Mice lacking multidrug resistance protein 1a (mdr1a) are protected from methylenedioxymethamphetamine (MDMA)-induced neurotoxicity, suggesting mdr1a might play an important role in this phenomenon. We characterized MDMA pharmacokinetics in murine plasma and brain to determine if mdr1a alters MDMA distribution. Wild-type (mdr1a +/+ ) and mdr1a knock-out (mdr1a −/− ) mice received i.p. 10, 20 or 40 mg/kg MDMA. Plasma and brain specimens were collected 0.3–4 h after MDMA, and striatum were dissected. MDMA and metabolites were quantified in plasma and striatum by gas chromatography–mass spectrometry. MDMA maximum plasma concentrations (C_{max}) for both strains were 916–1363, 1833–3546, and 5979–7948 µg/L, whereas brain C_{max} were 6673–14,869, 23,428–29,433, and 52,735–66,525 µg/kg after 10, 20, or 40 mg/kg MDMA, respectively. MDMA and metabolite striatum/plasma AUC ratios were similar in both strains, inconsistent with observed MDMA neuroprotective effects in mdr1a −/− mice. Ratios of methylenedioxyamphetamine (MDA) and 4-hydroxy-3-methoxymethamphetamine (HMMA) AUCs exceeded 18% of MDMA's in plasma, suggesting substantial MDMA hepatic metabolism in mice. MDMA, MDA, HMMA, and 4-hydroxy-3-methoxymethamphetamine maximum concentrations and AUCs exhibited nonlinear relationships during dose-escalation studies, consistent with impaired enzymatic demethylation. Nonlinear increases in MDMA plasma and brain concentrations with increased MDMA dose may potentiate MDMA effects and toxicity.

Introduction

Methylenedioxymethamphetamine (MDMA, ecstasy) is a ring-substituted amphetamine possessing stimulant and entactogenic properties with enhanced feelings of empathy, understanding and euphoria (1,2). In most species, MDMA's effects are serotonin-mediated with MDMA-induced depletions of serotonin and serotonin transport protein in brain (2). MDMA-induced toxicity in humans remains unclear (3), although clinical research suggests long-term learning and memory impairment in heavy users (4,5).

Multidrug resistance protein 1a (mdr1a, also known as p-glycoprotein) is a membrane-bound transport protein belonging to the ATP-binding cassette protein family. mdr1a facilitates excretion of xenobiotics (6,7) in kidney tubules, bile ducts and intestinal tract. mdr1a also is highly expressed in the blood-brain barrier (BBB) endothelium, where it transports xenobiotics out of the brain, thereby minimizing exposure and protecting brain cells from potential damage (6,7). Although MDR1a has wide substrate specificity, compounds are typically lipid soluble, positively charged at physiologic pH, and of 300 to 1000 molecular weight (7).

Our previously reported studies in mice lacking MDR1a protein (mdr1a −/− ) versus wild-type mice expressing MDR1a protein (mdr1a +/+ ), suggest that MDR1a plays a role in neurotoxicity caused by MDMA and that individuals with elevated MDR1a expression could be at increased risk of neurotoxicity (8,9). After 20 mg/kg MDMA, mdr1a −/− mice were protected from MDMA-induced decreases in dopamine uptake sites (DAT) in striatum compared to mdr1a +/+ mice (9). These results are inconsistent with MDR1a's well-characterized drug efflux action within the BBB (6), suggesting that MDR1a may be causing MDMA uptake across the BBB via a novel mechanism.

Upreti and Eddington (10) reported that MDR1a does not alter...
whole brain concentrations of MDMA and its metabolite methylenedioxyamphetamine (MDA) (10); however, MDMA effects are region specific. In a previous preliminary study, we found that MDR1a did not alter MDMA and MDA striatal distribution after MDMA (8); however, we were unable to collect plasma to evaluate potentially altered MDMA metabolism in mdr1a –/– mice. The primary objective of this study was to evaluate whether MDR1a alters MDMA metabolism and whether altered metabolism may have confounded interpretation of striatal MDMA and MDA concentrations during our preliminary studies.

MDMA metabolism is thought to play an important role in MDMA-induced neurotoxicity (11) (see Figure 1 for MDMA metabolic scheme). Peripheral MDMA administration produced neurotoxicity whereas direct injection into rat brain did not produce damage (11). In humans, 3,4-dihydroxymethamphetamine (HHMA) is a major metabolite of MDMA, formed mainly via cytochrome P450 2D6 (CYP2D6) (2). HHMA is converted via catechol-O-methyltransferase to 4-hydroxy-3-methoxymethamphetamine (HMMA) (12,13). MDMA displays nonlinear pharmacokinetics in human (14,15), squirrel monkey (16), and rat (17,18) MDMA dose-escalation studies, yielding higher maximum MDMA plasma concentrations (C_{max}) and area under the curves (AUCs) than predicted. Furthermore, HHMA and HMMA C_{max} did not increase as expected with increasing dose (14–17). MDMA auto-inhibition of CYP2D6 is the proposed mechanism for nonlinear MDMA pharmacokinetics (19). Nonlinear pharmacokinetics may play an important role in MDMA toxicity because MDMA C_{max} and AUC were correlated to serotonergic toxicity in rats (20).

