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PAPER

TOXICOLOGY

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Interaction of 3,4-Methylenedioxymethamphetamine and Methamphetamine During Metabolism by *In Vitro* Human Metabolic Enzymes and in Rats*

ABSTRACT: Illicit amphetamine-type stimulant (ATS) tablets commonly contain one or more active ingredients, which have hallucinogenic and/or stimulant effects. Because components such as 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (MA) in ATS tablets have similar chemical structures, they could be metabolized by common metabolic enzymes. To investigate potential metabolic interactions of ATS tablet components, we studied the *in vitro* metabolism of MDMA and MA using human metabolic enzymes. MDMA and MA were mainly metabolized by cytochrome P450 2D6 (CYP2D6) and mutually inhibited the production of their main metabolites. *In vivo* experiments were also performed using intravenous administration of MDMA, MA, or their mixture to rats. The plasma concentrations of MDMA and MA after co-administration were higher than those after administration of MDMA or MA alone. The results in this study imply that multiple components in ATS tablets can interact to mutually inhibit their metabolism and potentially enhance the toxicity of each component.

KEYWORDS: forensic science, toxicology, interaction, methamphetamine, 3,4-methylenedioxymethamphetamine, metabolism

The amphetamine-type stimulant (ATS) family includes compounds such as methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxyethylamphetamine (MDEA). These compounds have similar chemical structures, and pharmacological and toxicological effects on the central nervous system (1–4). Abuse of ATS tablets has become a serious social problem worldwide (1,5,6). In addition to the ATS, ATS tablets can contain other active ingredients such as ketamine and caffeine, which have hallucinogenic and/or stimulant effects (2,7–11). The components in these tablets might interact with each other *in vivo*, which could enhance toxicity. Therefore, investigations of interactions of ATS and related compounds are important for public health and forensic toxicology.

The only structural difference between MDMA and MA is the presence or absence, respectively, of the methylenedioxy moiety. Most ATS, including MDMA and MA, are metabolized by *N*-demethylation and hydroxylation of their aromatic moieties. MDMA is mainly metabolized to 4-hydroxy-3-methoxymethamphetamine (HMMA) and MDA, while MA is mainly metabolized to *p*-hydroxymethamphetamine (OHMA) and amphetamine (AP) in humans and rats (Fig. 1) (12–16). It has been reported that cytochrome P450 2D6 (CYP2D6) is the dominant metabolic enzyme contributing to the metabolism of MDMA and MA (17–21).

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MDMA and MA have some pharmacological differences such as the hallucinogenic effects of MDMA (2,3). MDA and AP themselves, which are metabolites of MDMA and MA respectively, have hallucinogenic and/or stimulant effects and are abused as illicit drugs. HMMA, which is a main metabolite of MDMA, reportedly affects the release of neurohypophysial hormones (22,23), Therefore, the changes of metabolic patterns by co-administration of drugs can bring unexpected adverse health effects.

We previously investigated interactions of ATS and related compounds during absorption both *in vitro* using human intestinal Caco-2 cells and *in vivo* using rats (24,25). MDMA and MA were transported into Caco-2 cells via a common transporter, and the uptake and permeation were mutually inhibited. Although metabolic interactions of AP analogs have been reported, there are few reports concerning direct interaction between MDMA and MA, and these reports have focused on *in vitro* metabolism (17–19).

In this study, *in vitro* experiments with human metabolic enzymes were used to study the interaction between MDMA and MA. In addition, *in vivo* drug administration experiments were performed using rats to examine the interactions of MDMA and MA because we cannot perform *in vivo* experiments in humans. The correlation between the *in vitro* and *in vivo* metabolic results was examined.

Materials and Methods

Materials

d-MA hydrochloride (MA·HCl) and *d*-OHMA·HCl were purchased from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan) and Sigma-Aldrich Co. (St. Louis, MO), respectively. *dl*-HMMA and 3,4-dihydroxymethamphetamine (DHMA) were purchased as methanol solutions from Cerilliant Co. (Austin, TX) and

Lipomed Co. (Arlesheim, Switzerland), respectively. *dl*-MDMA·HCl, *dl*-MDA·HCl, and *dl*-AP sulfate were synthesized as reported previously (26,27). All other reagents and solvents were purchased from Wako Pure Chemical Co. (Osaka, Japan). Acetonitrile was of liquid chromatograph (LC)–mass spectrometer (MS) grade.

Nicotinamide adenine dinucleotide phosphate (NADPH) regeneration system solution (1.3 mM $NADP^+$, 3.3 mM glucose 6-phosphate, 0.4 U/mL glucose 6-phosphate dehydrogenase, 3.3 mM $MgCl_2$) was purchased from Daiichi Pure Chemicals Co. Ltd. (Tokyo, Japan). Human liver and intestinal microsomes, human liver cytosol, and microsomes from human B-lymphoblastoid cell lines that stably express specific human CYPs and microsomes from baculovirus-infected insect cells that stably express human flavine-containing monooxygenase (FMO) or monoamine oxidase (MAO) were also purchased from Daiichi Pure Chemicals Co. Ltd.

