Non-linear Pharmacokinetics of MDMA ("Ecstasy") and its Major Metabolites in Squirrel Monkeys at Plasma Concentrations of MDMA that Develop After Typical Psychoactive Doses

Melanie Mueller, Frank T. Peters, Hans H. Maurer, Una D. McCann and George A. Ricaurte

Department of Neurology (MM, GR) and Psychiatry and Behavioral Sciences (UM), Johns Hopkins University School of Medicine, Baltimore, MD 21224; Department of Experimental and Clinical Toxicology (MM, FP, HM), Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, D-66421 Homburg (Saar), Germany
Running Title: Dose-related changes in MDMA pharmacokinetics in primates

Corresponding Author:

George A. Ricaurte, M.D., Ph.D.
Department of Neurology
Johns Hopkins Medical Institutions
5501 Hopkins Bayview Circle, Rm. 5B.71E
Baltimore, MD 21224
Tel: 410-550-0993
E-mail: Ricaurte@jhmi.edu
Fax: 410-550-2005

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List of nonstandard abbreviations:

- MDMA – 3, 4-methylenedioxyamphetamine
- HHMA - 3, 4-dihydroxymethamphetamine
- HMMA - 4-hydroxy-3-methoxymethamphetamine
- HHA - 3, 4-dihydroxyamphetamine
- MDA - 3, 4-methylenedioxyamphetamine
- $C_{\text{max}}$ - maximal concentration
- AUC - Area under the curve
- $T_{1/2}$ - Half-life

Recommended section assignment: Metabolism
Abstract

At certain doses, the psychoactive drug (±) 3, 4-methylenedioxymethamphetamine (MDMA, “Ecstasy”) destroys brain serotonin axon terminals. By causing increases in plasma MDMA concentrations that exceed those predicted by the increase in dose, non-linear pharmacokinetics has the potential to narrow the range between safe and neurotoxic doses of MDMA. The present study sought to determine if the pharmacokinetics of MDMA in non-human primates are non-linear and, if they are, to identify plasma concentrations of MDMA at which non-linear accumulation of MDMA occurs. Four different oral doses of MDMA were tested in the same six squirrel monkeys, in random order. At each dose, pharmacokinetic parameters for MDMA and its metabolites 3, 4-dihydroxymethamphetamine (HHMA), 4-hydroxy-3-methoxymethamphetamine (HMMA) and 3, 4-methylenedioxyamphetamine (MDA) were determined. Doses were selected to be equivalent to 0.4, 0.8, 1.6 and 2.8 mg/kg doses in humans. The maximal concentration (Cmax) and area under the curve (AUC) of MDMA increased non-linearly with dose, whereas the Cmax and AUC of HHMA and HMMA remained relatively constant. Non-linear MDMA pharmacokinetics occurred at plasma MDMA concentrations of 100 to 300 ng/ml and above. The half-life (T1/2) of MDMA and its metabolites also increased with dose. These results firmly establish non-linear pharmacokinetics for MDMA in squirrel monkeys and indicate that non-linear MDMA accumulation occurs at plasma MDMA concentrations that develop in humans taking typical doses. By raising MDMA concentrations and prolonging its action, non-linear pharmacokinetics and T1/2 prolongation, respectively, may influence the likelihood and severity of MDMA toxicities (including brain serotonin neurotoxicity).
Introduction

Despite a large body of research demonstrating that the popular psychoactive drug, (±) 3, 4-methylenedioxyamphetamine (MDMA, “Ecstasy”) has the potential to destroy brain serotonin (5-HT) axon terminals (see Green et al., 2003; Quinton and Yamamoto, 2006, for reviews), recreational use of MDMA continues and, in recent years, numerous laboratories have begun testing various pharmacologic effects of MDMA in humans (see Dumont and Verkees, 2006, for review). Four clinical trials involving MDMA use are also underway (clinicaltrials.gov identifiers NCT00252174, NCT00090064, NCT00402298, and NCT00353938). MDMA use and abuse continue, at least in part, because of uncertainty regarding the clinical relevance of much of the animal research on MDMA-induced serotonin neurotoxicity. As we (Mechan et al., 2006) and others (Easton and Marsden, 2006) have discussed, this uncertainty stems from the fact that the majority of animal studies have used multiple high doses, have given these doses systemically rather than orally (as taken by humans) and, most often, have used rodents (rats and mice), which metabolize MDMA differently than primates (Cho and Kumangai, 1994) (see Figure 1).

