotoxic potential of MDMA in humans (e.g., positron emission tomography) (McCann et al., 2005; Kish et al., 2010). In this regard, baboons offer significant promise because they are known to be susceptible to MDMA neurotoxicity (Scheffel et al., 1998; Szabo et al., 2002) and because positron emission tomography imaging identical to that used in recreational MDMA users has already been shown to reliably detect MDMA neurotoxicity in baboons (Scheffel et al., 1998; Szabo et al., 2002).

Because pharmacokinetic equivalence is a key feature of a good predictive laboratory animal model (Mahmood and Balian, 1999), the present studies were undertaken to characterize the metabolism and disposition of MDMA in baboons and compare them with those in humans. Plasma MDMA and metabolite concentrations in baboons were determined using the same liquid chromatographic-mass spectrometric procedure recently used to measure MDMA and metabolites in humans (Mueller et al., 2009b). MDMA was administered orally at doses selected to be equivalent to those used by humans. To our knowledge, this is the first study to characterize the pharmacokinetic profile of MDMA and its metabolites [3,4-dihydroxymethamphetamine (HHMA), 4-hydroxy-3-methoxymethamphetamine (HMMA), and 3,4-methylenedioxyamphetamine (MDA)] in the baboon.

Materials and Methods

Animals. Four (MO, BR, BO, and BS) adult male baboons (*Papio hamadryas anubis*) served as subjects. Body weights of the animals were as follows: MO, 35.4 kg; BR, 32.3 kg; BO, 46.7 kg; and BS, 27.3 kg. Three of the baboons (MO, BR, and BO) had a history of participation in previous behavioral pharmacology experiments, which, for some of the animals, included exposure to cocaine, alcohol, γ -hydroxybutyric acid, tryptamine derivatives, benzodiazepines, or various nonbenzodiazepine hypnotic sedatives (e.g., zolpidem). The other baboon (BS) was drug-naïve at the beginning of the present study. For the animals with a drug history, at least 4 weeks had elapsed between participation in prior studies and the start of the present

Fig. 1. Metabolic pathways of MDMA, along with the associated microsomal enzymes.

study. Animals were housed singly, with free access to food and water. Animal care and use were in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals and the U.S. Animal Welfare Act Regulations.

Drugs and Reagents. Racemic MDMA hydrochloride was obtained from Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse (Rockville, MD). Racemic HHMA hydrochloride and methanolic solutions (1000 mg/l) of racemic MDMA hydrochloride and racemic MDA hydrochloride were purchased from Lipomed (Cambridge, MA). Methanolic solutions (1000 mg/l) of racemic HHMA and methanolic solutions (100 mg/l) of racemic MDMA- d_5 and MDA- d_5 were obtained from Ceriliant Corporation (Round Rock, TX). 4-Hydroxymethamphetamine (pholedrine), 4-methylcatechol, and EDTA disodium salt were obtained from Sigma-Aldrich (St. Louis, MO). Sodium metabisulfite was obtained from E. Merck (Darmstadt, Germany). Perchloric acid was obtained from Mallinkrodt Baker, Inc. (Phillipsburg, NJ). J. T. The authenticity of the MDMA, HHMA, HMMA, and MDA samples was confirmed using liquid chromatographic-mass spectrometric methods to determine the corresponding pseudomolecular ions and at least one fragment ion for each compound. Analysis was performed in full scan (mass range from 100–1000) to check for the presence of possible impurities.