It has been postulated that minimal MDMA metabolism or rapid urinary MDMA excretion in mice accounts for predominant dopamine toxicity in mice opposed to serotonergic toxicity in other species (21–23). MDMA distribution in mice is incompletely characterized; MDMA and metabolite excretion in mouse urine (21) and plasma distribution studies were evaluated at only two time-points or less (10,24,25). The most complete MDMA distribution study to date collected specimens for only 80 min after MDMA dosing (25). Therefore, the secondary aim of this research was to characterize MDMA pharmacokinetics in mouse plasma and striatum to determine if metabolism in mice differs from other species, and whether nonlinear pharmacokinetics also can be demonstrated in mice. Because there is not any clear evidence of neurotoxicity in humans (2), forensic toxicologists must extrapolate from preclinical studies during MDMA forensic cases. Our pharmacokinetic data in mice will assist toxicologists attempting to extrapolate MDMA exposure during pre-clinical mouse studies to forensic MDMA cases.

### Materials and Methods

#### Mice

Ten to 12-week-old male FVB (mdr1a +/+) and mdr1a –/– mice weighing 30–50 g were purchased from Taconic (Germantown, NY). Mice were housed individually in a temperature and humidity controlled environment with a 12 h light-dark cycle and ad libitum access to food and water. All animal use and care procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the National Institute on Drug Abuse, Intramural Research Program Animal Care and Use Committee.

#### Chemicals

Racemic MDMA-hydrochloride administered to mice was purchased from Lipomed (Cambridge, MA). Racemic mixtures of MDMA and methylenedioxyamphetamine (MDA) (1 g/L in methanol) and internal standards MDMA-d_{5} and MDA-d_{5} (100 mg/L) were from Cerilliant (Round Rock, TX). Racemic HMMA and 4-hydroxy-3-methoxymethamphetamine (HMA) (1 g/L in methanol) were obtained from Lipomed. Heptfluorobutyric acid anhydride and triethylamine were purchased from Pierce Chemical (Rockford, IL). All other reagents and solvents were reagent- or HPLC-grade obtained from JT Baker (Phillipsburg, NJ).

#### Experimental design

MDMA hydrochloride was dissolved in sterile saline at 1, 2, and 4 mg/mL concentrations (as hydrochloride salt) and
mice received 300–500 µL solution intraperitoneally (i.p.) to achieve the appropriate administered dose. mdr1a +/+ and mdr1a −/− mice were administered 10, 20, or 40 mg/kg MDMA-hydrochloride i.p. and euthanized via cervical dislocation 0.3, 1, 2, 3, or 4 h after MDMA administration (five mice per time point). Brains were removed, dissected on an ice-cold steel plate, immediately frozen on dry ice, and stored at −80°C. Trunk blood was collected from mice with a pasteur pipet, transferred to a heparinized tube, centrifuged at 1500 × g for 7 min, and plasma transferred to 1.5-mL screw-top polypropylene tubes for storage at −20°C.

**MDMA and metabolite analysis in plasma and striatum**

MDMA, MDA, HMMA, and HMA in plasma and striatum were quantified by gas chromatography–mass spectrometry (26). Plasma specimens (100 µL) were vortex mixed briefly after internal standard addition, and 800 µL of ice-cold 0.388 M trichloroacetic acid in water was added prior to centrifugation at 3000 × g at 4°C for 10 min. Supernatants were decanted into 10 mL reacti-vials. Pellets were re-suspended in 0.2 M trichloroacetic acid in water prior to centrifugation and supernatants were decanted into the same reacti-vials. Each striatum specimen was weighed, thawed and homogenized in ice-cold 0.05 M trichloroacetic acid/0.025 M thiourea. An aliquot containing the equivalent of 7.5 mg tissue was removed and diluted to 800 µL with ice-cold 0.05 M trichloroacetic acid/0.025 M thiourea. Supernatants were collected after centrifugation. Brain tissue pellets were re-suspended in 0.05 M trichloroacetic acid prior to centrifugation and supernatants were combined with the first fraction. Following acid precipitation of plasma and brain specimens, 100 µL of 12 M hydrochloric acid was added and samples incubated at 100°C for 45 min to hydrolyze glucuronidated and sulfated metabolites. These hydrolysis conditions achieved 60% efficiency for cleaving conjugated HMMA and HMA in mouse plasma relative to enzyme hydrolysis with β-glucuronidase from *Helix pomatia* (26). Therefore, total plasma concentrations of HMMA and HMA in our study are approximately 40% lower than reported here. Specimens were extracted and derivatized as described in Scheidweiler et al. (26).