To begin bridging the gap between MDMA neurotoxicity studies in animals and human MDMA use patterns, we recently characterized the pharmacokinetic profile of oral doses of MDMA in nonhuman primates, and compared results in squirrel monkeys to those in humans (Mechan et al., 2006). As might be expected, the biologic half-life ($T_{1/2}$) of MDMA in squirrel monkeys was shorter than in humans, due to the much smaller body mass of the squirrel monkey. However, other aspects of the pharmacokinetics and metabolism of MDMA in squirrel monkeys
resembled those in humans, including the ratio of 3, 4-methylenedioxyamphetamine (MDA) to MDMA (3–5 / 100). Notably, plasma concentrations of MDMA in squirrel monkeys that developed neurotoxicity were only two to three times higher than those that develop in humans given single 100–150 mg doses (Mechan et al., 2006), suggesting that the margin of safety of MDMA (at least with respect to brain serotonin neurotoxicity) is narrow.

Cytochrome P<sub>450</sub> 2D6 (CYP<sub>450</sub> 2D6) isoenzymes participates in the oxidative metabolism of MDMA (Tucker et al., 1994; Ramamoorthy et al., 2002). In particular, CYP<sub>450</sub> 2D6 isoenzymes are chiefly responsible for the demethylation of MDMA to HHMA (Figure 1). Approximately 7-10% of Caucasians have deficient CYP<sub>450</sub> 2D6 activity (Ingelman-Sundberg et al., 2007). Whether or not such individuals are more (or less) susceptible to effects of MDMA has been a subject of discussion (see Yang et al., 2006).

An interesting and potentially very important feature of MDMA is its apparent tendency to accumulate in a non-linear fashion (i.e., have non-linear pharmacokinetics). The reason that this feature is extremely important is because seemingly small or trivial increases in dose could translate into large increases in plasma concentrations and, thus, a greater likelihood of toxicity (e.g., hyperthermia, serotonin neurotoxicity, etc.). We recently noted a tendency for non-linear MDMA accumulation in squirrel monkeys (Mechan et al., 2006), and others have previously made similar observations in rats (Chu et al., 1996) and humans (de la Torre et al., 2000). However, our own studies in squirrel monkeys, like those of Chu et al., (1996) in rats, did not allow for accurate measurement of pharmacokinetic parameters, nor did they include measurements of its major metabolites 3,4-dihydroxymethamphetamine (HHMA) and 4-
hydroxy-3-methoxymethamphetamine (HMMA) (Mechan et al., 2006). The study by de la Torre and colleagues (2000) involved testing of different doses of MDMA in different individuals, and thus left open the possibility that apparent non-linear MDMA accumulation in humans due to individual differences rather than non-linear pharmacokinetics.

The purpose of the present studies was to determine if the pharmacokinetics of MDMA in non-human primates (squirrel monkeys) are non-linear and, if they are, to identify plasma concentrations of MDMA at which non-linear accumulation of MDMA occurs.

**Methods**

**Animals:** Six male adult squirrel monkeys (*Saimiri sciureus*) ranging in weight from 0.743 - 1.329 kg were used. Animals were housed in pairs (except during MDMA treatment, when they were housed singly) in standard steel cages, at an ambient temperature of 26 ± 3 °C and 20-40 % humidity, with free access to food and water. The colony room in which the animals were housed was maintained on a 14:10 h light:dark cycle (lights on: 07:00 h). The facilities for housing and care of the animals are accredited by the American Association for the Assessment and Accreditation of Laboratory Animal Care. Animal care and experimental 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.

**Drugs and Reagents:** Racemic MDMA hydrochloride was obtained through the National Institute on Drug Abuse drug supply program (Rockville, MD, USA). Racemic HHMA
hydrochloride and methanolic solution of racemic MDMA hydrochloride and racemic MDA hydrochloride were purchased from Lipomed (Cambridge, MA, USA). Methanolic solutions (1000 mg/l) of racemic HMMA and methanolic solutions (100 mg/l) of racemic MDMA-\(d_5\) and MDA-\(d_5\) were obtained from Cerilliant (Round Rock, TX, USA). 4-Hydroxymethamphetamine (pholedrine), 4-methylcatechol, and ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Sodium metabisulfite (SMBS) was obtained from E. Merck (Darmstadt, Germany). The authenticity of the MDMA, HHMA, HMMA and MDA samples used in the present studies was confirmed using liquid chromatographic/mass spectrometric (LC/MS) methods.