Drug Treatment. MDMA was administered orally. To facilitate comparison with doses administered to humans, interspecies dose scaling (Mordenti and Chappell, 1989) was used to calculate doses for each baboon estimated to be equivalent doses of 0.5, 1.6, 3.2, and 5.0 mg/kg in humans. Actual doses in each baboon depended on the weight of each animal and were approximately 0.6, 2.0, 3.9, and 6.1 mg/kg for MO, 0.6, 2.0, 4.0, and 6.3 mg/kg for BR, 0.6, 1.6, 3.2 and 5.7 mg/kg for BO, and 0.7, 2.1, 4.3, and 6.5 mg/kg for BS. These doses were selected because they bracket doses previously tested in humans (de la Torre et al., 2000a,b; Kolbrich et al., 2008a,b). For interspecies dose scaling, the allometric equation, $D_{\text{human}} = D_{\text{animal}} (W_{\text{human}}/W_{\text{animal}})^{0.7}$, was used, where D is the dose in milligrams, W is the weight of the animal in kilograms, and 0.7 is a commonly used (empirically derived) exponent. All doses were calculated as the salt form. To achieve reliable oral dosing, baboons were habituated to the taste of quinine sulfate dissolved in the readily consumed orange drink TANG by gradually increasing the concentration in 60 ml of TANG presented at approximately the same time each morning (training phase) (Turkkan et al., 1989). The solution was delivered to the baboon from the end of a 60-ml syringe held at the bars of the cage by a research technician familiar to the animal. The quinine solution was followed by delivery of 10 ml of unadulterated TANG. Once a baboon was reliably consuming 60 ml of 0.325 mg/ml quinine solution in less than 15 min (usually 5 min), MDMA dosing began (testing phase). At least 24 h lapsed between the end of the training phase and the beginning of the testing phase. MDMA was dissolved in 60 ml of TANG (without quinine). Doses were administered in a mixed order within and across baboons; at least 8 and typically >14 days elapsed between doses.

Blood Sampling. To obtain blood samples, baboons were anesthetized with intramuscular injections of either ketamine hydrochloride or the commercially available solution of 50:50 tiletamine hydrochloride and zolazepam hydrochloride (Telazol); atropine sulfate was given to control secretions. Preliminary studies showed no difference in MDMA metabolism after use of either anesthetic. In particular, a baboon was administered 1.3 mg/kg MDMA orally on two occasions, with an interval of 2 weeks between each treatment. Blood was collected as described below, the first time using ketamine hydrochloride as anesthetic and the second time using Telazol. No differences were observed (data not shown). After all doses tested, blood samples were taken at 0.125, 0.25, 0.5, 1, 2, 4, 8, and 12 h after MDMA administration. The first four blood draws (i.e., those within the 1st h) were collected after the first anesthetic dose; all subsequent blood draws took place after separate anesthetic dosages. At each time point, at least 1.5 ml of blood was collected, via either the

saphenous or cephalic vein into a tube containing EDTA as an anticoagulant. Plasma was processed and stored as described previously (Mueller et al., 2009).

Measurement of Plasma MDMA and Metabolite Concentrations. Plasma MDMA, MDA, HHMA, and HMMA concentrations were determined as described recently (Mueller et al., 2009b). The linear range for the method used in the present study was 20 to 1000 ng/ml for HHMA, HMMA, and MDMA and 10 to 500 ng/ml for MDA. If values were found, after initial plasma analysis, to be above the calibration range, the corresponding plasma samples were diluted in the same way as samples for the determination of the above-calibration-range quality control samples during the method validation procedure (Mueller et al., 2007) and were reanalyzed. Values below the limit of quantification [20 ng/ml (HHMA, HMMA, and MDMA) or 10 ng/ml (MDA)] were assumed to be 0 and were treated as such for calculation of pharmacokinetics.

Calculation of Pharmacokinetic Parameters. Peak plasma concentration (C_{max}), time of peak plasma concentration (T_{max}), elimination half-life ($t_{1/2}$), and area under the concentration-time curve (AUC) were calculated using the pharmacokinetic functions for Microsoft Excel (developed by J. I. Usansky, A. Desai, and D. Tang-Liu, <http://www.boomer.org/pkin/xcel/pkf/pkf.doc>). AUC was calculated using the linear trapezoidal rule starting at time 0 and finishing at the last quantifiable point.

