Direct Comparison of (±) 3,4-Methylenedioxymethamphetamine (''Ecstasy'') Disposition and Metabolism in Squirrel Monkeys and Humans

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Abstract: The present study compared the disposition and metabolism of the recreational drug (±) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") in squirrel monkeys and humans because the squirrel monkey has been extensively studied for MDMA neurotoxicity. A newly developed liquid chromatography–mass spectrometric procedure for simultaneous measurement of MDMA, 3,4-dihydroxymethamphetamine, 4-hydroxy-3-methoxymethamphetamine, and 3,4-methylenedioxyamphetamine was employed. In both humans and squirrel monkeys, a within-subject design permitted testing of different doses in the same subjects. Humans and squirrel monkeys were found to metabolize MDMA in similar, but not identical, pathways and proportions. In particular, amounts of 3,4-dihydroxymethamphetamine (after conjugate cleavage) and 3,4-methylenedioxyamphetamine were similar in the 2 species, but formation of 4-hydroxy-3-methoxymethamphetamine was greater in squirrel monkeys than in humans. Both species demonstrated nonlinear MDMA pharmacokinetics at comparable plasma MDMA concentrations (125–150 ng/mL and above). The elimination half-life of MDMA was considerably shorter in squirrel monkeys than in humans (2–3 versus 6–9 hours). In both species, there was substantial individual variability. These results suggest that the squirrel monkey may be a useful model for predicting outcomes of MDMA exposure in humans, although this will also depend on the degree to which MDMA pharmacodynamics in the squirrel monkey parallels that in humans.

Key Words: MDMA, nonlinear pharmacokinetics, MDMA metabolites, neurotoxicity, serotonin, squirrel monkey

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INTRODUCTION

Animal models play an important role in drug abuse research. However, the extent to which drug effects in animals provide insight into effects in humans depends critically upon whether or not actions of the drug in animals parallel those in humans. In this regard, pharmacokinetic issues are especially important because they provide basic information regarding duration of exposure to the parent drug and its various metabolites. In situations where species differences result in markedly different pharmacokinetics, results from animal studies may have little relevance to their human counterpart.

In the case of (±) 3,4-methylenedioxymethamphetamine (MDMA), a few interspecies pharmacokinetic differences have already been identified. For instance, the elimination half-life ($T_{1/2}$) of MDMA is considerably shorter in rats than in humans (1 versus 7–9 hours),[1] no doubt because of the much smaller size of the rat (0.25 versus 70 kg) and subsequent differences in metabolic rates. Another difference is that rodents metabolize a much larger fraction of MDMA to 3,4-methylenedioxyamphetamine (MDA) than humans, approximately 50% compared with less than 5% in humans.[2–4]

Whether such pharmacokinetic differences influence pharmacologic and/or toxic effects of MDMA in different species has yet to be systematically investigated. However, neurotoxic effects of MDMA are known to differ among species. For instance, nonhuman primates (squirrel monkeys, rhesus monkeys, cynomolgus monkeys), like rats, sustain neurotoxic effects that are largely selective for brain serotonin axon terminals, whereas mice incur selective dopaminergic neurotoxicity.[5,6] As yet, it is not clear which, if any, of these animal models best predicts possible effects of MDMA in humans, although available data suggest that humans, like rats and nonhuman primates, are susceptible to brain serotonin neurotoxicity but not brain dopamine neurotoxicity.[7]

The purpose of the present study was to directly compare MDMA disposition and metabolism in humans and squirrel monkeys, with the same analytical procedure and

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a similar experimental design in the 2 species. The squirrel monkey was selected for comparison because this nonhuman primate species has been extensively studied for MDMA neurotoxicity. Although drug administration and specimen collection occurred in 2 independent laboratories, human and squirrel monkey specimens were processed in parallel in 1 laboratory (G.A.R.). Portions of these data have been published elsewhere.[4,8]

MATERIALS AND METHODS

Subjects

Six male adult squirrel monkeys and 9 (7 males, 2 females, age range 18–24 years) human subjects participated. The characteristics of each subject group have been presented in detail in recent publications.[4,8] During the study, squirrel monkeys were housed in standard steel cages, at an ambient temperature of 26 ± 3°C, with free access to food and water in a room maintained on a 14:10 h light:dark cycle. Animal care and manipulations were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University School of Medicine, and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Human studies were carried out in the closed residential research unit of the National Institute on Drug Abuse (NIDA) Intramural Research Program and were approved by the NIDA Institutional Review Board. Participants provided written informed consent. To be eligible, they were required to have used at least 5 MDMA tablets, including a minimum of once in the past 90 days and be free of significant medical problems at the time of enrollment.