**MDMA dosing**: Each monkey received one of four doses of MDMA (on average, 6 weeks between doses), in random order. MDMA was given orally, and dose was expressed as the salt. For oral drug administration, animals were placed in a plexiglass restraining chair, and a number 8 French feeding tube was inserted and used to administer the drug by gavage. Doses used for this experiment were calculated to be equivalent to 0.4, 0.8, 1.6 and 2.8 mg/kg doses in a 70 kg human, using interspecies dose scaling methods, as below (for discussion of these methods, including their limitations, see Mordenti and Chappell, 1989; Mahmood, 1996; 1999). Each monkey was administered a dose calculated using their individual weight at the time of administration. Animal equivalents of doses of MDMA used by humans were calculated using a standard allometric equation, shown below, where \(D = \) dose in mg and \(W = \) weight of the animal in kg and 0.7 is a commonly used (empirically derived) exponent:

\[
D_{\text{human}} = D_{\text{animal}} \left(\frac{W_{\text{human}}}{W_{\text{animal}}}\right)^{0.7} 
\]
Dose in Human (70 kg) | ‘Equivalent’ dose in squirrel monkey (1 kg)
---|---
0.4 mg/kg | 1.4 mg/kg
0.8 mg/kg | 2.8 mg/kg
1.6 mg/kg | 5.7 mg/kg
2.8 mg/kg | 10.0 mg/kg

**Blood sampling:** For determination of plasma concentrations of MDMA (and its metabolites) and their pharmacokinetic profiles, blood was sampled at 0.75, 1.5, 3.0, 4.5, 6, 7, 9, 11, 23, and 25 h after MDMA administration. At each time point, approximately 0.7 ml of blood was collected as previously described (Mechan et al., 2006). Blood samples were dispensed into 5 ml Vacutainer7 hematology tubes, containing 0.057 ml 15 % EDTA solution (Becton-Dickinson, Franklin Lakes, NJ, USA), and stored on ice for up to 30 min, until centrifuged. Samples were centrifuged at 1100 x g for 10 min at 4 °C (Sorvall RC2-B, Kendro Laboratory Products, Newtown, CT, USA). Plasma was withdrawn using a 5 3/4 Pasteur pipette and decanted into a 1.5 ml microcentrifuge tube and SMBS (250 mM) was added at a volume of 30μl/ml plasma to minimize oxidation of the compounds of interest. Plasma samples were stored at -20 °C until assay.

**Plasma sample preparation:** Aliquots (100 μl) of squirrel monkey plasma were preserved with 20 μl of SMBS (250 mM) and 10 μl of EDTA (250 mM). After addition of 100 μl of an aqueous solution of the racemic internal standards MDMA-\(d_5\), MDA-\(d_5\), and pholedrine (1.0 μg/ml, each) and 300 μl of 0.5 M HCl, samples were mixed (15 s) on a rotary shaker and left at 100°C for 80 min to perform conjugate cleavage. After cooling to room temperature, 20 μl of 4-methylcatechol (1 mg/ml) was added, and samples were briefly vortexed. Then, perchloric
acid (10 µl) was added and the samples were mixed again on a rotary shaker for 15 s to perform protein precipitation. The samples were centrifuged (16 000g for 5 min), and the supernatant was transferred to autosampler vials. Aliquots (5 µl) were injected into the LC-MS system.

**Determination of MDMA, HHMA, HMMA and MDA Concentrations:** Plasma concentrations of MDMA, HHMA, HMMA and MDA were determined using a recently described LC-MS method modified to include acidic hydrolysis (Mueller et al., 2007). Values for HHMA and HMMA represent total free amounts (i.e., amounts measured after cleavage of sulfate and glucuronic acid conjugates).

**Calculation of Pharmacokinetic Parameters:** Peak plasma concentrations ($C_{\text{max}}$), times of peak plasma concentration ($T_{\text{max}}$), area under the concentration-time curve (AUC), and the elimination half-lives ($T_{1/2}$) were calculated using the software program WinNonlin™ (Pharsight Co., Mountain View, CA). At least three points in the declining portion of the curve were included in the calculation of $T_{1/2}$. A noncompartmental model with first order output and elimination was used.