Male Wistar rats weighing 200–250 g were purchased from the Biomaterial Center of Japan Inc. (Tokyo, Japan). A polyethylene tube for cannulation was purchased from Natsume Seisakusho Co. (Tokyo, Japan).

In Vitro Metabolism Experiments

Drug solutions of MDMA·HCl and MA·HCl at various concentrations were prepared by dissolving the compounds in 100 mM phosphate buffer (pH 7.4). Drug solution (30 μL) was mixed with 140 μL of NADPH regeneration system solution and 10 μL of aqueous 11.6 mM *S*-adenosyl methionine (SAM). To this mixture, 20 μL of a solution containing metabolic enzymes (1 mg/mL protein) was added. The mixture was incubated at 37°C, and 10-μL aliquots of the solution were removed at 15, 30, 60, and 120 min. Each of these aliquots was mixed with 90 μL of the LC mobile phase as described in the LC-MS/MS Conditions section containing 0.2 μg/mL dibenzylamine (DBA) as an internal standard. After vortex mixing for 5 min and centrifugation (15,000 × *g*, 5 min), the supernatant was filtered through a 0.45-μm filter. The filtrate (5 μL) was injected into a liquid chromatograph-tandem mass spectrometer (LC-MS/MS).

Animal Experiments

The animal experiments were carried out under approval of an ethics committee at the National Research Institute of Police Science (Kashiwa, Japan). Polyethylene tubing (1.1 mm o.d.) for administration of solutions of MDMA·HCl and MA·HCl in 0.9% aqueous NaCl was inserted in the femoral vein of each rat under diethyl ether anesthesia. After intravenous administration of MDMA·HCl (10 mg/kg), MA·HCl (10 mg/kg), or a mixture of MDMA (10 mg/kg) and MA (10 mg/kg), the rat was placed in a cage. Blood (about 0.2 mL) was periodically collected (0.1, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 10 h) from the tail vein in a micro test tube containing heparin and immediately centrifuged (2000 × *g*, 10 min). The plasma was transferred to another tube and stored at −30°C until use.

Preparation of Analytical Sample from Plasma

Fifty microliters of plasma and 100 μL of 3 M hydrochloric acid were poured into a 1.5-mL micro tube, and then the tube was placed in a heat block at 120°C for 30 min for hydrolysis of the conjugated compounds. Five hundred microliters of acetonitrile containing 0.2 μg/mL DBA was added to the sample after hydrolysis. After vortex mixing for 5 min and centrifugation (15,000 × *g*, 10 min), the supernatant was transferred to another tube and evaporated to dryness under a nitrogen flow. The residue was dissolved in the LC mobile phase as described in the LC-MS/MS Conditions section. After filtration, 5 μL of the sample solution was injected into the LC-MS/MS.

LC-MS/MS Conditions

The LC (Shiseido Nanospace SI-2, Tokyo, Japan) was coupled to a MS (Thermo Electron TSQ Quantum Ultra; Thermo Electron Corp., San Jose, CA). Chromatographic separation was achieved using a polymer-coated strong cation-exchange column (Shiseido CAPCELL PAK SCX UG 80, 75 × 1.5 mm i.d.; Tokyo, Japan) at 40°C. The mobile phase was 25 mM ammonium acetate (pH 4.0) and acetonitrile (1:3). The flow rate was maintained at 0.3 mL/min. The switch valve was changed from a waste line to the MS line at 1 min. Positive mode of electrospray ionization and selected reaction monitoring were used for quantification of compounds. The parameters for MS were as follows: spray voltage, 5000 V; sheath gas pressure, 6 Pa; auxiliary gas pressure, 0.5 Pa; capillary temperature, 300°C; tube lens offset, 50 V; collision pressure, 0.2 Pa; and collision energy, 15 eV. Precursor and product ions were monitored at *m/z* 194 → 163 (MDMA), *m/z* 150 → 119 (MA), *m/z* 196 → 165 (HMMA), *m/z* 180 → 163 (MDA), *m/z* 182 → 151 (DHMA), *m/z* 166 → 135 (OHMA), *m/z* 136 → 91 (AP), and *m/z* 198 → 91 (DBA). Data acquisition and instrument control were performed using Thermo Electron Xcalibur software.