Analysis was performed on an Agilent 6890 GC (Agilent Technologies, Wilmington, DE) with mass selective detector (Agilent 5975) operated in electron impact mode. See Scheidweiler et al. (26) for instrument parameters. To extend the linear dynamic range, calibrators, controls, and specimens were injected in pulsed splitless and pulsed split (1:5) modes to produce two calibration curves. In plasma, MDMA and MDA were linear from 10 to 1000 µg/L on the low curve and for HMMA and HMA from 20 to 1000 µg/L. High curves for MDMA and HMMA in plasma extended linearity from 1000 to 20,000 µg/L with split injections. In brain, linearity for MDMA, MDA, and HMMA were 100 to 10,000 and 200 to 10,000 µg/kg for HMA. The linear range for the MDMA high striatum curve with split injections was 10,000 to 200,000 µg/kg. Extraction efficiencies of MDMA, MDA, HMMA, and HMA from plasma and striatum were greater than 90%. Interassay analytical recovery (bias) for MDMA, MDA, HMMA, and HMA from plasma and striatum were between 90% and

![Figure 2](Image)
110% of target concentrations ($n = 14$). Interassay imprecision was less than 10% coefficient of variation for all four analytes ($n = 14$).

Pharmacokinetic and statistical analysis

One-half the limit of quantification was employed in all statistical analyses for specimens with concentrations below the assay limit of quantification. Bailer’s method for determining area under the concentration versus time curve (AUC$_{0–\text{last}}$) and standard error of AUC was employed to analyze results from this non-repetitive sampling study design (27). Comparisons between AUCs were made with $z$-tests calculated with Microsoft Excel, employing Bonferroni adjusted $p$-values equivalent to $p < 0.05$. Elimination half-lives ($t_{\text{1/2}}$) were calculated by non-compartmental analysis of mean concentration time data with WinNonlin (version 5.2, Pharsight, Mountain View, CA). To evaluate nonlinearity for $C_{\text{max}}$ and AUC, concentrations and AUCs following 10 mg/kg MDMA doses were multiplied by two or four to compare to results from 20 and 40 mg/kg MDMA doses, respectively, with one-sample $z$-tests calculated with Microsoft Excel.

**Results**

Elimination profiles of MDMA and metabolites in plasma

Figures 2 (circles) and 3 depict plasma elimination profiles of MDMA, MDA, HMMA and HMA in mdr1a $+/+$ and $–/–$ mice after a single i.p. dose of 10, 20 or 40 mg/kg MDMA. It should be noted that a specimen collected from one mdr1a $–/–$ mouse 3 h after 40 mg/kg MDMA appeared to contain abnormally elevated concentrations of MDMA, MDA, HMMA, and HMA (Figures 2 and 3). Plasma and striatal data from this mouse were not included in our calculations because plasma concentrations of all analytes differed from the mean by greater than three times the standard deviation. $C_{\text{max}}$ for MDMA and HMMA occurred prior to or in the first plasma specimen collected 0.3 h following all MDMA doses (Figures 2 and 3). MDMA and HMMA concentrations declined rapidly with half-lives ranging from 0.4 to 0.8 and 0.9 to 1.1 h, respectively (Table I). MDA $C_{\text{max}}$ occurred 1–2 h after MDMA administration (Figure 2) with half-lives ranging from 0.5 to 1.2 h. HMA concentrations never exceeded 140 µg/L, with $C_{\text{max}}$ 1–3 h after MDMA administration (Figure 3). Because of the low HMA concentrations and prolonged HMA excretion observed during this study, HMA $t_{\text{1/2}}$ could not be accurately determined.

Plasma AUCs increased significantly with escalating dose. MDMA and MDA AUCs after 40 mg/kg MDMA were 6.3–7.0 and 7.2–8.5 times greater than AUCs after 10 mg/kg MDMA, respectively, in both mdr1a $+/+$ and $–/–$ mice (Table II). Also, HMMA and HMA plasma AUCs were lower than expected after 40 mg/kg MDMA in both strains (only 2.6–2.8 and 3.2–3.5 times greater than AUCs after 10 mg/kg MDMA, respectively). Apparent MDMA and MDA plasma $C_{\text{max}}$ after 40 mg/kg MDMA were higher than predicted, as compared to those after 10 mg/kg MDMA (5.5–6.2 and 6.1–6.7 times greater; Table III), while apparent HMMA and HMA $C_{\text{max}}$ were lower than predicted in both strains (2.3–2.5- and 2.6–2.8-fold increases relative to 10 mg/kg MDMA) (Table III).
Elimination profiles of MDMA and MDA in striatum

Figure 2 (triangles) illustrates elimination profiles for MDMA and MDA in striatum of mdr1a +/+ and mdr1a –/– mice. Elimination profiles in striatum were similar to plasma, with apparent MDMA C_{max} observed in the first specimen collected 0.3 h after MDMA (Figure 2). MDMA striatal concentrations decreased rapidly with half-lives from 0.5 to 0.8 h. MDA striatal C_{max} occurred 1–2 h after MDMA (Figure 2), with MDA rapidly eliminated with half-lives of 0.5–1.2 h. HMMA and HMA striatal concentrations did not exceed 200 µg/kg in either mdr1a +/+ or –/– mice.