Results

MDMA. Plasma time-concentration profiles of orally administered MDMA and its metabolites in baboons and humans are shown in Fig. 2. The human data, shown for comparative purposes, are from previously published studies (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a). As indicated above, doses in baboons are expressed as human equivalent doses (calculated using interspecies dose scaling methods; see *Materials and Methods*). We were surprised to find that after the two lower MDMA doses (0.5 and 1.6 mg/kg), no MDMA was detected in baboon plasma (Fig. 2). After the two higher doses (3.2 and 5.0 mg/kg), some MDMA was detected, but levels were much lower than those found in humans given lower doses (Fig. 2; Table 1). For example, after administration of 3.2 mg/kg MDMA to baboons, the C_{max} of MDMA was approximately 100 ng/ml, whereas the C_{max} of MDMA in humans receiving half the dose of MDMA (1.6 mg/kg) was more than twice as high (approximately 250 ng/ml). In addition to these differences in C_{max} , there were marked differences between AUC values of MDMA in baboons and humans. This result is illustrated by the fact that a 5-fold higher dose of MDMA was required to generate comparable AUC values in baboons (1201 ng/ml after 5.0 mg/kg MDMA) and humans (1389 ng/ml after 1.0 mg/kg MDMA) (Table 1). The time required to reach peak MDMA concentrations (T_{max}) also differed between the two species: 2.3 h in humans compared with 7 h in baboons (Table 1).

HHMA. After all MDMA doses, including the two doses that gave rise to no detectable MDMA in baboon, HHMA was readily detected in plasma of baboons (Fig. 2; Table 1). Levels of HHMA in baboons were substantially higher than those in humans given an identical MDMA dose. For example, after administration of the 1.6 mg/kg dose, the C_{max} of HHMA in the baboon was nearly 3-fold higher than that in human (Table 1). As shown in Fig. 2, HHMA levels in baboons rose as the dose of MDMA was increased from 0.5 to 3.2 mg/kg. However, despite a dose increase of approximately 60% (from

Fig. 2. Relative proportions of MDMA and its O-demethylenated metabolites (HHMA and HMMA) in baboons and humans at various times after administration of different oral doses of MDMA. Doses given to baboons were calculated to be equivalent to 0.5, 1.6, 3.2, and 5.0 mg/kg in humans (see *Materials and Methods*). Human data are from Mueller et al., 2009a (1.0 and 1.6 mg/kg; same analytic method) and de la Torre et al., 2000a (2.0 mg/kg; different analytic method). Values shown are the mean. For the sake of clarity, error bars are not shown. *, subjects in the study performed by de la Torre et al., 2000a received 150 mg of MDMA, which equals a 2.0 mg/kg dose, assuming an average body weight of 70 kg.

3.2 to 5.0 mg/kg), the $C_{\max}$ of HHMA remained relatively constant (Fig. 2; Table 1). Similar to $C_{\max}$, after administration of equivalent doses (namely, 1.6 mg/kg), the AUC of HHMA in baboons was twice as high as that in humans (Table 1). The $t_{1/2}$ of HHMA in baboons was substantially shorter than that in humans (approximately 3 h versus 10 h). The $T_{\max}$ of HHMA was approximately 2-fold longer in baboons than in humans (Table 1).

HMMA. The $C_{\max}$ of HMMA increased as the dose was increased from 0.5 to 3.2 mg/kg but then remained constant despite a further increase in MDMA dose from 3.2 to 5.0 mg/kg (Fig. 2; Table 1). After administration of equivalent doses (namely 1.6 mg/kg), the $C_{\max}$ of HMMA in baboons was only approximately one-half of that in humans, whereas its AUC was one-fourth of that in humans (Fig. 2; Table 1). The relative proportion of HMMA to HHMA also differed between

baboons and humans, plasma levels of HMMA in baboons being only approximately 1/10 of those of HHMA across the dose range tested (Fig. 2; Table 1). Similar to the $T_{\max}$ of HHMA, the $T_{\max}$ of HMMA was approximately 2-fold longer in baboons than in humans (Table 1).