**Statistics:** $C_{\text{max}}$ and AUC values for each analyte (MDMA, HHMA, HMMA and MDA) were normalized (by dividing by the dose of MDMA administered), then compared using repeated measures analysis of variance (ANOVA) and subsequent Tukey's multiple comparison test. $T_{1/2}$ and $T_{\text{max}}$ values at each dose were also compared using repeated measures analysis of variance (ANOVA) and subsequent Tukey's multiple comparison test. Expected versus observed $C_{\text{max}}$ and AUC values of MDMA and its major metabolites (HHMA and HMMA) at various
MDMA doses were compared by means of paired t-test. Expected values were calculated by multiplying the observed C\text{max} or AUC values at the lowest dose (0.4 mg/kg) by the proportionate increase in dose. Statistical analyses were performed using GraphPad Prism Version 3.02. Differences were considered significantly different if P < 0.05 (two-tailed).

**Results**

Results of pharmacokinetic studies in six squirrel monkeys, each of which received four different oral doses of MDMA in random order (with an average interval of 6 weeks between each dose) are shown in *Table 1*. As might be expected, absolute C\text{max} and AUC values of MDMA increased with dose. To determine if the observed increases were linear or non-linear, the values (C\text{max} and AUC) were normalized by dividing by the corresponding dose. If increases in C\text{max} and AUC were linear, normalized C\text{max} and AUC values would be expected to remain constant across dose (because, under linear conditions, plasma levels would rise in direct proportion to dose). As can be seen in *Table 1*, normalized C\text{max} and AUC values of MDMA did not adhere to this expectation. Instead, normalized C\text{max} and AUC values of MDMA increased significantly with dose. *T*\text{max} values of MDMA did not change as a function of dose. The T\text{1/2} of MDMA was significantly longer at the highest dose tested (2.8 mg/kg) (*Table 1*).

In sharp contrast to what was observed with MDMA, normalized C\text{max} and AUC values of HHMA decreased significantly with dose (*Figure 2*, *Table 1*). The *T*\text{max} of HHMA did not change with dose. Similar to MDMA, the T\text{1/2} of HHMA was longer at the highest dose tested (2.8 mg/kg) (*Table 1*).
Results with HMMA paralleled those with HHMA (Figure 2, Table 1).

Results with MDA were unique in that some aspects paralleled those with HHMA and HMMA and others paralleled those with MDMA. In particular, as with normalized C\textsubscript{\text{max}} values of HHMA and HMMA, normalized C\textsubscript{\text{max}} values of MDA decreased significantly with dose (Table 1), whereas normalized AUC values of MDA (like those of MDMA) increased significantly with dose. The T\textsubscript{\text{max}} and T\textsubscript{1/2} of MDA remained constant across dose.

Figure 3 shows the relative proportions of MDMA and its major metabolites at various times after administration of different oral doses of MDMA. At the lowest dose of MDMA tested (0.4 mg/kg), levels of MDMA were only one-half to one-sixth of those of HHMA and HMMA at comparable time points, respectively. As the dose of MDMA was increased, there was a clear shift in this pattern, with the relative proportion of MDMA increasing sharply, while levels of HMMA and HHMA remained relatively constant (Figure 3).

Notably, once levels of approximately 100 ng/ml of MDMA were achieved, plasma concentrations of HHMA and HMMA remained relatively constant, even though levels of MDMA rose sharply (Figure 3).

Discussion

The present results are the first to provide unequivocal evidence of non-linear pharmacokinetics for MDMA in non-human primates. Our findings in squirrel monkeys are in good agreement with those of Chu and colleagues (1996) in rats and speak to the species
generality of non-linear MDMA pharmacokinetics. Species generality of non-linear MDMA pharmacokinetics is further demonstrated by findings in humans published while this paper was under review (Kolbrich et al., 2008). Unlike the previous study that had reported non-linear pharmacokinetics of single doses of MDMA in humans (de la Torre et al., 2000), the study by Kolbrich and colleagues (2008) tested the same subjects at two different doses, and thus eliminated the possibility that non-linear MDMA accumulation might be due to individual differences rather than non-linear pharmacokinetics. Taken together, these results indicate that the phenomenon of non-linear MDMA pharmacokinetics has broad species generality.