AUCs increased significantly with escalating doses in striatum, but similar to observations in plasma, MDMA and MDA AUCs after 40 mg/kg MDMA were greater than predicted (5.0–6.0 and 5.8–6.7 times, respectively, than AUCs after 10 mg/kg MDMA) in mdr1a +/+ and –/– mice (Table IV). Apparent MDMA striatal C_{max} also increased in a nonlinear manner during dose-escalation studies (Table V).

Evaluation of MDR1a-facilitated transport of MDMA and metabolites across BBB

Apparent MDMA C_{max} were significantly higher in mdr1a –/– as compared to +/+ mouse plasma after 20 and 40 mg/kg MDMA (Table III). Apparent MDA and HMMA plasma C_{max} were significantly elevated in mdr1a –/– compared to +/+ mice after 10 and 20 mg/kg MDMA (Table III). In striatum, the only statistically significant interstrain difference in apparent striatal C_{max} was observed for MDMA after the 20 mg/kg dose (Table V).

Interstrain comparisons of plasma AUCs revealed significantly higher MDMA and HMMA concentrations in mdr1a –/– mice after 20 and 40 mg/kg MDMA, while MDA plasma AUCs were significantly elevated in mdr1a –/– mice following 10 and 20 mg/kg MDMA (Table II). Following 20 mg/kg MDMA, HMA plasma AUCs were elevated in mdr1a –/– versus +/+ mice (Table II). In striatum, the only statistically significant interstrain difference for AUCs were higher MDMA AUCs in mdr1a –/– versus +/+ mice after 40 mg/kg MDMA (Table IV).

To help clarify the role of MDR1a in distribution of MDMA and metabolites into brain, striatum/plasma AUC ratios were calculated to normalize for altered circulating MDMA and metabolite concentrations in mdr1a –/– mice. These results are detailed in Table VI. Striatum/plasma ratios for both MDMA and MDA appear similar in mdr1a –/– and +/+ mice.

### Table I. Elimination Half-Lives Determined from Mean Plasma and Striatal Concentrations of Methylenedioxymethamphetamine (MDMA), Methylenedioxyamphetamine (MDA), and 4-Hydroxy-3-methoxy-3-methamphetamine (HMMA) Collected from Wild-Type (mdr1a +/+) and mdr1a Knock-Out (mdr1a –/–) Mice following 10, 20, or 40 mg/kg i.p. MDMA*

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Strain</th>
<th>10 mg/kg</th>
<th>Half-life (h)</th>
<th>20 mg/kg</th>
<th>40 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>mdr1a +/+</td>
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<td>0.6</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mdr1a –/–</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>mdr1a +/+</td>
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<td>0.8</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>mdr1a –/–</td>
<td>0.7</td>
<td>0.8</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>HMMA</td>
<td>mdr1a +/+</td>
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<td>0.9</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mdr1a –/–</td>
<td>1.0</td>
<td>0.8</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Striataum</td>
<td>mdr1a +/+</td>
<td>0.5</td>
<td>0.6</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mdr1a –/–</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

* n = 5 mice per time-point for mean plasma and striatal elimination profiles, except n = 4 mdr1a –/– mice 3 h after 40 mg/kg MDMA. Half-lives were not calculated for 4-hydroxy-3-methoxyamphetamine in plasma due to low concentrations.

### Table II. Area Under the Curve (AUC) for Methylenedioxymethamphetamine (MDMA), Methylenedioxyamphetamine (MDA), 4-Hydroxy-3-methoxy-4-methamphetamine (HMMA), and 4-Hydroxy-3-methoxyamphetamine (HMA) in Plasma from Wild-Type (mdr1a +/+) and mdr1a Knock-Out (mdr1a –/–) Mice following 10, 20, or 40 mg/kg i.p. MDMA*

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Strain</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>40 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>mdr1a +/+</td>
<td>1233 †,‡(53)</td>
<td>2611 ‡,§,&quot;(86)</td>
<td>7763 †,§,&quot;,#(228)</td>
</tr>
<tr>
<td></td>
<td>mdr1a –/–</td>
<td>1350 †,‡(56)</td>
<td>3826 ‡,§,&quot;(206)</td>
<td>9419 †,§,&quot;(386)</td>
</tr>
<tr>
<td>MDA</td>
<td>mdr1a +/+</td>
<td>226 †,‡,&quot;(10)</td>
<td>599 ‡,§,&quot;,#(24)</td>
<td>1910 †,§,&quot;(92)</td>
</tr>
<tr>
<td></td>
<td>mdr1a –/–</td>
<td>295 †,‡,&quot;(17)</td>
<td>836 ‡,§,&quot;,#(33)</td>
<td>2127 †,§,&quot;(118)</td>
</tr>
<tr>
<td>HMMA</td>
<td>mdr1a +/+</td>
<td>606 †,‡(25)</td>
<td>852 †,§,&quot;,#(47)</td>
<td>1575 †,§,&quot;,#(39)</td>
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<tr>
<td></td>
<td>mdr1a –/–</td>
<td>638 †,‡(31)</td>
<td>1124 †,§,&quot;,#(58)</td>
<td>1814 †,§,&quot;,#(67)</td>
</tr>
<tr>
<td>HMA</td>
<td>mdr1a +/+</td>
<td>88 †,‡(7)</td>
<td>160 †,§,&quot;(8)</td>
<td>308 †,§,&quot;(10)</td>
</tr>
<tr>
<td></td>
<td>mdr1a –/–</td>
<td>109 †,‡(7)</td>
<td>210 †,§,&quot;(9)</td>
<td>345 †,§,&quot;(13)</td>
</tr>
</tbody>
</table>