Drugs and Reagents

Racemic MDMA hydrochloride, obtained through the NIDA drug supply program (Rockville, MD), was administered to squirrel monkeys. Racemic MDMA hydrochloride for human administration was purchased from Lipomed (Arlesheim, Switzerland). Racemic 3,4-dihydroxymethamphetamine (HHMA) hydrochloride and a methanolic solution of racemic MDMA hydrochloride and racemic MDA hydrochloride were purchased from Lipomed (Cambridge, MA). Methanolic solutions (1000 mg/L) of racemic 4-hydroxy-3-methoxymethamphetamine (HMMA) and methanolic solutions (100 mg/L) of racemic MDMA-d_5 and MDA-d_5 were obtained from Cerilliant (Round Rock, TX). 4-Hydroxymethamphetamine (pholedrine), 4-methylcatechol, and EDTA disodium salt dihydrate were obtained from Sigma–Aldrich (St. Louis, MO). Sodium metabisulfite was obtained from E. Merck (Darmstadt, Germany). The authenticity of MDMA, HHMA, HMMA, and MDA samples were confirmed by liquid chromatography–mass spectrometry (LC-MS).

Dosing

Monkeys

Each squirrel monkey received different oral doses (expressed as the salt) of MDMA, with an average of 6 weeks between each dose, in random order. Oral administration was accomplished by means of gavage, as detailed elsewhere.[8] Doses administered to squirrel monkeys were calculated to be equivalent to 0.4, 0.8, 1.6, and 2.8 mg/kg doses in a 70 kg human, according to a standard interspecies dose-scaling equation:

$$D_{\text{human}} = D_{\text{animal}}(W_{\text{human}}/W_{\text{animal}})^{0.7},$$

where D = dose in mg and W = weight of the animal in kg and 0.7 is a commonly used exponent.[9] Absolute doses administered to monkeys and the human equivalent doses (assuming a weight of 1 kg for squirrel monkeys and 70 kg for humans), calculated with the above allometric equation, are shown in Table 1.

Humans

Participants received oral placebo, 1.0 and 1.6 mg/kg (expressed as the salt) MDMA, in a double-blind, randomized, within-subject design. Doses were separated by 1 week or more. Subjects remained on the research unit for 2–7 days after each dose, as detailed elsewhere.[4]

Blood Collection

In squirrel monkeys, approximately 0.7 mL of blood was obtained at 0.75, 1.5, 3.0, 4.5, 6, 7, 9, 11, 23, and 25 hours after MDMA administration, as recently described.[8] In humans, blood was collected at −0.25, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.5, 4.0, 4.5, 5.0, 7.0, 9.0, 11, 13, 15, 23, 29, 34, and 39 hours after each dose. Specimens were stored on ice and centrifuged, and plasma was separated within 2 hours and frozen at −20°C until analysis. On average, analysis of squirrel monkey plasma samples was performed 5 days after sample collection. Repeated analysis of some of the plasma samples 3 months later showed levels within a ±20% range of the first results for each analyte. Human plasma samples were stored for 16–30 months before analysis for the present study. Comparison of the gas chromatography–mass spectrometry data[4] with the here presented LC-MS data (obtained about 6 months later) showed for 92.4% of all samples MDMA, HMMA, and MDA levels within a ±20% range and for 98% of all samples levels within a ±25% range. Currently there is no information on HHMA stability in human plasma samples because it was not measured in the study by Kolbrich et al.[4] However, as HHMA is present in its conjugated form in human plasma, one could assume similar stability than that found for the HMMA conjugate.

TABLE 1. Absolute Doses Administered to Monkeys and the Human Equivalent Doses, Calculated With the Allometric Equation

Absolute Dose Administered (mg/kg)	Human Equivalent Dose (mg/kg)
1.4	0.4
2.8	0.8
5.7	1.6
10.0	2.8

Sample Preparation

Plasma samples from squirrel monkeys and humans were processed identically as previously described.[10] Briefly, 20 μL of sodium metabisulfite (250 mM); 10 μL of EDTA (250 mM); 100 μL of racemic internal standards MDMA-d_5, MDA-d_5, and pholedrine (1.0 μg/mL each) in distilled water; and 300 μL of 0.5 M HCl were added to 100 μL aliquots of plasma. Samples were mixed (15 seconds) on a rotary shaker and heated at 100°C for 80 minutes for conjugate cleavage. After cooling samples to room temperature, 20 μL of 4-methylcatechol (1 mg/mL) was added, and briefly vortexed. Perchloric acid (10 μL) was added, and samples were mixed again on a rotary shaker for 15 seconds to perform protein precipitation. After centrifugation (16,000 *g* for 5 minutes), supernatants were transferred to autosampler vials, and 5 μL injected into the LC-MS system.