In addition to documenting non-linear pharmacokinetics of MDMA, we demonstrate that the pharmacokinetics of HHMA and HMMA, the two major phase I metabolites of MDMA in primates (Cho and Kumangai, 1994; de la Torre et al., 2004), are altered. In particular, despite disproportionate increases in plasma MDMA concentration, plasma concentrations of HHMA and HMMA remained relatively constant (Figure 3) and, once $C_{\text{max}}$ and AUC values of HHMA and HMMA were dose-normalized, significant decreases in $C_{\text{max}}$ and AUC values of HHMA and HMMA became evident (Table 1). This contrasts sharply with dose-normalized pharmacokinetic parameters for MDMA, which increase with dose (Figure 2). Considered together, these findings suggest that the non-linear pharmacokinetics of MDMA are probably related to inhibition (or saturation) of the metabolic step that converts MDMA to HHMA: CYP$_{450}$ 2D6-mediated ring demethylation (Tucker et al, 1994; Kreth et al., 2000; Maurer et al., 2000; Ramamoorthy et al., 2002) (Figure 1), recognizing that the nomenclature for CYP$_{450}$ 2D6 enzymes is not necessarily the same for squirrel monkeys as humans. This suggestion is in keeping with observations that MDMA has the potential to inhibit CYP$_{450}$ 2D6 isoenzymes in
*vitro* (Heydari et al., 2004; Yang et al., 2006; Van et al., 2007). Inhibition or saturation of 
CYP<sub>450</sub> 2D6-mediated ring demethylenation is not the only possible way that non-linear MDMA 
pharmacokinetics could occur, although other metabolic mechanisms that may be involved 
remain to be identified.

MDA, a relatively minor metabolite of MDMA in primates (Cho and Kumangai, 1994; 
de la Torre et al., 2004; Mechan et al., 2006), displayed unusual dose-related pharmacokinetic 
changes. In particular, while the normalized C<sub>max</sub> of MDA decreased with dose, its normalized 
AUC increased with dose (*Table 1*). The decrease in normalized C<sub>max</sub> of MDA parallels the 
decrease in normalized C<sub>max</sub> of the other metabolites of MDMA (HHMA and HMMA), and may 
be due to decreased MDA formation. Whether this is due to impaired \(N\)-demethylation of 
MDMA to MDA remains to be determined. If it is, this would suggest that MDMA is relatively 
non-specific in its ability to inactivate or saturate CYP<sub>450</sub> enzyme systems responsible for 
MDMA metabolism, because separate and distinct CYP<sub>450</sub> enzyme systems are believed to be 
responsible for \(N\)-demethylation and ring demethylenation (Kreth et al., 2000). In contrast to the 
decrease in normalized C<sub>max</sub>, there was an increase in the normalized AUC of MDA with dose, 
suggesting that, over time, metabolism of MDA to 3, 4-dihydroxyamphetamine (HHA) (possibly 
by the same CYP<sub>450</sub> enzyme system that converts MDMA to HHMA) is impaired. Assuming 
this that occurs, the accumulation of MDA would become non-linear, much in the same way that 
the accumulation of MDMA becomes non-linear. Additional studies are needed to investigate 
these possibilities.
A key question regarding non-linear MDMA pharmacokinetics is: at what dose or plasma concentration of MDMA does it occur? The present results indicate that non-linear MDMA accumulation occurs at plasma concentrations of MDMA of 100 to 300 ng/ml and above. Once these plasma MDMA concentrations are achieved, plasma concentrations of HHMA and HMMA cease to increase (Figure 3). Of note, plasma concentrations in the range of 100 to 300 ng/ml of MDMA are the norm after doses of MDMA that produce psychoactive effects in humans (Hemlin et al., 1996; de la Torre et al., 2000; Pacifici et al., 2001; Peters et al., 2003), suggesting that nonlinear MDMA accumulation takes place within the range of doses typically used by humans, either on the street or in the research laboratory.