Data Analysis

The initial production rates of metabolites were estimated using linear regression analysis of a linear portion of the amount of metabolite versus time. The data were fitted to the Michaelis–Menten equation by an iterative nonlinear least-squares method:

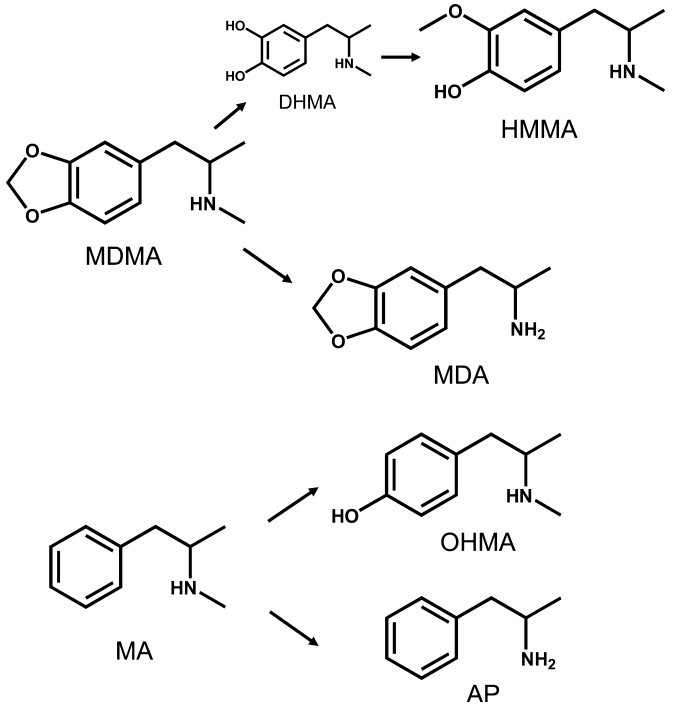

FIG. 1—*Metabolism of 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (MA).*

$$V = V_{max}[S]/(K_m + [S])$$

where V represents the initial production rate of the metabolite and [S] the initial concentration of substrate. To examine the type of inhibition, a Lineweaver-Burk plot of the reciprocal of substrate concentration (x-axis) and the reciprocal of metabolite production rate (y-axis) with or without inhibitor was depicted. The inhibition constant (K_i) was determined from the intersection of lines for two different concentrations of substrate on a Dixon plot of inhibitor concentration (x-axis) and the reciprocal of metabolite production rate (y-axis).

The interactions between MDMA and MA in drug administration experiments were evaluated using moment analysis. The area under the plasma concentration–time curve (AUC) and the mean residence time (MRT) were calculated based on the trapezoidal areas of the plasma concentration–time curve from 0 h to infinity after intravenous administration of drugs.

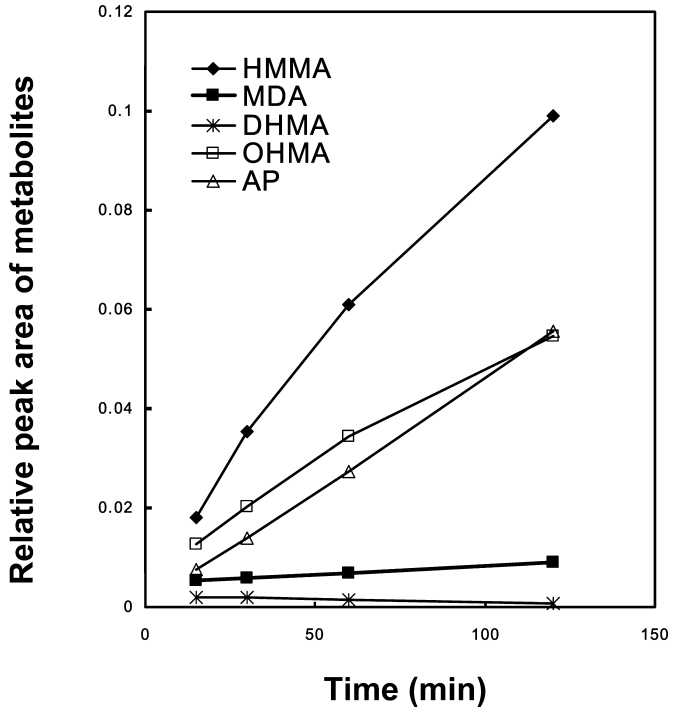

FIG. 2—*Typical time courses of metabolite production from 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (MA).* In vitro *metabolic experiments of MDMA and MA were performed using media with human liver microsomes. The relative peak areas of each metabolite to internal standard are indicated at each incubation time.*

Results and Discussion

Metabolism of MDMA and MA by Various Metabolic Enzymes

The typical time courses from metabolite production of MDMA and MA (Fig. 2) were recorded in media with human liver microsomes. The concentrations of the main metabolites of MDMA (HMMA and MDA) and MA (OHMA and AP) linearly increased with incubation time up to 60 min. The concentration of DHMA, which is a metabolic intermediate in the conversion of MDMA to HMMA (Fig. 1) (28), plateaued early in the incubation. DHMA is produced by cleavage of the methylenedioxy moiety of MDMA. It is subsequently metabolized to HMMA by *O*-methylation of catechol by catechol *O*-methyl transferase using SAM as a methyl supplier (29). We previously demonstrated that SAM addition was indispensable to reproduce MDMA *in vivo* metabolism because the amount of SAM in microsomes was insufficient during *in vitro* experiments (30). Therefore, SAM was added to *in vitro* experiments in this study. DHMA was detected after 15 min of incubation and the amount slowly decreased until 120 min. The results imply the production of HMMA by fast cleavage of the methylenedioxy moiety of MDMA and subsequent *O*-methylation of DHMA.