Determination of MDMA, HHMA, HMMA, and MDA Concentrations

Plasma concentrations of MDMA, HHMA, HMMA, and MDA were determined by a recently described, fully validated LC-MS procedure.[10] Values for HHMA and HMMA represent total free amounts (ie, amounts after cleavage of sulfate and glucuronic acid conjugates). The linear range for MDMA, HHMA, and HMMA was 20–1000 ng/mL and 10–500 ng/mL for MDA. Method accuracy was greater than 80%. The lowest point of the calibration curve was the limit of quantification of the method (20 ng/mL for MDMA, HHMA, and HMMA each, and 10 ng/mL for MDA).

Calculation of Pharmacokinetic Parameters

$T_{1/2}$, peak plasma concentration (C_{max}), time of peak plasma concentrations (T_{max}), and area under the concentration–time curve (AUC) for MDMA, HHMA, HMMA, and MDA were calculated using noncompartmental modeling (WinNonlin v 5.2; Pharsight Corp, Mountain View, CA). Three or more time points of the declining plasma concentration–time curve were used for calculation of $T_{1/2}$. C_{max} values were obtained from the plasma concentration–time curve based on the appearance of the corresponding T_{max}. AUC was determined using the trapezoidal rule.

Statistics

Statistical analyses were performed with GraphPad Prism Version 3.02 (GraphPad Software Inc, San Diego, CA). $T_{1/2}$, T_{max}, C_{max}, and AUC in humans and squirrel monkeys were compared by a Student *t* test. For within-subject comparisons in squirrel monkeys, repeated-measures analysis of variance with subsequent Tukey multiple comparison tests were employed. For within-subject comparisons in humans, paired *t* tests were applied. Differences were considered significantly different if $P < 0.05$ (2-tailed).

RESULTS

Figure 1 shows the pharmacokinetic profile of a single oral dose of MDMA in humans and squirrel monkeys. Doses selected for this comparison were ones that produced roughly comparable C_{max} values in each species (1.6 mg/kg for humans and 2.8 mg/kg for monkeys, which translates to a human equivalent dose of 0.8 mg/kg after interspecies dose scaling—see Materials and Methods). The most notable difference that such a comparison reveals is the considerably longer $T_{1/2}$ of MDMA in humans than in squirrel monkeys. The MDMA $AUC_{0–23}$ in squirrel monkeys was approximately one half that in humans. Also, the T_{max} of MDMA was shorter in squirrel monkeys than in humans (Fig. 1).

Parameter	Human (n=9)	Squirrel monkey (n=6)
C_{max} *(ng/ml)*	254.7 † 60.4	312.7 † 92.8
$AUC_{0\text{-}23h}$*(ng/ml · h)*	3070.6 † 673.4	1314.2 † 581.5*
T_{max}*(h)*	2.4 † 0.6	1.1 † 0.4*
$T_{1/2}$*(h)*	8.4 † 1.6	2.1 † 0.8*

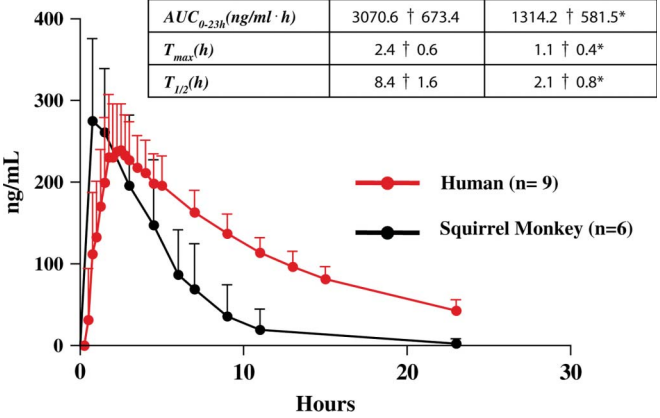

FIGURE 1. Pharmacokinetic parameters [peak plasma concentration (C_{max}), AUC, time of peak plasma concentration (T_{max}), and half-life ($T_{1/2}$)] of MDMA in humans and squirrel monkeys receiving single oral doses of MDMA producing comparable peak concentrations. Dose of MDMA in humans was 1.6 mg/kg; absolute dose of MDMA in the squirrel monkey was 2.8 mg/kg, which translates to a human equivalent dose of 0.8 mg/kg, after interspecies dose scaling (see Materials and Methods for interspecies dose-scaling procedure). Values shown are the mean ± SD. n = 9 for humans and 6 for squirrel monkeys. *Significant difference between the 2 species ($P < 0.05$, Student *t* test).