In addition to demonstrating non-linear MDMA pharmacokinetics and identifying plasma concentrations of MDMA at which non-linear accumulation occurs, the present studies are the first reveal that the T1/2 of MDMA in the squirrel monkey lengthens with dose. Although lengthening of the T1/2 of MDMA was only significant at the highest dose tested (Table 1), there was a clear trend toward lengthening at lower doses as well. This was also the case for HHMA and HMMA (Table 1). A longer T1/2 of MDMA at high dosage is noteworthy because it would effectively prolong the length of time that target sites are exposed to potentially toxic drug concentrations. Although direct confirmatory data are still needed, we suspect that non-linear MDMA accumulation and T1/2 prolongation are both related to impairment of MDMA demethylenation (Figure 1). Consistent with this proposal is the observation that paroxetine, which impairs MDMA demethylenation (apparently by competing for CYP450 2D6 enzymes), increases both peak concentrations and T1/2 of MDMA in humans (Segura et al., 2005).
The present results allow comment on the accuracy of interspecies dose scaling. Principles of interspecies dose scaling dictate that to arrive at comparable doses in animals and humans, it is important to take into account differences in body mass (see Mordenti and Chappell, 1989; Mahmood, 1996, 1999). Therefore, as in past studies, we used interspecies dose scaling to calculate doses for squirrel monkeys that would be comparable to 0.4, 0.8, 1.6 and 2.8 mg/kg doses in humans. Comparison of plasma concentrations achieved in squirrel monkeys to those reported in humans (Hemlin et al., 1996; de la Torre et al., 2000; Peters et al., 2003; Kolbrich et al., 2008) reveals that, on average, peak plasma MDMA concentrations in squirrel monkeys were approximately two-fold higher. This observation indicates that interspecies dose-scaling procedures, while useful for compensating for differences in body mass, are not perfect, and underscores the importance of measuring actual drug plasma concentrations rather than relying solely on estimated dose equivalents.

Basic and clinical implications of the present findings remain to be determined. If the parent compound (MDMA) is chiefly responsible for pharmacological and toxic effects of MDMA (e.g., increased blood pressure, elevated body temperature, serotonin neurotoxicity), the present results suggest that seemingly small or trivial increases in dose could result in unexpected toxicities, because of dose-disproportionate increases in plasma MDMA concentrations. Alternatively, if major metabolites (HHMA, HMMA) are chiefly responsible for the effects of MDMA, the present results suggest that MDMA accumulation per se is not a source of concern, but that increases in dose would lead to increased exposure to potentially toxic levels of HHMA and HMMA, due to $T_{1/2}$ prolongation of both of these metabolites (Table 1). However, if metabolite toxicity is a function of their Cmax or AUC to a greater extent than
duration of exposure (T1/2), then the dose-dependent kinetics reported may not aggravate toxicities because the AUC of HHMA and HMMA did not increase, and their Cmax decreased (Table 1). With specific reference to brain serotonin neurotoxicity, it remains to be determined if MDMA or one of its metabolites is primarily responsible. Some findings point to (but do not establish) the importance of the parent compound (dose-dependency, high correlation between MDMA levels and subsequent serotonin neurotoxicity - see Mechan et al., 2006), while others suggest a possible role for metabolites (Monks et al., 2004; Erives et al., 2008). By exploring the relationship among MDMA, its metabolites and serotonin neurotoxicity in the same animal in the context of non-linear MDMA accumulation, it should be possible to begin discerning the relative importance of MDMA versus metabolites in the neurotoxic process.

In summary, the results of this study are the first to firmly establish non-linear pharmacokinetics for MDMA in non-human primates (squirrel monkeys) and to show that the half-lives of MDMA and its major metabolites (HHMA and HMMA) increase with dose. Whether these dose-related pharmacokinetic changes (non-linear accumulation and T1/2 prolongation) influence the likelihood and severity of MDMA toxicities remains to be determined. It also remains to be determined if in the context of non-linear pharmacokinetics there is preferential metabolism of the S-(+)- or R-(-)-enantiomer of MDMA. Of particular concern is the possibility that non-linear MDMA pharmacokinetics, by causing disproportionate increases in plasma MDMA concentrations, narrows the already small gap that appears to exist between safe and neurotoxic doses of MDMA in primates. Additional studies are needed to explore relationship between pharmacokinetic parameters of the parent drug (MDMA) and its metabolites (HHMA, HMMA) and brain serotonin neurotoxicity, to identify threshold neurotoxic
doses (and associated plasma drug concentrations) of MDMA, and to assess the influence of non-linear MDMA accumulation on the development of brain serotonin neurotoxicity.
Acknowledgments

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References


Erives GV, Lau SS, Monks TJ (2008) Accumulation of neurotoxic thioether metabolites of


Footnotes

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Legends for Figures

Figure 1: MDMA and its metabolism to HHMA, HMMA, MDA and HHA. In primates (humans and squirrel monkeys) ring-demethylenation predominates (as depicted by the larger arrow); in rodents (rats and mice), N-demethylation is more prominent (see Cho and Kumangai, 1994). Also shown are the CYP_{450} isoenzymes involved in these metabolic conversions in humans (see Kreth et al., 2000; Maurer et al., 2000).