* Mean and (standard error), n = 5 per time-point for mean plasma and striatal elimination profiles, except n = 4 mdr1a –/– mice 3 h after 40 mg/kg MDMA.Half-lives were not calculated for 4-hydroxy-3-methoxyamphetamine in plasma due to low concentrations.

### Table VI. Striatum/plasma AUC ratios for MDMA and metabolites in mice with wild-type (mdr1a +/+) and mdr1a Knock-Out (mdr1a –/–) genotypes following 10, 20, or 40 mg/kg i.p. MDMA

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Strain</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>40 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>mdr1a +/+</td>
<td>1233 †,‡(53)</td>
<td>2611 ‡,§,&quot;(86)</td>
<td>7763 †,§,&quot;,#(228)</td>
</tr>
<tr>
<td></td>
<td>mdr1a –/–</td>
<td>1350 †,‡(56)</td>
<td>3826 ‡,§,&quot;(206)</td>
<td>9419 †,§,&quot;(386)</td>
</tr>
<tr>
<td>MDA</td>
<td>mdr1a +/+</td>
<td>226 †,‡,&quot;(10)</td>
<td>599 ‡,§,&quot;,#(24)</td>
<td>1910 †,§,&quot;(92)</td>
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<td></td>
<td>mdr1a –/–</td>
<td>295 †,‡,&quot;(17)</td>
<td>836 ‡,§,&quot;,#(33)</td>
<td>2127 †,§,&quot;(118)</td>
</tr>
<tr>
<td>HMMA</td>
<td>mdr1a +/+</td>
<td>606 †,‡(25)</td>
<td>852 †,§,&quot;,#(47)</td>
<td>1575 †,§,&quot;,#(39)</td>
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<td>mdr1a –/–</td>
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<tr>
<td>HMA</td>
<td>mdr1a +/+</td>
<td>88 †,‡(7)</td>
<td>160 †,§,&quot;(8)</td>
<td>308 †,§,&quot;(10)</td>
</tr>
<tr>
<td></td>
<td>mdr1a –/–</td>
<td>109 †,‡(7)</td>
<td>210 †,§,&quot;(9)</td>
<td>345 †,§,&quot;(13)</td>
</tr>
</tbody>
</table>

* Mean (standard error), n = 5 per time-point; 25 mice per AUC determination, except 24 mice for 40 mg/kg MDMA dose in mdr1a –/– mice. p < 0.005 significance threshold for all comparisons (Bonferroni-adjusted, equivalent to p < 0.05).

† Significantly different from 20 mg/kg AUC.
‡ Significantly different from 40 mg/kg AUC.
§ Significantly different from 10 mg/kg AUC.
# Significantly lower than AUC predicted by multiplying 10 mg/kg AUC by 2 or 4.
** Significantly lower than AUC predicted by multiplying 10 mg/kg AUC by 2 or 4.
Discussion

MDR1a's role in effects of MDMA

Our previous research demonstrated that deletion of mdr1a affords protection from MDMA-induced decreases in dopamine and DAT binding in mouse striatum suggesting that individuals with elevated MDR1a expression would be at higher risk of neurotoxicity (9). We also demonstrated that MDR1a does not alter striatal MDMA and MDA concentrations (8). The current research investigated the role of MDR1a-mediated MDMA metabolism and distribution in mdr1a +/+ and –/– mice and whether altered MDMA metabolism in mdr1a –/– mice confounded our previous results. We found that mean plasma and striatum MDMA AUCs were generally elevated in mdr1 –/– compared to +/+ mice, which is inconsistent with our previous observations of mdr1a –/– mice being protected from MDMA-induced dopaminergic effects (8,9). Furthermore, we observed similar striatum/plasma AUC ratios in mdr1a +/+ and –/– mice, thereby demonstrating that MDR1a-mediated MDMA distribution into striatum does not potentiate MDMA's effect in mdr1a +/+ mice.

Our results confirm that MDMA is a weak substrate for MDR1a mediated transport across the BBB (10). However, we demonstrated interstrain differences between MDMA-induced alterations of dopamine concentrations and dopamine transport protein expression in mdr1a +/+ and –/– mice (8,9). We propose that MDR1a functions through a novel non-efflux mechanism or that compensatory changes in protein expression/activity account for observed protection in mdr1a –/– mice.

