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Distribution of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) stereoisomers in a fatal poisoning

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Abstract

This communication presents the quantitation and differential distribution of the enantiomers of 3,4-methylenedioxymethamphetamine (MDMA) and its physiologically active metabolite 3,4-methylenedioxyamphetamine (MDA) in a fatal poisoning following insufflation of MDMA, cocaine and heroin. Animal studies have demonstrated the stereoselective pharmacokinetics and neurotoxicity of these compounds; however, enantiomeric distributions have not been reported in humans. Quantitation of MDMA and MDA enantiomer was by gas chromatography/mass spectrometry (GC/MS) following chiral derivatization with *N*-trifluoroacetyl-L-triproyl chloride (LTPC). The decedents' blood concentration of S(+)-MDMA was slightly less than that of R(−)-MDMA (1.3 vs. 1.6 mg/l, respectively), while the S(+)- and R(−)-MDA blood concentrations were identical (0.8 mg/l). Both primary routes of excretion, bile and urine, had greater concentrations of R(−)-MDMA than the S(+) isomer. These fluids also contained twice the concentration of

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S(+)-MDA than the R(−)-isomer. These data indicate that S(+)-MDMA is metabolized and eliminated faster than R(−)-MDMA. The results appear to support the findings in animals regarding stereoselective metabolism of MDMA.

Keywords: 3,4-Methylenedioxymethamphetamine (MDMA); 3,4-Methylenedioxyamphetamine (MDA); Fatal poisoning; Stereoisomers

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) and its demethylated analog/metabolite 3,4-methylenedioxyamphetamine (MDA) are psychotropic agents chemically and pharmacologically related to amphetamine and mescaline [1,2]. Both MDA and MDMA were first synthesized early in this century. MDA was initially tested as an anorexic agent, antitussive or ataractic, although it was never successfully brought to market as such [2]. MDMA was first synthesized by Merck in 1914 as an appetite suppressant, but never marketed. Both drugs have been associated with illicit drug use [3].

Clinically both drugs display marked sympathomimetic activity similar to amphetamine demonstrated by peripheral vasoconstriction, tachycardia, pupillary dilation and effects on other smooth muscles. Central nervous system stimulatory effects also mimic those of amphetamine and in cases of overdose convulsions, hyperthermia and behavior changes may occur [4,5]. Additionally, severe complications of MDMA overdose include rhabdomyolysis, intravascular coagulation, acute renal failure and hepatonecrosis [6]. Both MDA and MDMA produce perceptional distortions and pronounced subject effects including intensification of feelings, a facilitation of self-insight, an overwhelming desire to communicate, profound empathy and euphoria [2,3]. Both have been used to treat mental disorders such as depression and have been used as an adjunct to psychotherapy [2,3,7]. Presently, both drugs are classified a Schedule 1 controlled substances by the US Federal Drug Enforcement Agency.

MDA was a popular 'love drug' in the USA during the late 1960s and throughout the 1970s [3]; however, the drug is seldom encountered today. In the USA, MDMA, as 'Ecstasy', 'XTC' and 'X', has become a widely used drug of abuse beginning in the mid-1980s especially on college campuses [8,9]. In Europe, the drug is associated with the 'rave Culture'. MDMA's reputed popularity derives from effects such as positive mood changes, enhanced communication and intimacy, improved interpersonal relationships, increased self-esteem and elevated mood attributed to its usage. Following doses of 150–225 mg, MDMA users report experiencing positive changes in mood, attitude, beliefs, relationships, occupation, spiritual-physical condition, and a transient decrease in substance abuse following MDMA-assisted therapy sessions [10]. However, MDMA ingestion has also been reported to cause tachycardia, an occasional 'wired' feeling, jaw clenching, nystagmus, a nervous desire to be in motion, transient anorexia, panic attacks, nausea and vomiting, ataxia, urinary urgency, diplopia, insomnia, tremors, inhibition of ejaculation, and rarely, transient hallucinations [11].

MDA and MDMA contain an asystemic carbon and exist as a racemic mixture. While both isomers of MDMA are mild hallucinogens, the S(+)-isomer of MDA is thought to be more amphetamine-like while R(−)-MDA is hallucinogenic. Several investigators have shown MDMA to be a selective serotonin (5-HT) neurotoxin, causing retrograde destruction of 5-HT neurons following large doses [12–14]. The S(+)-isomer of MDMA has been reported to be a more potent neurotoxin than the R(−)-isomer [15]. Unlike MDMA, however, both isomers of MDA cause long-term serotonin neurotoxicity [16].