Figure 2 displays plasma concentration profile of MDMA relative to each of its major metabolites (HHMA, HMMA, and MDA) in humans and squirrel monkeys. For this comparison, humans and squirrel monkeys received single oral doses estimated to be equivalent in the 2 species by the interspecies dose-scaling equation (human subjects received 1.6 mg/kg MDMA, whereas squirrel monkeys received 5.7 mg/kg MDMA, which translates to 1.6 mg/kg after interspecies dose scaling). These doses produced AUC values that were not significantly different (humans: 3070 ± 673 ng/mL·h; squirrel monkeys: 3866 ± 891 ng/mL·h), but C_{max} values were higher in squirrel monkeys (squirrel monkeys: 723.6 ± 93 ng/mL; humans: 254.7 ± 20.1 ng/mL). The relative proportion of MDMA to HHMA was roughly comparable in the 2 species (Fig. 2, Table 2). In contrast, the relative proportion of MDMA to HMMA was different, with squirrel monkeys generating more HMMA than humans (Fig. 2, Table 2). In the squirrel monkey, HHMA showed a later T_{max} than HMMA (Table 2). In both humans and squirrel monkeys, the fraction of MDMA converted to MDA was relatively small (less than 5%) (Fig. 2).

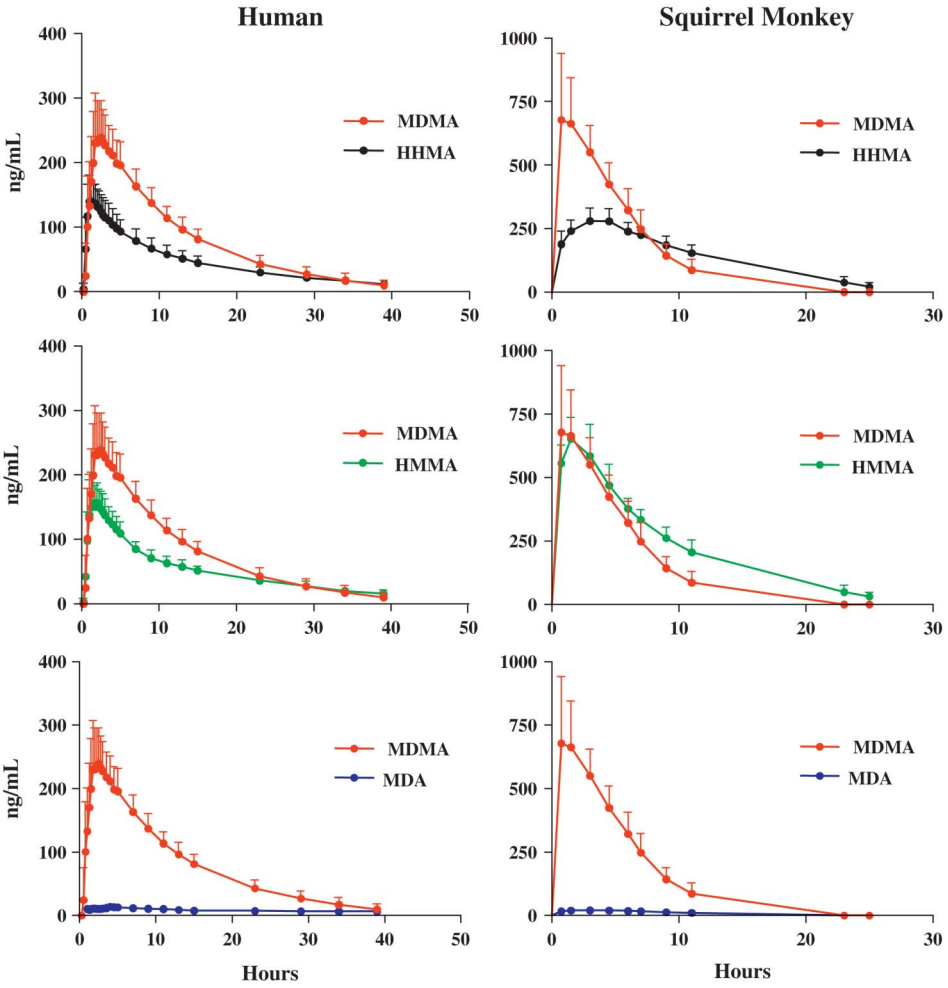

FIGURE 2. Plasma profiles of MDMA and HHMA (top panels) and HMMA (middle panels) and MDA (lower panels) in humans and squirrel monkeys after an oral dose of MDMA estimated to be equivalent in the 2 species. Humans received 1.6 mg/kg; squirrel monkeys received 5.7 mg/kg, which is equivalent to 1.6 mg/kg in humans after interspecies dose scaling (see Materials and Methods for interspecies dose-scaling procedure). Note that relative amounts of HHMA and MDA are roughly comparable in the 2 species, whereas relative amounts of HMMA are higher in the squirrel monkey than in human. Values shown are the mean ± SD. n = 9 for humans and 6 for squirrel monkeys.