We reported increased monoamine oxidase (MAO)-mediated metabolism of dopamine to 3,4-dihydroxyphenylacetic acid (DOPAC) in mdr1a +/+ mice 24 h after MDMA, while observing little change in mdr1a –/– dopamine metabolism (8). MAO-mediated metabolism of dopamine produces hydrogen peroxide and hydroxyl radicals that are neurotoxic oxygen species (ROS) (2). A possible explanation for observed mdr1a +/+ and –/– interstrain disparity in dopaminergic responses to MDMA could be due to altered MAO expression in mdr1a +/+ and –/– mice. Sanchez-Carbayo et al. (28) observed increased MAO-A in MDRI-trans-
MDMA metabolites distributed into brain (29,30), inhibited 3,4-dihydroxyamphetamine. These thioether conjugated cysteine conjugated species of MDMA metabolites HHMA and detailing neurotoxic effects of glutathione (GSH) and our mice studies. Monks et al. (11) published a series of studies minimizing likelihood of HHMA-induced neurotoxicity during with minimal distribution of hydroxy metabolites into brain; HMMA brain concentrations in both mice strains consistent mice. Similar to Mueller et al. (20), we observed minimal potential role of this analyte in the observed neurotoxicity in termine HHMA concentrations and to directly evaluate the method development making it impossible to accurately de-

Also, HHMA reference standards were unavailable during 476

Further-

related neurotransmitter of Methylenedioxymethamphetamine (MDMA) and Methylenedioxyamphetamine (MDA) in mdr1a Knock-Out (mdr1a –/–) and Wild-Type (mdr1a +/+). Monoamine oxidase (MAO) is a mitochondrial enzyme that catalyzes the oxidative deamination of monoamines. In addition, MDMA is a weak MDR1a substrate (10,34–36).

We present valuable data demonstrating that MDR1a transport of MDMA into brain does not play a role in MDMA neurotoxicity. MDR1a expression is variable throughout the human population (6). Our previous reports demonstrating that MDR1a potentiates MDMA neurotoxicity suggests that individuals with elevated MDR1a expression would be at increased risk of neurotoxicity (8,9). Considering results from our previous studies lead us to hypothesize that MDR1a facilitates transport of MDMA into brain and that MDMA brain concentrations would be elevated in mdr1a +/- mice. Inconsistent with this hypothesis, we showed mdr1a +/- mice had slightly lower brain concentrations than mdr1a –/– mice and that altered metabolism does not confound interpretation of MDMA brain concentrations between the two mice strains. The mechanism for altered distribution and/or impaired MDMA elimination in mdr1a –/– mice remains unknown.

MDMA metabolism in mice compared to rats

Altered MDMA metabolism in mice compared to other species was proposed as a potential mechanism for predominant dopamine toxicity in mice and serotonergic toxicity in

Table VI. Striatum/Plasma Ratios Based Upon Areas Under the Curves (AUC) for of Methylenedioxymethamphetamine (MDMA) and Methylenedioxyamphetamine (MDA) in mdr1a Knock-Out (mdr1a –/–) and Wild-Type (mdr1a +/+). 

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Dose (mg/kg)</th>
<th>Strain</th>
<th>Ratio†</th>
<th>Ratio‡</th>
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<tbody>
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<td>MDMA</td>
<td>10</td>
<td>mdr1a –/–</td>
<td>11.1</td>
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</tr>
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*25 mice per AUC determination, except 24 mice for 40 mg/kg MDMA dose in mdr1a –/– mice.
† Mean AUC measured in striatum divided by mean AUC measured in plasma.
‡ Striatum/plasma AUC ratios in mdr1a –/– mice divided by striatum/plasma ratios in mdr1a +/+ mice.
other species (21–23). This study was primarily designed to investigate MDR1a's role in MDMA and metabolites' distribution into striatum of mdr1a +/+ and −/− mice, preventing repetitive plasma time-course sampling limiting the extent of pharmacokinetic analysis. MDMA metabolism and distribution in mice is incompletely characterized, with only MDMA and metabolite excretion in mouse urine (21) and less extensive plasma distribution studies (10,24,25) available. Results of our current study provide the most complete characterization of MDMA plasma pharmacokinetics and simultaneous characterization of MDMA distribution into brain.

It must be noted that HMMA and HMA concentrations in mouse specimens that were acid hydrolyzed reflect approximately 60% of total concentrations. We conducted hydrolysis efficiency studies with plasma collected from mice after MDMA administration that was combined and mixed into a single pool. These studies indicated hydrolysis for 45 min at 100°C after addition of 100 µL 12 M hydrochloric acid was consistently 60% as effective as overnight, 37°C hydrolysis with 5000 units of glucuronidase from Helix pomatia for deconjugating HMMA and HMA (26). Results obtained via both acidic and enzyme hydrolysis were reproducible with % CVs less than 4.5% for triplicates following acidic and enzyme hydrolysis (26). Increased matrix interferences produced elevated analyte LOQs, leading us to select acidic rather than enzyme hydrolysis. Our observation of more efficient enzyme than acidic hydrolysis contrasts with observations in humans (38,39) and suggests interspecies differences in HMMA and HMA conjugation. We hypothesize that HMMA and HMA glucuronides predominate over sulfate conjugates in mice and that glucuronidase is more effective at hydrolyzing HMMA and HMA glucuronides than acid hydrolysis. Shima et al. (40) directly measured HMMA-glucuronide and HMMA-sulfate in human and rat plasma by liquid chromatography–MS, finding that glucuronides exceeded sulfates in rats while sulfates exceeded glucuronides in humans, which is consistent with our hypothesis. Total HMMA and HMA concentrations presented in this manuscript are likely 40% lower than actual total concentrations due to incomplete acidic hydrolysis of glucuronides. Previously, we investigated hydrolysis techniques for deconjugating HMMA and HMA conjugates in human, monkey, and rat plasma specimens and found that acidic hydrolysis was more effective than enzyme for human and monkey specimens and enzyme was optimal for rats (39). All of these observations indicate the importance of optimizing hydrolysis for each species.