MDA. Like MDMA, its N-demethylated metabolite, MDA, was only detected in baboon plasma after administration of the two higher MDMA doses (3.2 and 5.0 mg/kg) (Fig. 3; Table 1). As in humans, levels of MDA in the baboon were lower than those of MDMA. However, the relative proportion of MDA to MDMA in baboons was greater than that in humans (approximately 20–60% versus 3–5%) (Fig. 3; Table 1). Because MDA was not detected in baboons after treatment with 1.6 mg/kg MDMA, $C_{\max}$ and AUC could not be directly compared between the two species. In baboons, the $T_{\max}$ of MDA was approximately 2 h longer than that of

TABLE 1
Comparison of pharmacokinetic parameters C_{max} , AUC, T_{max} , and $t_{1/2}$ for MDMA and its metabolites in baboons and humans after administration of different oral doses of MDMA

Doses consumed orally by baboons were equivalent to 0.5, 1.6, 3.2, and 5.0 mg/kg in humans, which were, respectively, approximately 0.6, 2.0, 4.0, and 6.0 mg/kg for each baboon. Values shown are the means $\pm$ S.E.M., except for the 2.0 mg/kg dose, for which individual pharmacokinetic data are presented. Human data are from Mueller et al. (2009) and Kolbrich et al. (2008b) (1.0 and 1.6 mg/kg) and from de la Torre et al. (2000a) (2.0 mg/kg).

Dose	Human				Analyte	Baboon				Dose
	C_{max}	AUC	T_{max}	$t_{1/2}$		C_{max}	AUC	T_{max}	$t_{1/2}$	
	ng/ml	ng · h/ml	h	h		ng/ml	ng · h/ml	h	h	
1.0 mg/kg	147 $\pm$ 10	1389 $\pm$ 119	2.3 $\pm$ 0.2	7.2 $\pm$ 0.6	MDMA	0	0	0	0	0.5
	146 $\pm$ 12	1471 $\pm$ 146	1.3 $\pm$ 0.1	10.7 $\pm$ 0.9	HHMA	331 $\pm$ 91	1589 $\pm$ 575	3.1 $\pm$ 0.9	2.5 $\pm$ 0.2	
	180 $\pm$ 18	1759 $\pm$ 92	1.4 $\pm$ 0.1	9.8 $\pm$ 1.1	HMMA	28 $\pm$ 11	123 $\pm$ 53	3.0 $\pm$ 1.0	N.A.	
	8 $\pm$ 0.5	188 $\pm$ 13	7.5 $\pm$ 0.4	11 $\pm$ 1.1	MDA	0	0	0	0	
1.6 mg/kg	255 $\pm$ 20	3071 $\pm$ 225	2.4 $\pm$ 0.2	8.4 $\pm$ 0.5	MDMA	0	0	0	0	1.6
	152 $\pm$ 11	1801 $\pm$ 130	1.6 $\pm$ 0.2	11.9 $\pm$ 0.9	HHMA	694 $\pm$ 104	4000 $\pm$ 644	3.0 $\pm$ 0.6	2.6 $\pm$ 0.3	
	168 $\pm$ 14	2060 $\pm$ 109	1.7 $\pm$ 0.1	13.7 $\pm$ 1.3	HMMA	85 $\pm$ 17	505 $\pm$ 95	3.0 $\pm$ 0.6	4.0 $\pm$ 0.6	
	14 $\pm$ 1.0	352 $\pm$ 27	7.6 $\pm$ 0.6	12 $\pm$ 0.9	MDA	0	0	0	0	
2.0 mg/kg ^a	442–487	5133–5232	1.5–2.0	6.9–7.2	MDMA	97 $\pm$ 49	631 $\pm$ 357	7.0 $\pm$ 1.0	N.A.	3.2
	Not analyzed				HHMA	1059 $\pm$ 87	6896 $\pm$ 465	4.5 $\pm$ 1.3	4.6 $\pm$ 1.6	
	Values not reported				HMMA	135 $\pm$ 22	859 $\pm$ 109	5.5 $\pm$ 1.5	2.9 $\pm$ 0.5	
	34–31	590–374	4–10	37.3–23.2	MDA	59 $\pm$ 16	334 $\pm$ 96	9.0 $\pm$ 1.9	N.A.	
					MDMA	249 $\pm$ 86	1201 $\pm$ 458	7.1 $\pm$ 2.5	N.A.	5.0
					HHMA	988 $\pm$ 233	6081 $\pm$ 1211	6.5 $\pm$ 2.2	2.6 $\pm$ 0.9	
					HMMA	131 $\pm$ 31	723 $\pm$ 143	7.0 $\pm$ 1.9	2.8 $\pm$ 0.3	
					MDA	58 $\pm$ 27	256 $\pm$ 147	10.7 $\pm$ 1.3	N.A.	