The production rates of the metabolites differed greatly depending on the kind of enzymes (Table 1). With liver microsome incubation, HMMA production was greater than MDA production from MDMA, while OHMA production was lower than AP production from MA. The production rates of the metabolites in liver microsomes were greater than those in liver cytosol and intestinal microsomes. Among the CYP isozymes tested, CYP2D6 dominantly contributed to metabolism of MDMA and MA. MDA and AP were also produced by other isozymes and enzymes such as FMO and MAO.

After addition of MDMA or MA alone, production of MDA from *N*-demethylation of MDMA was inferior to HMMA production, while AP production from *N*-demethylation of MA was superior to OHMA production. The difference in *N*-demethylation reaction efficiency implies that in addition to CYP2D6, some other enzymes contribute to *N*-demethylation. Baba et al. (31) proposed that both *N*-hydroxymethyl-amphetamine production by CYP and *N*-hydroxyl-methamphetamine production by FMO contribute to

TABLE 1—*Metabolism of MDMA and MA by various metabolic enzymes.*

	Production Rate of Metabolite (pmol/min/mg Protein)			
Metabolic Enzyme	MDMA → HMMA	MDMA → MDA	MA → OHMA	MA → AP
Liver microsome	75.1 ± 0.9	1.78 ± 0.13	23.8 ± 1.3	30.4 ± 0.7
Liver cytosol	2.25 ± 0.31	0.37 ± 0.21	0.13 ± 0.04	3.32 ± 0.87
Intestine microsome	6.03 ± 1.00	0.99 ± 0.55	1.59 ± 0.11	1.65 ± 0.63
CYP1A2	0.26 ± 0.14	0.32 ± 0.07	N.D.	Trace
CYP2B6	Trace	Trace	N.D.	Trace
CYP2C9	Trace	Trace	Trace	Trace
CYP2D6	6.76 ± 0.60	0.41 ± 0.10	25.3 ± 1.2	37.8 ± 2.9
CYP3A4	Trace	Trace	N.D.	Trace
FMO	N.D.	0.57 ± 0.08	N.D.	5.91 ± 0.60
MAO_A	N.D.	0.22 ± 0.16	N.D.	1.64 ± 0.45
MAO_B	N.D.	0.24 ± 0.05	N.D.	2.35 ± 1.18

Each (20 μM) of MDMA and MA was incubated with various metabolic enzymes. Each value represents the mean ± SD from three independent experiments. "Trace" indicates the concentration was lower than the limit of quantification.

MDMA, 3,4-methylenedioxymethamphetamine; MA, methamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; OHMA, *p*-hydroxymethamphetamine; AP, amphetamine; CYP, cytochrome P450; FMO, flavine-containing monooxygenase; MAO, monoamine oxidase (MAO); N.D., not detected.

TABLE 2—*Interaction between MDMA and MA in metabolism with human liver microsomes.*

	Production Rate of Metabolite (pmol/min/mg Protein)		
		Inhibitor	
Substrate → Metabolite	Inhibitor (−)	MDMA	MA
MDMA → HMMA	75.1 ± 0.9	–	30.8 ± 0.5*
MDMA → MDA	1.78 ± 0.13	–	0.82 ± 0.04*
MA → OHMA	23.8 ± 1.3	2.82 ± 0.21*	–
MA → AP	30.4 ± 0.7	16.7 ± 0.3*	–

Each (20 μM) of MDMA and MA was incubated with or without inhibitor (MA or MDMA, 400 μM).

MDMA, 3,4-methylenedioxymethamphetamine; MA, methamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; OHMA, *p*-hydroxymethamphetamine; AP, amphetamine.

*A significant difference between amounts with and without inhibitor using Student's *t*-test ($P < 0.05$). Each value indicates the mean ± SD from three independent experiments.

N-demethylation of MA. The opinion corresponds to the fact that production of MDA and AP was high with FMO in this study.

In Vitro Interaction of MDMA and MA

When the mixture of MDMA and MA was incubated with human liver microsomes, MDMA and MA mutually inhibited the production of their metabolites (Table 2). HMMA production was inhibited more than MDA production in MDMA metabolism, while OHMA production was inhibited more than AP production in MA metabolism.

The concentration dependences of production of the main metabolites of MDMA and MA were examined using microsomes from cells that stably express human CYP2D6, which dominantly contributed to metabolism of MDMA and MA. The curves for the four main metabolites were fitted to Michaelis–Menten equation (Fig. 3). When the mixture of MDMA and MA was added to the medium, production of each of the four metabolites was inhibited. The types of inhibition were competitive for each of four metabolites because the lines with and without inhibitor on a Lineweaver-Burk plot were intersected around *y*-axis (Fig. 4). The interaction between MDMA and MA was depicted using a Dixon plot (Fig. 5) and K_i was calculated (Table 3).