We found that MDMA and metabolites are rapidly eliminated from mouse plasma with MDMA t½ less than 1 h. We recently reported MDMA t½ in Sprague-Dawley rat plasma of 1.1 h compared to 0.4 h in mdr1a +/+ mice following 10 mg/kg MDMA i.p., suggesting that MDMA is eliminated more rapidly in mice than rats (18). Similarly, MDA and HMMA also were more rapidly eliminated. In rat plasma following 10 mg/kg MDMA i.p., MDA and HMMA t½ were 2.2 and 2.7 h, respectively (18), compared to our observed t½ in mice of 0.5 and 0.9 h, respectively. During MDMA i.p. dosing studies in mice, we did not observe delayed HMMA tmax, as was seen in rats. Apparent maximum concentrations of HMMA (Tmax) were observed in the first plasma specimen collected 0.3 h after MDMA administration in mice, more consistent with our human observations of 1.1–2.8 h after 1.6 mg/kg oral MDMA (15), than in rats with Tmax of 4–6 h after 2 and 10 mg/kg oral MDMA (18).

MDMA is typically taken orally by humans, with single doses typically ranging from 75 to 125 mg (14,41–44). Our controlled administration study administered up to 150 mg oral MDMA (15). MDMA, MDA, and HMMA t½ were less than 1.5 h after all MDMA doses in mice while following 1.6 mg/kg oral dose to humans t½ were 5–13, 7–21, and 8–19 h for MDMA, MDA, and HMMA, respectively (15). Thus, mice appear to metabolize MDMA more rapidly than humans. Mueller et al. (20) observed that in rats MDMA plasma Cmax and AUC were correlated to decreases in brain serotonin while not observing correlations for HMMA or HMA Cmax and AUC. Shorter half-lives in mice suggest that higher doses are needed in this species as compared to humans to achieve similar Cmax or AUC, although matching both pharmacokinetic parameters simultaneously is not possible, as also noted for primates by Mueller et al. (45). To date, it is unknown whether Cmax or AUC is a better predictor of neurotoxicity.

We observed, for the first time, that HMMA and MDA are significant metabolites in mice, similar to observations in rat. These data contradict the postulation by de la Torre and Farre (22) that minimal hepatic metabolism of MDMA in mice accounts for minimal MDMA serotonergic effects. Following 10 mg/kg MDMA, plasma HMMA/MDMA and MDA/MDMA AUC ratios in mdr1a +/+ mice were 49.1% and 18.3%, respectively, compared to 72% and 28% AUC ratios observed in rats after the same dose, respectively. (18) suggesting HMMA and MDA are significant MDMA metabolites in mice. We observed HMMA/MDMA and MDA/MDMA AUC ratios of 4–7% after 10, 20, and 40 mg/kg MDMA suggesting that HMA is a minor metabolite in mice, similar to rats (18). (15). MDMA, MDA, HMMA, and HMMA/MDMA ratios were 67%, 10%, and 17%, respectively, after 1.6 mg/kg MDMA oral administration to humans (15), suggesting similar overall exposure to putative neurotoxic metabolites may occur in mice and humans, although dose disparity and known nonlinear pharmacokinetics necessitate caution extrapolating between these two studies. It should also be noted that significant HMMA plasma concentrations observed during our studies with FVB-1 mice contrast with Fantegrossi and co-workers' observation of minimal HMMA concentrations in Swiss-Webster mice following enzyme hydrolysis (25). The disparity in HMMA plasma concentrations could be explained by metabolic differences between mouse strains.

We observed trace HMMA and HMA concentrations in striatum, indicating HMMA and HMA (and glucuronide/sulfate conjugates) are not efficiently transferred across the BBB in mice and are not metabolically formed in brain, similar to observations in rats (20). Similar to observations in rats, MDMA and MDA striatal concentrations exceeded plasma concentrations (20), but the elimination profiles paralleled those in plasma. Unfortunately, no primate studies detailing distribution of MDMA and metabolites into brain after MDMA administration exist to improve our understanding of the roles of metabolism and distribution on MDMA neurotoxicity in a species more closely related to humans. A postmortem case re-
port detailed finding 11,700–17,400 and 220–362 µg/kg MDMA and MDA, respectively, in a brain of an overdose victim (46). They also reported aortic serum concentrations of 8200 and 178 µg/L of MDMA and MDA, respectively (46). These post-mortem brain concentrations were similar to our observations in mice 0.3–1 h after 10 mg/kg MDMA; serum concentrations also were similar to our observations in mice plasma 0.3–1 h after 40 mg/kg MDMA i.p.