N.A., not available.
^a Subjects in the study performed by de la Torre et al. (2000a) received 150 mg of MDMA, which equals a 2.0 mg/kg dose, assuming an average body weight of 75 kg.

Fig. 3. Relative proportion of MDMA to its N-demethylated metabolite, MDA, in baboons and humans. Data shown are from baboons that received MDMA at a dose of 3.2 mg/kg and humans that received MDMA at a dose of 1.0 mg/kg. These doses were compared because they produced comparable MDMA levels. Human data are from Kolbrich et al. (2008a). Values shown are the mean. For the sake of clarity, error bars are not shown.

MDMA (9–10 h versus 7 h, respectively) (Table 1). As already seen with the parent compound and the other metabolites, the T_{max} of MDA was longer in baboons compared with that of humans (Table 1).

Extensive first-pass metabolism severely limiting the systemic bioavailability of MDMA was evident in all four baboons used in this study, including the animal (BS) without a prior drug history (Fig. 4).

Discussion

The major finding of the present study is that MDMA metabolism in baboons differs markedly from that in humans. In particular, after oral ingestion of MDMA, first-pass metabolism of MDMA to HHMA in the baboon appears to be much more rapid than in humans. In baboons, MDMA doses that engender MDMA plasma levels ranging from 100 to 300 ng/ml in humans [0.5–1.6 mg/kg (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a)] give rise to no detectable MDMA but high HHMA levels. An interesting and noteworthy consequence of the rapid first-pass O-demethylenation of MDMA in baboons is that, despite receiving MDMA, this animal is exposed to little or no MDMA, at

least not after doses that engender substantial amounts of MDMA in humans. Extensive first-pass metabolism occurring after oral administration, which limits the systemic bioavailability of MDMA in baboons, may render this animal ideal for determining which MDMA actions are related to the parent compound (MDMA) and which are related to its major metabolite HHMA and other downstream metabolites (e.g., HMMA).

As alluded to above, the present data indicate that HHMA is the major metabolite of MDMA in baboons. HHMA levels in baboons far exceeded those of other MDMA metabolites (HMMA and MDA) (Fig. 2). Of note, HHMA levels in baboons were 4- to 10-fold higher than those of MDMA (Fig. 2; Table 1). This result differs from what is seen in humans, in whom HHMA levels are typically lower than those of MDMA (Fig. 2). One potential reason for the far more rapid conversion of MDMA to HHMA in baboons could be greater activity of the cytochrome P450 2D6 ortholog in baboons relative to that in humans. Other possible explanations include higher amounts of the enzyme and/or the involvement of other cytochrome P450 subtypes in catalyzing the O-demethylenation of MDMA in baboons.