It was revealed that the metabolism of MDMA and MA was mutually inhibited with CYP2D6 as well as with liver microsomes. OHMA production was strongly inhibited by MDMA in media with liver microsomes (Table 2), while the inhibition of OHMA production by MDMA was not so high as that of HMMA production by MA in media with CYP2D6 (Table 3). The differences in degree of inhibition between in liver microsomes and in CYP2D6 imply that other components in liver microsomes complexly affect the metabolism of MDMA and MA, although CYP2D6 greatly contributes to the metabolism. The values of K_i calculated were comparatively high (μg/mL levels) when they were converted to plasma concentration. It is, however, considered that the concentrations are probable levels even in humans, because the contents of ATS tablets are uncertain and tablets containing MDMA and MA in wide ranges of contents appear (11).

Interaction Between MDMA and MA in Rats

MDMA and MA were intravenously administered to rats and the concentration of each drug was measured in plasma until 10 h after administration (Fig. 6). The plasma concentration–time curves were obtained for MDMA or MA administered individually and co-administered (Table 4). The AUC of MDMA after co-administration of MDMA and MA was significantly higher

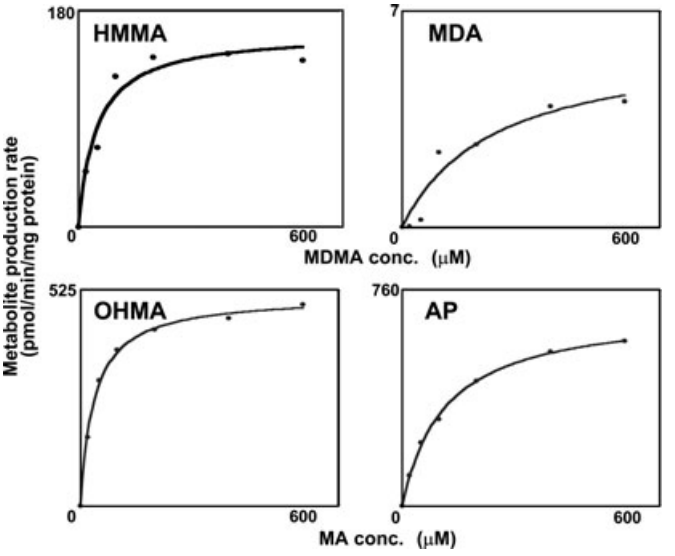

FIG. 3—*Concentration-dependent increase of metabolite production in the metabolism of 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (MA) with cytochrome P450 2D6 (CYP2D6). Various concentrations of substrates (MDMA or MA) were added in media with CYP2D6. The metabolite production rate was determined using the slope of the line from a plot of amount of metabolite versus time.* V_{max} and K_m *were determined by fitting to the Michaelis–Menten equation using the iterative nonlinear least-squares method. HMMA, 4-hydroxy-3-methoxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; OHMA, p-hydroxymethamphetamine; AP, amphetamine.*

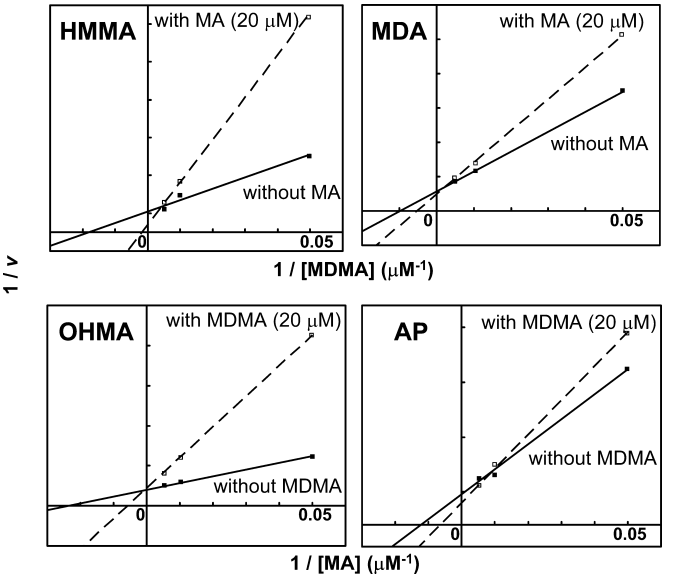

FIG. 4—*Types of inhibition between 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (MA) in metabolite production with cytochrome P450 2D6 (CYP2D6). Various concentrations of substrates with or without inhibitors (20 μM) were added in media with CYP2D6. The metabolite production rate was determined using the slope of the line from a plot of amount of metabolite versus time. Types of inhibition were evaluated using the two lines obtained from plots of the reciprocal of substrate concentration (x-axis) and the reciprocal of metabolite production rate (y-axis) with and without inhibitor. HMMA, 4-hydroxy-3-methoxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; OHMA,* p*-hydroxymethamphetamine; AP, amphetamine.*