Nonlinear MDMA metabolism in mice

Although nonlinear MDMA metabolism in mice was suggested (25), the present results are the first clearly demonstrating nonlinear pharmacokinetics in this species. Both mdr1a −/− and +/+ mice exhibited similar patterns of nonlinear pharmacokinetics; however, the nonlinearity in mdr1a +/+ mice will be discussed. Following 40 mg/kg MDMA, apparent plasma C\text{max} and AUCs for MDMA, MDA, HMMA, and HMA were not linearly correlated to C\text{max} and AUCs after 10 mg/kg MDMA. MDMA and MDA apparent C\text{max} and AUCs were significantly higher than predicted, whereas HMMA and HMA apparent C\text{max} and AUCs were significantly lower than predicted, suggesting impaired demethylation (19).

Similar to plasma, nonlinearity also was observed in mouse striatum. MDMA and MDA apparent C\text{max} and AUC following 40 mg/kg MDMA were higher than predicted as compared to those after 10 mg/kg MDMA. Our observations of MDMA and MDA nonlinearity in mice brain are consistent with observations in rats (17).

Nonlinear C\text{max} and/or AUCs for MDMA and metabolites during dose-escalation studies are not species specific, as also demonstrated in rats (17,18), primates (16), and humans (14,15). MDMA auto-inhibition of human CYP2D6 is proposed as the mechanism producing higher than predicted MDMA concentrations and reduced HMMA concentrations (19,47). Mice do not express CYP2D6, having several CYP2D homologues (25), which might be inhibited by MDMA, in a similar manner to human CYP2D6. Prolonged exposure to MDMA and its metabolites caused by MDMA nonlinear pharmacokinetics may play a role in MDMA toxicity. Recently, Mueller et al. (20) reported significant correlations between MDMA C\text{max} and AUC with serotonergic deficits in rats. If acute effects of MDMA are directly causing neurotoxicity, then MDMA nonlinear pharmacokinetics yielding dose-disproportionate increases in MDMA plasma and brain concentrations would lead to potentiation of MDMA toxicity (16,20). Alternatively, if neurotoxic MDMA metabolites play a key role in MDMA neurotoxicity, then prolonged excretion of thioether conjugated metabolites could cause potentiation of MDMA toxicity (16).

It is useful to consider the potential impact of MDMA nonlinear pharmacokinetics on our previously published observations in mdr1a +/+ and −/− mice. Nonlinear pharmacokinetics may explain why we observed significant interstrain differences in nucleus accumbens DAT binding after 5 and 10 mg/kg MDMA demonstrating that mdr1a −/− mice were protected from MDMA-induced reductions in DAT binding (9). However, we observed similar reductions in nucleus accumbens DAT binding in both strains after 20 mg/kg MDMA (9). Alternatively, the higher dose MDMA may simply overwhelm the still uncharacterized protective mechanism present in mdr1a −/− mice.

It is sometimes difficult to translate effects observed in preclinical MDMA studies, including the one presented here, to effects observed in humans due to dose and administration route differences. However, we previously reported more rapid MDMA and metabolite elimination from rats than humans after administering MDMA via oral, subcutaneous, and i.p. routes at doses similar to those used by humans (18). Our latest observations suggest MDMA is eliminated more rapidly in mice than rats when comparing 10 mg/kg i.p. dosing in both species, and possibly also more rapidly than in humans. There is debate regarding whether MDMA is neurotoxic in humans and furthermore, if MDMA itself or thioether conjugated metabolites produce neurotoxicity (2). If MDMA itself is neurotoxic, evidence of toxicity following administration of clinically relevant MDMA doses to mice and rats could heighten concern for MDMA-induced neurotoxicity in humans, because rapid MDMA elimination in these species limits exposure. If metabolites play a key role in MDMA neurotoxicity, further studies are necessary to identify MDMA metabolites that are neurotoxic employing clinically relevant dosing strategies.

In summary, although MDMA and metabolite concentrations were generally elevated in mdr1a −/− compared to +/+ mice, striatum/plasma ratios were similar in both strains; inconsistent with a hypothesis that MDR1a protein is facilitating MDMA and metabolites transfer into brain. We also observed that mdr1a +/+ mice metabolize MDMA more efficiently and HMMA forms more quickly than in rats. HMMA and MDA are major metabolites of MDMA in mdr1 +/+ mice, inconsistent with observations of low HMMA concentrations in Swiss-Webster mice. Finally, we observed nonlinear pharmacokinetics for MDMA and metabolites in plasma and striatum of mdr1a +/+ mice, which may potentiate MDMA toxicity during dose-escalation studies.

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References

4. R. Thomassius, P. Zapletalova, K. Petersen, R. Buchert, B. Andresen, L. Wartberg, B. Nebeling, and A. Schmoldt. Mood, cognition and