Figure 2: Expected versus observed C_{max} (top panels) and AUC (bottom panels) values of MDMA and its major phase I metabolites, HHMA and HMMA (after conjugate cleavage), at various MDMA doses. Each dose, expressed as the human equivalent dose, was tested in the same six squirrel monkeys, as detailed in Methods. Expected values were calculated by multiplying the observed C_{max} or AUC values at the lowest dose (0.4 mg/kg) by the proportionate increase in dose. Note that while observed C_{max} and AUC values of MDMA are greater than those expected, C_{max} and AUC values of HHMA and HMMA are less than those expected. Values are the mean ± SD. *Designates significant difference at P < 0.05; **Designates significant difference at P < 0.01; ***Designates significant difference at P < 0.001 (paired t-test).

Figure 3: Relative proportions of MDMA and its metabolites HHMA and HMMA (after conjugate cleavage) at various times after administration of different oral doses of MDMA to squirrel monkeys. Values are the mean ± SD (n=6). Neither weighting functions nor statistical objectivity were used to decide on the best fit lines. Note that the time course and concentration
of each compound is dependent on dose of MDMA administered and that, despite marked increases in plasma MDMA concentrations with dose, plasma concentration of HHMA and HMMA remain relatively constant. Also note that once levels of approximately 100 ng/ml of MDMA are achieved, plasma concentrations of HHMA and HMMA remain relatively constant, while MDMA concentrations continue to rise, suggesting inhibition or saturation of MDMA metabolism.
Table 1: Pharmacokinetic parameters of MDMA and its metabolites in the same squirrel monkeys (n=6) given different oral doses of MDMA. On average, there was an interval of 6 weeks between the testing of each dose. Doses are expressed as human equivalents of 0.4, 0.8, 1.6 and 2.8 mg/kg (see Methods). At the lowest dose of MDMA tested (0.4 mg/kg), MDA concentrations were below the level of quantitation (LOQ: 10 ng/ml) and are, therefore, not shown. Values represent the mean (± SD). C_{max} and AUC values were normalized by dividing by the dose administered.