Fig. 4. Individual plasma-concentration-time profiles of O-demethylenated MDMA metabolites (HHMA and HMMA) after administration of equivalent doses of MDMA (1.6 mg/kg) to different baboons. Listed along with the individual plasma profiles is the history of prior drug exposure of each animal. Three of the animals had drug histories (subjects 1–3); one had had no prior drug exposure (subject 4). Note that MDMA and MDA were not detected in the plasma of any of the baboons after the 1.6 mg/kg dose of MDMA. Values shown are the mean. For the sake of clarity, error bars are not shown. NBZ, non-benzodiazepine.

The pharmacokinetic profile of HMMA, the O-methylated product of HHMA, also differed between baboons and humans. In particular, in baboons, HMMA plasma levels are substantially lower than those that have been reported in humans exposed to similar dosages of MDMA (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a), possibly because of the decreased conversion of HHMA to HMMA in baboons. Over the dose range tested in the current study, approximately 10% of HHMA was converted to HMMA in baboons, whereas in humans approximately 50% of HHMA undergoes metabolism to HMMA (resulting in a 1:1 ratio of HHMA to HMMA in humans) (Mueller et al., 2009a). These observations suggest that catechol-O-methyltransferase (COMT)-mediated metabolism of HHMA to HMMA is less efficient in baboons than in humans (Fig. 2). One possible explanation for the reduced formation of HMMA in baboons is the very high levels of the HHMA saturate COMT (Meyer and Maurer, 2009). However, in the present circumstance, this explanation seems unlikely because HMMA levels would be expected to remain constant across the MDMA dose range tested, and this was not observed. Rather, as the dose of MDMA was increased from 0.5 to 3.2 mg/kg, levels of HMMA also increased (Fig. 2), suggesting that overall decreased COMT activity, rather than COMT saturation, is the basis for the lower levels of HMMA in baboons than in humans.

As in humans, MDA appears to be a relatively minor metabolite of MDMA in baboons. In fact, MDA was only detected in baboons after the two higher doses of MDMA tested (3.2 and 5.0 mg/kg) (Fig. 3). Of note, however, N-demethylation appears to be more efficient in baboon than in humans because, in baboons, approximately 20 to 60% of MDMA was converted to MDA, whereas in humans it is only 3 to 5% (Kolbrich et al., 2008b) (Fig. 3). Higher enzyme activity and/or a greater amount of the enzyme system responsible for the formation of MDA in baboons may be the basis for this species difference.

As in other species, the pharmacokinetics of MDMA in baboons appears to be nonlinear. That is, MDMA plasma levels rose to a higher degree than predicted by the increase in dose. In particular, when the dose of MDMA was further increased from 3.2 to 5 mg/kg, plasma MDMA levels rose by a factor of 2.56 rather than by the dose-predicted factor of

1.56. This observation, coupled with the fact that HHMA levels remained constant (despite approximately a 60% increase in MDMA dose), points to nonlinear MDMA pharmacokinetics in baboons. CYP2D6, the enzyme responsible for O-demethylation of MDMA to HHMA in humans, has been shown to be susceptible to saturation and/or inhibition by MDMA (Ramamoorthy et al., 2002; Heydari et al., 2004), and this saturation/inhibition is thought to be responsible for the nonlinear MDMA pharmacokinetics observed in humans (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a) as well as in rats (Chu et al., 1996) and squirrel monkeys (Mueller et al., 2008). The present observations indicate that the CYP2D6 ortholog in baboons is also susceptible to MDMA saturation and/or inhibition.

Of note, the plasma concentration of MDMA at which MDMA metabolism to HHMA becomes saturated and/or inhibited (and MDMA pharmacokinetics become nonlinear) is approximately the same plasma concentration at which nonlinear MDMA pharmacokinetics becomes evident in humans (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a), rats (Chu et al., 1996), and squirrel monkeys (Mueller et al., 2008). An important consequence of MDMA pharmacokinetics becoming nonlinear at plasma levels engendered by typical pharmacological doses of MDMA is that high doses are not required to produce unexpectedly high MDMA plasma concentrations. That is, seemingly small increases in dose may result in unexpectedly high plasma MDMA concentrations. Such disproportionate increases in plasma MDMA concentration may give rise to unexpected toxicities.