Of note, the relative proportion of MDMA to metabolites was highly dependent on dose in both species (Fig. 3). At the lowest dose tested in the squirrel monkeys, the C_{max} and AUC of HMMA significantly exceeded those of MDMA ($P < 0.0001$). In humans, the C_{max} of HMMA after 1.0 mg/kg MDMA also was higher, but the difference did not achieve significance. At the higher doses tested, the amount of MDMA exceeded that of HMMA and HHMA in both species. At all doses, the relative proportion of HMMA to MDMA was greater in squirrel monkeys than in humans. The HHMA: HMMA ratio in both species did not change with dose.

To determine if the observed increases were linear or nonlinear, C_{max} and AUC values were normalized by dividing by the corresponding dose administered per kg of body weight. As shown in Table 3, C_{max} and AUC of MDMA increased nonlinearly with dose in squirrel monkeys, as reflected by the significant differences in dose-normalized C_{max} and AUC values. In humans, only the dose-normalized

TABLE 2. Comparison of Pharmacokinetic Parameters C_{max}, AUC, T_{max}, and $T_{1/2}$ for MDMA and Its Metabolites HHMA and HMMA in Humans (n = 9) and Squirrel Monkeys (n = 6) Given an Oral Dose of MDMA Estimated to Be Equivalent in the 2 Species

	C_{max} (ng/mL)		AUC (ng/mL·h)		T_{max} (h)		$T_{1/2}$ (h)	
	Human	Monkey	Human	Monkey	Human	Monkey	Human	Monkey
MDMA	254.7 ± 60.4	723.6 ± 228*	3070.6 ± 673.4	3866.2 ± 891	2.4 ± 0.6	1.3 ± 0.9*	8.4 ± 1.6	2.6 ± 0.7*
HHMA	151.8 ±33.5	294.8 ± 51.8*	1801.2 ± 390.5	3617.2 ± 581*	1.6 ± 0.5	3.8 ± 0.8*	11.9 ± 2.8	5.3 ± 1.3*
HMMA	167.7 ± 41.6	653.0 ± 83*	2060.2 ± 327.5	6064.5 ± 744.1*	1.7 ± 0.4	1.5 ± 0	13.7 ± 4.0	5.5 ± 1.2*

Equivalent dosing was attempted by means of interspecies dose scaling (see Materials and Methods for interspecies dose-scaling procedure). Humans received 1.6 mg/kg; the absolute dose for squirrel monkeys was 5.7 mg/kg. Values represent mean ± SD.

*Significant difference between the analyte parameter in 2 species ($P < 0.05$, Student t test).