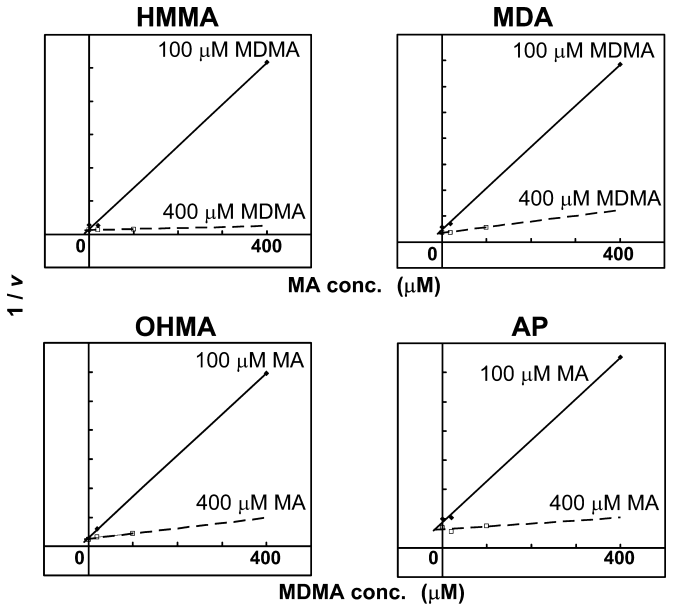

FIG. 5—*Interaction between 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (MA) in metabolite production with cytochrome P450 2D6 (CYP2D6). Various concentrations of substrates and inhibitors were added in media with CYP2D6. The metabolite production rate was determined using the slope of the line from a plot of amount of metabolite versus time.* K_i *was determined from the intersection of the two lines obtained from plots of inhibitor concentration (*x*-axis) and the reciprocal of metabolite production rate (*y*-axis) for two different concentrations of substrate. HMMA, 4-hydroxy-3-methoxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; OHMA,* p*-hydroxymethamphetamine; AP, amphetamine.*

TABLE 3—*Kinetic parameters of MDMA and MA metabolism with CYP2D6.*

			K_i(μM)	
Substrate → Metabolite	V_{max} (pmol/min/mg Protein)	K_m (μM)	Inhibitor MDMA	MA
MDMA → HMMA	153	53	2.1	–
MDMA → MDA	3.9	247	10	–
MA → OHMA	500	37	–	45
MA → AP	544	95	–	17

MDMA, 3,4-methylenedioxymethamphetamine; MA, methamphetamine; CYP2D6, cytochrome P450 2D6; HMMA, 4-hydroxy-3-methoxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; OHMA, *p*-hydroxymethamphetamine; AP, amphetamine.

than that after a single administration of MDMA. The AUC of MA after co-administration of MA and MDMA was also significantly higher than that after a single administration of MA. Significant differences were not observed for the MRT.

Because various enzymes related to MDMA and MA metabolism are present *in vivo*, the *in vivo* interaction between MDMA and MA is complicated. To simply analyze the *in vivo* interaction, the moment analysis, which is model-independent analysis, is used. In this study, the values of AUC were significantly changed between a single administration and co-administration, while the values of MRT did not indicate significant differences. One of the reasons is that MRT tends to be calculated with a larger error than AUC when a plasma concentration–time curve is extrapolated to infinity (32). Co-administration of drugs at different doses may delay the elimination of drugs from plasma and enhance toxicity of each drug. Because users do not know the exact components and their amounts in illicit ATS tablets, serious toxicity might arise because of intake of these tablets. Reports have detailed unexpected death and adverse effects because of *in vivo* interaction of ATSs (33,34) and *in vitro* toxicity of MDMA and MA (35). We found previously that similar compounds with amine moiety such as MDMA and MA interact during intestinal absorption both *in vitro* with human intestinal Caco-2 cells and *in vivo* in rats (24). In this study, it is revealed that MDMA and MA interact during metabolism.

Although the polymorphisms of enzymes and the differences of species can affect some metabolic patterns, it is important for public health and forensic toxicology to inform potential toxicity by the interaction between compounds with similar chemical structures. There are various designer drugs including phenethylamine derivatives such

TABLE 4—*Pharmacokinetic parameters of MDMA and MA on plasma concentration–time curve.*

	AUC (mg·h/mL)	MRT (h)
MDMA alone	2.4 ± 0.3	1.7 ± 0.1
MDMA with MA	3.5 ± 0.1*	1.6 ± 0.2
MA alone	3.0 ± 0.4	2.1 ± 0.4
MA with MDMA	4.3 ± 0.2*	2.4 ± 0.1

MDMA, 3,4-methylenedioxymethamphetamine; MA, methamphetamine; AUC, area under the plasma concentration–time curve; MRT, mean residence time.

*A significant difference between values for individual administration and co-administration using Student's *t*-test ($P < 0.05$). Each value indicates the mean ± SD from four independent experiments.