<table>
<thead>
<tr>
<th>Dose of MDMA</th>
<th>Analyte</th>
<th>C_{max} (ng/mL)</th>
<th>Normalized C_{max}</th>
<th>AUC (ng/mL*h)</th>
<th>Normalized AUC</th>
<th>T_{max} (h)</th>
<th>T_{1/2} (h)</th>
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</thead>
<tbody>
<tr>
<td>0.4 mg/kg</td>
<td>MDMA</td>
<td>100.2 ± 51.5</td>
<td>250.5 ± 128.8</td>
<td>340.3 ± 248.4</td>
<td>850.8 ± 621.0</td>
<td>1 ± 0.4</td>
<td>1.8 ± 0.9</td>
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<td></td>
<td>HHMA</td>
<td>247.6 ± 58.1</td>
<td>619 ± 145.3</td>
<td>1505.5 ± 450.0</td>
<td>3763.8 ± 1125.0</td>
<td>2.3 ± 1.3</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>HMMA</td>
<td>576.9 ± 91.2</td>
<td>1442.3 ± 228</td>
<td>2455.5 ± 440.3</td>
<td>6138.8 ± 1100.8</td>
<td>1.3 ± 0.4</td>
<td>2.5 ± 0.8</td>
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<tr>
<td>0.8 mg/kg</td>
<td>MDMA</td>
<td>312.7 ± 92.8</td>
<td>390.9 ± 116</td>
<td>1314.2 ± 581.5</td>
<td>3763.8 ± 1125.0</td>
<td>1.1 ± 0.4</td>
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<tr>
<td></td>
<td>HHMA</td>
<td>222.4 ± 31.1</td>
<td>278 ± 38.9^{1}</td>
<td>2766.9 ± 325.6</td>
<td>3458.6 ± 407.0</td>
<td>4 ± 0.8</td>
<td>4.2 ± 0.5</td>
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<tr>
<td></td>
<td>HMMA</td>
<td>574.7 ± 55.6</td>
<td>718.4 ± 69.5^{1}</td>
<td>4601.1 ± 1053</td>
<td>5751.4 ± 1316.3</td>
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<tr>
<td></td>
<td>MDA</td>
<td>12.0 ± 4.0</td>
<td>15.0 ± 5.0</td>
<td>83.8 ± 36.8</td>
<td>104.8 ± 46.0</td>
<td>3 ± 1.3</td>
<td>6.6 ± 4.6</td>
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<tr>
<td>1.6 mg/kg</td>
<td>MDMA</td>
<td>723.6 ± 228^{1,2}</td>
<td>452.3 ± 142.5</td>
<td>3866.2 ± 891.0^{1,2}</td>
<td>2416.4 ± 556.9^{1}</td>
<td>1.3 ± 0.9</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>HHMA</td>
<td>294.8 ± 51.8</td>
<td>184.3 ± 32.4^{1}</td>
<td>3617.2 ± 581.0</td>
<td>2260.8 ± 363.1^{12}</td>
<td>3 ± 0.8</td>
<td>5.3 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>HMMA</td>
<td>653.0 ± 83.0</td>
<td>408.1 ± 51.9^{1,2}</td>
<td>6064.5 ± 744.1</td>
<td>3790.3 ± 465.1^{12}</td>
<td>1.5 ± 0.0</td>
<td>5.5 ± 1.2^{1}</td>
</tr>
<tr>
<td></td>
<td>MDA</td>
<td>21.9 ± 6.4^{2}</td>
<td>13.7 ± 4.0</td>
<td>185.7 ± 60.3</td>
<td>116.1 ± 37.7</td>
<td>3 ± 0.9</td>
<td>7.9 ± 2.5</td>
</tr>
<tr>
<td>2.8 mg/kg</td>
<td>MDMA</td>
<td>1594.5 ± 295.6^{1,2,3}</td>
<td>569.5 ± 105.6^{1}</td>
<td>12839.2 ± 2144.6^{1,2,3}</td>
<td>4585.4 ± 765.9^{1,2,3}</td>
<td>1.3 ± 0.9</td>
<td>4.2 ± 1.5^{1,2}</td>
</tr>
<tr>
<td></td>
<td>HHMA</td>
<td>260.1 ± 41.5</td>
<td>92.9 ± 51.9^{1,2}</td>
<td>4336.7 ± 732.3^{1,2}</td>
<td>1548.8 ± 261.5^{1,2}</td>
<td>3.8 ± 0.8</td>
<td>12.9 ± 4.4^{1,2,3}</td>
</tr>
<tr>
<td></td>
<td>HMMA</td>
<td>564 ± 113.9</td>
<td>201.4 ± 40.7^{1,2,3}</td>
<td>6568.9 ± 1347.1^{1,2,3}</td>
<td>2346 ± 481.1^{1,2}</td>
<td>1.8 ± 0.6</td>
<td>9.4 ± 2.5^{1,2,3}</td>
</tr>
<tr>
<td></td>
<td>MDA</td>
<td>29.7 ± 8.4^{2}</td>
<td>10.6 ± 3.0^{1,2}</td>
<td>452.4 ± 110.1^{2,3}</td>
<td>161.6 ± 39.3^{2,3}</td>
<td>5.7 ± 1.0^{2,3}</td>
<td>8.9 ± 2.9</td>
</tr>
</tbody>
</table>

1Significantly different from 0.4 mg/kg; repeated measures ANOVA followed by Tukey's Multiple Comparison test.
2Significantly different from 0.8mg/kg; repeated measures ANOVA followed by Tukey's Multiple Comparison test.
3Significantly different from 1.6 mg/kg; repeated measures ANOVA followed by Tukey's Multiple Comparison test.
Figure 1

MDMA → CYP2D6/CYP1A2/CYP3A4 → HHMA → COMT → HMMA

MDA → CYP1A2/CYP2B6 → HHA

CYP2D6/CYP1A2/CYP3A4 → HHA