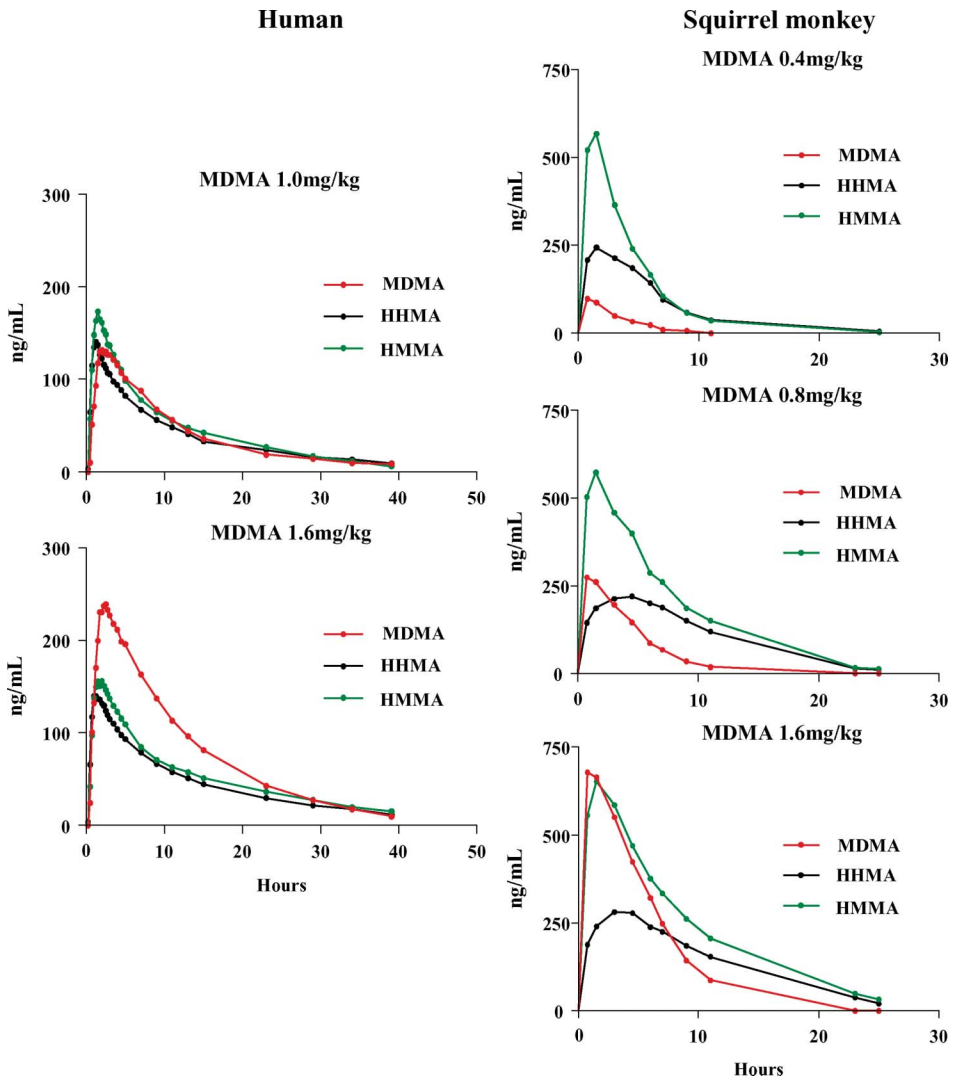

FIGURE 3. Dose dependency of relative proportions of MDMA and metabolites (HHMA and HMMA) in humans (left panels) and squirrel monkeys (right panels). Values shown are the mean ± SD. n = 9 for humans and 6 for squirrel monkeys. For clarity, error bars are omitted.

change in AUC achieved significance. As also shown in Table 3, nonlinear MDMA accumulation in both species was associated with significant relative decreases in HHMA and HMMA formation, as evidenced by significant decreases in dose-normalized values of these 2 metabolites.

In both humans and squirrel monkeys, there was a high degree of intersubject variability, as reflected by the relatively large standard deviations of kinetic parameters in both species.

DISCUSSION

This is the first study to directly compare and contrast the metabolism and disposition of MDMA in squirrel monkeys and humans. Direct head-to-head comparisons between animal and human pharmacokinetics are important if a goal of animal studies is to accurately predict MDMA effects in humans. Although previous studies have evaluated MDMA pharmacokinetics in humans[3,4] and squirrel monkeys,[8,11] the drug regimens employed, the metabolic outcome measures assessed, and the assays employed for their detection have differed in the 2 species, making direct comparisons impossible.

The present results underscore the challenge of estimating "equivalent" doses across species through the use of interspecies dose scaling (see Materials and Methods). As shown in Table 2, doses estimated to be equivalent in humans and squirrel monkeys generate similar AUC values but different C_{max} values. Conversely, dosages that are not equivalent (1.6 mg/kg in humans and 0.8 mg/kg in squirrel monkeys) generate comparable C_{max} values but different AUC values (Fig. 1). Because of the shorter $T_{1/2}$ of MDMA in the squirrel monkey, it is impossible to achieve comparable C_{max} and AUC values simultaneously in the 2 species. Thus, after single oral doses of MDMA, it is not feasible to produce identical "MDMA exposure" profiles in humans and squirrel monkeys, despite the use of interspecies dose scaling. Given this fundamental difference in MDMA pharmacokinetics in humans and squirrel monkeys (and other small laboratory animals), animal studies that seek to predict MDMA effects in humans on the basis of findings in smaller species must take into account the reduced drug exposure that results from the shorter elimination half-lives in smaller animals.