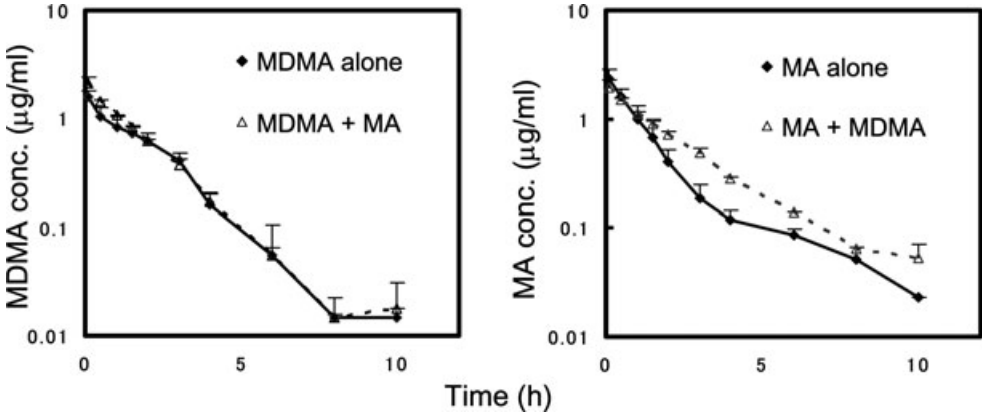

FIG. 6—*Plasma concentrations of 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (MA; 10 mg/kg individually or as a mixture) after intravenous administration to rats. Each value indicates the mean ± SD from three independent experiments.*

as *N*-hydroxy MDMA and methylone that are synthesized in clandestine laboratories and are available on the street (36,37). These designer drugs as well as MDMA and MA may alter the pharmacokinetics with each other.

Conclusion

In this study, *in vitro* experiments with human metabolic enzymes were performed to study the interaction between MDMA and MA. MDMA and MA were mainly metabolized by CYP2D6, and mutually inhibited the production of their main metabolites. In addition, *in vivo* drug administration experiments were performed using rats to examine the interactions of MDMA and MA. The plasma concentrations of MDMA and MA after co-administration were higher than those after administration of MDMA or MA alone. Because compounds with similar chemical structures can be metabolized by the same metabolic enzyme, interactions may occur with other combinations of ATSs as well.