Differences in pharmacokinetics between baboons and humans do not generalize to other nonhuman primates. In particular, squirrel monkeys metabolize MDMA in a manner that is highly similar to that in humans (Mueller et al., 2009a). One of the few differences between humans and squirrel monkeys is that the $t_{1/2}$ of MDMA in the squirrel monkey is shorter than that in humans (2–3 h versus 6–9 h). However, this difference is expected, given the substantial difference in body mass between the two species (approximately 1 kg versus 70 kg), and the known fact that smaller animals metabolize drugs faster than larger animals (Mordenti, 1986). The different pharmacokinetics of MDMA in the

baboon are not likely to be related to the fact that it is an “Old World” rather than a “New World” primate (like the squirrel monkey) because rhesus monkeys, which are also Old World primates, display MDMA pharmacokinetics that appear to resemble those in humans (Banks et al., 2007).

Potential limitations of the study should be acknowledged. Three of the four baboons used in the present study had previous drug exposure. In theory, prior drug exposure could have induced or altered hepatic enzyme activity in such a way as to contribute to the observed differences between baboons versus humans. However, mitigating this concern is the fact that the animals had been drug-free for at least 4 weeks. Furthermore, one baboon had no drug history and showed a profile of MDMA metabolism similar to that of the three baboons that had had prior drug exposure (Fig. 4). Another potential limitation is that use of anesthesia in the baboon (to facilitate collection of blood samples) could have altered the metabolism and disposition of MDMA. However, there is no evidence that either of the anesthetic agents used in this study (Telazol and ketamine) alters MDMA metabolism, and preliminary studies with Telazol and ketamine yielded similar findings with regard to the metabolic pattern of MDMA (M. Mueller, A. K. Goodwin, N. A. Ator, U. D. McCann, and G. A. Ricaurte, unpublished observations).

It remains to be determined how the extensive first-pass metabolism that limits systemic bioavailability of MDMA in baboons relates to previously reported biological effects of MDMA in this animal, namely, self-administration (Lamb and Griffiths, 1987) and neurotoxicity (Scheffel et al., 1998). In this regard, it is important to note that both of the aforementioned studies (Lamb and Griffiths, 1987; Scheffel et al., 1998) involved repeated MDMA dosing. With repeated dosing, we suspect that there is inhibition/saturation of CYP2D6 ortholog-mediated O-demethylation of MDMA to HHMA and that this leads to MDMA accumulation. Furthermore, Lamb and Griffiths (1987) used the intravenous route for their self-administration studies, thereby bypassing hepatic first-pass metabolism that occurs after oral administration. Thus, baboons in the above-mentioned studies were in all likelihood exposed to a combination of MDMA and metabolites, making it impossible to know whether the observed effects were primarily related to the parent compound or to one of its metabolites.

In sum, the present results suggest that baboons may not be ideal for modeling human exposure to MDMA and its metabolites because the metabolism and disposition of MDMA in the baboon differ markedly from those in humans. Nevertheless, the distinct pharmacokinetic profile of MDMA in baboons suggests that this laboratory animal may be useful for clarifying the relative role of parent compound versus metabolites in pharmacology and toxicology of MDMA.

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Authorship Contributions

Participated in research design: Mueller, Goodwin, Ator, McCann, and Ricaurte.

Conducted experiments: Mueller and Goodwin.

Contributed new reagents or analytic tools: Mueller and Goodwin.

Performed data analysis: Mueller.

Wrote or contributed to the writing of the manuscript: Mueller, Goodwin, Ator, McCann, and Ricaurte.

Other: Ator, McCann, and Ricaurte acquired funding for the research.

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