TABLE 3. Nonlinear Pharmacokinetics of MDMA, HHMA, and HMMA in Humans and Squirrel Monkeys

MDMA Dose (mg/kg)	Analyte	Normalized C_{max} (ng/mL)	Normalized AUC (ng/mL·h)
Humans			
1.0	MDMA	147.3 ± 31.2	1388.6 ± 356.7
	HHMA	146.4 ± 35.3	1470.5 ± 435.4
	HMMA	180.0 ± 53.2	1758.7 ± 275.2
1.6	MDMA	159.2 ± 37.7	1919.1 ± 420.8*
	HHMA	94.9 ± 20.9*	1125.7 ± 244.1*
	HMMA	104.8 ± 26.0*	1287.7 ± 204.7*
Squirrel monkeys			
0.4	MDMA	250.5 ± 128.8	850.8 ± 621.0
	HHMA	619.0 ± 145.3	3763.8 ± 1125.0
	HMMA	1442.3 ± 228.0	6138.8 ± 1100.8
0.8	MDMA	390.9 ± 116.0	1642.8 ± 726.9
	HHMA	278.0 ± 38.9†	3458.6 ± 407.0
	HMMA	718.4 ± 69.5†	5751.4 ± 1316.3
1.6	MDMA	452.3 ± 142.5	2416.4 ± 556.9†
	HHMA	184.3 ± 32.4†	2260.8 ± 363.1†‡
	HMMA	408.1 ± 51.9†‡	3790.3 ± 465.1†‡
2.8	MDMA	569.5 ± 105.6†	4585.4 ± 765.9†‡§
	HHMA	92.9 ± 51.9†‡	1548.8 ± 261.5†‡
	HMMA	201.4 ± 40.7†‡§	2346.0 ± 481.1†‡

C_{max} and AUC were normalized by dividing by the dose (mg/kg) of MDMA administered. Doses for squirrel monkeys shown below are expressed as human equivalents and correspond to absolute doses of 1.4, 2.8, 5.7, and 10.0 mg/kg (see Materials and Methods for interspecies dose scaling). Normalized pharmacokinetic parameters in humans were compared using a paired Student *t* test, whereas those in squirrel monkeys were compared by means of repeated-measures analysis of variance followed by Tukey multiple comparison test.

*Significantly different from corresponding analyte parameter after 1.0 mg/kg MDMA.
†Significantly different from corresponding analyte parameter after 0.4 mg/kg MDMA.
‡Significantly different from corresponding analyte parameter after 0.8 mg/kg MDMA.
§Significantly different from corresponding analyte parameter after 1.6 mg/kg MDMA.

The present findings also highlight the need to define which pharmacokinetic parameter is most important for the drug effect of interest. For example, in the case of MDMA-induced serotonin neurotoxicity, it will be important to determine whether AUC or C_{max} is a better predictor of serotonin neurotoxicity damage. On the one hand, if C_{max} is the best predictor of subsequent neurotoxicity, the present data suggest that there is a margin of safety between typical single recreational and neurotoxic doses, because peak plasma concentrations after single doses of MDMA that produce neurotoxicity in squirrel monkeys (700–800 ng/mL)[11] are 2 to 3 times higher than those that generally develop in humans after typical doses (200–300 ng/mL).[4,12] On the other hand, if AUC is a better predictor of MDMA-induced neurotoxicity, the present data indicate that the margin of safety may be narrower than that predicted by C_{max} values because AUCs in humans after typical single oral recreational doses (3070 ± 673 ng/mL·h) are not significantly different from those in squirrel monkeys after MDMA doses producing serotonin neurotoxic effects [(3866 ± 891 ng/mL·h) Mueller M, MS, Yuan J, MD, and Ricaurte G, MD, PhD, unpublished data]. Given that both a threshold peak plasma concentration (C_{max}) and a threshold duration of exposure (AUC) are likely to be important for MDMA-induced serotonin neurotoxicity, it may well be that no single pharmacokinetic parameter is an optimal predictor and that a combination of pharmacokinetic parameters (eg, C_{max} and AUC) will be more relevant.

Concentration profiles of MDMA metabolites, here determined after conjugate cleavage, indicate that squirrel monkeys and humans metabolize MDMA in similar, but not identical, pathways and proportions. In particular, proportions of HHMA and MDA after MDMA doses are similar in the 2 species, whereas formation of HMMA conjugates is greater in the squirrel monkey. This pattern suggests that activity of cytochrome P_{450} (CYP_{450}) enzymes that convert MDMA to HHMA (mainly CYP_{450} 2D6 in humans[13–16]; the squirrel monkey orthologue CYP_{450} 2D6 has yet to be identified) is comparable in the 2 species, whereas activity of catecholamine-*O*-methyltransferase, which converts HHMA to HMMA, is greater in squirrel monkeys than in humans. Low levels of MDA after MDMA doses in both humans and squirrel monkeys suggest that *N*-demethylation is limited in both species. The importance of these similarities and differences in metabolite profiles will depend upon which compounds (ie, MDMA, HHMA, HMMA, or MDA) are involved in the biological process under investigation. With regard to MDMA-induced serotonin neurotoxicity, some data suggest that MDMA is more important (there is a high correlation between MDMA concentrations and subsequent serotonin neurotoxicity),[11] whereas other data suggest that metabolites may be involved.[17,18]