References

1. Davidson C, Gow AJ, Lee TH, Ellinwood EH. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. Brain Res Brain Res Rev 2001;36:1–22.
2. Kalant H. The pharmacology and toxicology of ''ecstasy'' (MDMA) and related drugs. CMAJ 2001;165:917–28.
3. Lyles J, Cadet JL. Methylenedioxymethamphetamine (MDMA, ecstasy) neurotoxicity: cellular and molecular mechanisms. Brain Res Brain Res Rev 2003;42:155–68.
4. Hanson GR, Rau KS, Fleckenstein AE. The methamphetamine experience: a NIDA partnership. Neuropharmacology 2004;47(Suppl. 1):92–100.
5. National Police Agency of Japan. White paper on police 2009. Tokyo, Japan: National Police Agency of Japan, 2010.
6. United Nations Office on Drugs and Crime. World drug report 2010. New York, NY: United Nations Publications, 2010.
7. Dal Cason TA. An evaluation of the potential for clandestine manufacture of 3,4-methylenedioxyamphetamine (MDA) analogs and homologs. J Forensic Sci 1990;35:675–97.
8. Wolff K, Hay AW, Sherlock K, Conner M. Contents of ''ecstasy.'' Lancet 1995;346:1100–1.
9. Fineschi V, Masti A. Fatal poisoning by MDMA (ecstasy) and MDEA: a case report. Int J Legal Med 1996;108:272–5.
10. Ahmad K. Increased use of amphetamine-type stimulants threatens east Asian countries. Lancet 2002;359:1927.
11. Makino Y, Tanaka S, Kurobane S, Nakauchi M, Terasaki T, Ohta S. Profiling of illegal amphetamine-type stimulant tablets in Japan. J Health Sci 2003;49:129–37.
12. Caldwell J, Dring LG, Williams RT. Metabolism of (14 C)methamphetamine in man, the guinea pig and the rat. Biochem J 1972;129:11–22.
13. Helmlin HJ, Bracher K, Bourquin D, Vonlanthen D, Brenneisen R. Analysis of 3,4-methylenedioxymethamphetamine (MDMA) and its metabolites in plasma and urine by HPLC-DAD and GC-MS. J Anal Toxicol 1996;20:432–40.
14. de la Torre R, Farre M, Ortuno J, Mas M, Brenneisen R, Roset PN, et al. Non-linear pharmacokinetics of MDMA (''ecstasy'') in humans. Br J Clin Pharmacol 2000;49:104–9.
15. Shima N, Kamata HT, Katagi M, Tsuchihashi H. Urinary excretion of the main metabolites of methamphetamine, including p-hydroxymethamphetamine-sulfate and p-hydroxymethamphetamine-glucuronide, in humans and rats. Xenobiotica 2006;36:259–67.
16. Shima N, Katagi M, Kamata H, Zaitsu K, Kamata T, Nishikawa M, et al. Urinary excretion of the main metabolites of 3,4-methylenedioxymethamphetamine (MDMA), including the sulfate and glucuronide of 4-hydroxy-3-methoxymethamphetamine (HMMA), in humans and rats. Xenobiotica 2008;38:314–24.
17. Lin LY, Di Stefano EW, Schmitz DA, Hsu L, Ellis SW, Lennard MS, et al. Oxidation of methamphetamine and methylenedioxymethamphetamine by CYP2D6. Drug Metab Dispos 1997;25:1059–64.
18. Wu D, Otton SV, Inaba T, Kalow W, Sellers EM. Interactions of amphetamine analogs with human liver CYP2D6. Biochem Pharmacol 1997;53:1605–12.
19. Kreth K, Kovar K, Schwab M, Zanger UM. Identification of the human cytochromes P450 involved in the oxidative metabolism of ''ecstasy''-related designer drugs. Biochem Pharmacol 2000;59:1563–71.
20. Segura M, Farre M, Pichini S, Peiro AM, Roset PN, Ramirez A, et al. Contribution of cytochrome P450 2D6 to 3,4-methylenedioxymethamphetamine disposition in humans: use of paroxetine as a metabolic inhibitor probe. Clin Pharmacokinet 2005;44:649–60.
21. Meyer MR, Peters FT, Maurer HH. The role of human hepatic cytochrome P450 isozymes in the metabolism of racemic 3,4-methylenedioxy-methamphetamine and its enantiomers. Drug Metab Dispos 2008; 36:2345–54.
22. Forsling ML, Fallon JK, Shah D, Tilbrook GS, Cowan DA, Kicman AT, et al. The effect of 3,4-methylenedioxymethamphetamine (MDMA, ''ecstasy'') and its metabolites on neurohypophysial hormone release from the isolated rat hypothalamus. Br J Pharmacol 2002;135:649–56.
23. Fallon JK, Shah D, Kicman AT, Hutt AJ, Henry JA, Cowan DA, et al. Action of MDMA (ecstasy) and its metabolites on arginine vasopressin release. Ann NY Acad Sci 2002;965:399–409.
24. Kuwayama K, Inoue H, Kanamori T, Tsujikawa K, Miyaguchi H, Iwata Y, et al. Interactions between 3,4-methylenedioxymethamphetamine, methamphetamine, ketamine, and caffeine in human intestinal Caco-2 cells and in oral administration to rats. Forensic Sci Int 2007;170:183–8.
25. Kuwayama K, Inoue H, Kanamori T, Tsujikawa K, Miyaguchi H, Iwata Y, et al. Uptake of 3,4-methylenedioxymethamphetamine and its related compounds by a proton-coupled transport system in Caco-2 cells. Biochim Biophys Acta 2008;1778:42–50.
26. Inoue T, Suzuki S. Studies on metabolism of methamphetamine analogs 6. Analysis of dimethylamphetamine and its metabolites. Rep Natl Res Inst Police Sci 1986;39:228–34.
27. Tanaka K, Inoue T, Ohki H. Analysis of 3,4-methylenedioxymethamphetamine and its metabolites. Rep Natl Res Inst Police Sci 1988; 41:114–9.
28. de la Torre R, Farre M, Roset PN, Pizarro N, Abanades S, Segura M, et al. Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition. Ther Drug Monit 2004;26:137–44.
29. Zhu BT. Catechol-O-methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. Curr Drug Metab 2002;3:321–49.
30. Kuwayama K, Tsujikawa K, Miyaguchi H, Kanamori T, Iwata YT, Inoue H. Determination of 4-hydroxy-3-methoxymethamphetamine as a metabolite of methamphetamine in rats and human liver microsomes using gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. J Anal Toxicol 2009;33:266–71.
31. Baba T, Yamada H, Oguri K, Yoshimura H. Studies on N-demethylation of methamphetamine by means of purified guinea-pig liver flavin-containing monooxygenase. Biochem Pharmacol 1987;36:4171–3.
32. Yamaoka K, Nakagawa T, Uno T. Statistical moments in pharmacokinetics. J Pharmacokinet Biop 1978;6:547–58.
33. Pilgrim JL, Gerostamoulos D, Drummer OH, Bollmann M. Involvement of amphetamines in sudden and unexpected death. J Forensic Sci 2009;54:478–85.
34. Clemens KJ, Cornish JL, Hunt GE, McGregor IS. Repeated weekly exposure to MDMA, methamphetamine or their combination: long-term behavioural and neurochemical effects in rats. Drug Alcohol Depend 2007;86:183–90.
35. Escubedo E, Camarasa J, Chipana C, Garcia-Rates S, Pubill D. Involvement of nicotinic receptors in methamphetamine- and MDMA-induced neurotoxicity: pharmacological implications. Int Rev Neurobiol 2009;88:121–66.
36. Kikura-Hanajiri R, Kawamura M, Miyajima A, Sunouchi M, Goda Y. Determination of a new designer drug, N-hydroxy-3,4-methylenedioxy-methamphetamine and its metabolites in rats using ultra-performance liquid chromatography-tandem mass spectrometry. Forensic Sci Int 2010;198:62–9.
37. Meyer MR, Maurer HH. Metabolism of designer drugs of abuse: an updated review. Curr Drug Metab 2010;11:468–82.

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