Results from this research demonstrate nonlinear MDMA pharmacokinetics in both humans and squirrel monkeys. Chu et al[2] were the first to describe nonlinear MDMA pharmacokinetics in rats. Subsequently, a study by de la Torre et al[12] suggested nonlinear MDMA pharmacokinetics in humans, but results were not definitive. Most recently, the study by Kolbrich et al[4] provided evidence of nonlinear MDMA pharmacokinetics. However, HHMA, the metabolite directly affected by CYP_{450} 2D6 inhibition/saturation was not measured in that study[4] or in de la Torre's study.[12] In the present study, HHMA determination was included, and its pharmacokinetic profile at different MDMA doses is presented (Fig 2, Table 2). With HHMA pharmacokinetic parameters remaining constant despite increasing MDMA doses, the present findings provide the first clear-cut evidence of nonlinear MDMA pharmacokinetics in humans.

The relative proportion of MDMA to metabolites was highly dependent on dose. This was more evident in squirrel monkeys than in humans, no doubt because a broader dose range was tested in monkeys. At the lowest dose tested in the squirrel monkey (1.4 mg/kg, estimated to be equivalent to 0.4 mg/kg in humans), levels of HHMA and HMMA exceeded those of MDMA (Fig. 3). Then, as the dose of MDMA was increased, MDMA levels rose and surpassed those of HHMA and HMMA, which remained constant across doses. In humans, the pattern was somewhat different in that levels of HHMA and HMMA never significantly exceeded those of MDMA, at either of the doses tested (1.0 and 1.6 mg/kg) (Fig. 3). However, as the dose of MDMA was increased, MDMA levels rose and exceeded those of HHMA and HMMA, which again remained constant across doses. In all likelihood, had

a lower dose of MDMA been tested in humans, levels of HHMA and HMMA would have exceeded those of MDMA, as was the case in squirrel monkey (Fig. 3) and in humans given lower MDMA doses than those tested in this study.[12,19]

The present results suggest that plasma MDMA concentrations of 125–150 ng/mL are sufficient to inhibit metabolism of MDMA to HHMA and HMMA, in both humans and squirrel monkeys. This is evidenced by the fact that once these plasma MDMA concentrations (125–150 ng/mL) were achieved, plasma HHMA and HMMA concentrations remained constant despite increases in MDMA dose, leading to nonlinear increases in plasma MDMA concentrations in both species (Fig. 3). Rising levels of MDMA with little or no change in HHMA and HMMA concentrations are consistent with inhibition or impairment of CYP P_{450}-mediated conversion of MDMA to HHMA and HMMA. The precise plasma MDMA concentration at which impairment of MDMA metabolism begins remains to be determined. However, the observation that MDMA metabolism already appears impaired at plasma MDMA concentrations of 125–150 ng/mL indicates that nonlinear MDMA accumulation, a direct consequence of impaired MDMA metabolism, is not an overdose phenomenon. In other words, it suggests that inhibition of MDMA metabolism is likely to occur after typical human MDMA doses (1–2 mg/kg) because these are known to engender plasma MDMA concentrations of 150–400 ng/mL.[3,4] The fact that typical MDMA doses have, in effect, the potential to convert all MDMA consumers into "poor" MDMA metabolizers may help explain the apparent lack of impact of CYP_{450} 2D6 deficiency on the pharmacologic and toxic effects of MDMA in human users.[15] As discussed elsewhere,[8,12] nonlinear MDMA accumulation could have significant public health implications if adverse effects of MDMA are related to parent compound concentrations rather than its metabolites.

CONCLUSIONS

Comparison of MDMA metabolism and disposition in squirrel monkeys and humans indicates that the squirrel monkey is a good, but not perfect, model for predicting outcomes in humans. Positive aspects of the model include similar (although not identical) metabolic profiles, development of nonlinear MDMA pharmacokinetics, and the fact that nonlinear pharmacokinetics in squirrel monkeys occurs at similar plasma concentrations as in humans. The shorter $T_{1/2}$ of MDMA in the squirrel monkey is a drawback of the model. The utility of the squirrel monkey as a model for understanding MDMA effects in humans will depend upon the particular pharmacokinetic parameter of MDMA or its metabolites that most strongly influences the outcome measure of interest, and on the degree to which MDMA pharmacodynamics in the squirrel monkey parallels that in humans.

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