Animal studies have demonstrated the stereoselective pharmacokinetics and neurotoxicity of these compounds [3,17,18]; however, enantiomeric distributions have not been reported in humans. Additionally, while deaths due to MDMA have been reported, none of these reports have on focused on the presence or distribution of MDA as a metabolite of MDMA [6,9,19–21]. This communication presents the quantitation and differential distribution of the enantiomers of MDMA and its MDA metabolite in a fatal poisoning following insufflation of MDMA, cocaine and heroin.

2. Case history and autopsy findings

A 20-year-old, white male was staying with friends when he was found dead in bed at 14:00 h. He was last seen alive at approximately midnight the previous evening. There was no evidence of violence at the scene. The decedent had a history of polydrug abuse by insufflation. Postmortem examination of the body showed no evidence of assault type injuries. With the exceptions of petechial hemorrhages in the myocardium and pulmonary edema, gross and histological pathology findings were unremarkable. Toxicology analysis of blood revealed the following: 2.8 mg/l MDMA, 0.97 mg/l benzoylecgonine and 0.11 mg/l morphine. MDMA was also 'present' in liver, urine and vitreous. The cause of death was determined to be "acute poisoning due to insufflation of MDMA, cocaine and heroin" and the manner of death was ruled accidental. Specimens were then sent for chiral analysis and distribution studies of MDMA and its metabolite MDA.

3. Materials and methods

3.1. Reagents and chemicals

Racemic MDMA and MDA primary reference materials were purchased from Alltech Applied Science Labs (State College, PA). L-Triproyl chloride (LTPC) was purchased from Regis Technologies, Inc. (Morton Grove, IL). Solvents were HPLC grade, purchased from Fischer Scientific Products (Fair Lawn, New Jersey). *d,l*-Amphetamine-d5 and *d,l*-methamphetamine-d5 (Sigma Chemical Company, St. Louis, Missouri) were used as the internal standards for all GC/MS

procedures. 'CLEAN SCREEN' solid phase extraction columns (ZSDAU020) were purchased from United Chemical Technologies, Inc. (Bristol, PA).

3.2. Sample preparation

Drug-free autopsy tissue and fluids specimens were used to prepare MDMA and MDA calibrators. Liver samples were homogenized and suspended in three times their volume of deionized water. Bile and vitreous samples were diluted 1:3 with normal saline; 2.0-ml aliquots of liver homogenate, diluted bile and vitreous, urine or blood were used to prepare calibrators.

3.3. Liquid/liquid extraction

Aliquots of 2.0 ml of sample and calibrators containing 500 ng/ml *d,l*-amphetamine-d5 and *d,l*-methamphetamine-d5 as internal standards were made alkaline with 100 μl concentrated ammonium hydroxide and 4.0 ml of *n*-butylchloride extraction solvent were added. Following mixing and centrifugation, 3.0 ml of the *n*-butyl chloride layer was transferred to a 12×75 mm borosilicate test tube and evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was suspended in 400 μl of chloroform and 200 μl of LTPC derivatizing reagent were added. The mixture was vortexed for 3 min, then allowed to stand for 10 min at room temperature. Following derivatization, 1.0 ml of chloroform and 2.0 ml of sodium carbonate/bicarbonate buffer (pH 9.5) were added to each sample. The samples were mixed for 15 min and centrifugation at 3000 rev./min for 5 min. The upper (aqueous) layer was removed by aspiration and discarded. The remaining chloroform layer was evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was dissolved in 50 μl ethyl acetate. Two microliters were injected into the GC/MS for analysis.

3.4. Solid phase extraction

Two milliliters of urine, liver homogenate and diluted bile and vitreous containing 500 ng/ml *d,l*-amphetamine-d5 and *d,l*-methamphetamine-d5 as internal standards were acidified to pH 6.0 with 2.0 ml 0.1 M phosphate buffer in clean 16×125 mm test tubes. Blood samples, 2.0 ml, containing internal standards were diluted with 8.0 ml of deionized water, vortexed and allowed to stand for 5 min. Following centrifugation at 3000 rev./min for 5 min, the supernatants were removed to clean 16×125 mm test tubes and acidified to pH 6.0 with 3.0 ml 0.1 M phosphate buffer.

The extraction columns were conditioned by sequential rinsing with 3.0 ml methanol, 3.0 ml deionized water and 2.0 ml 0.1 M phosphate buffer. Aspiration was at ≤3 inchHg to prevent sorbent drying. Samples were then placed at head of the column and aspirated at 1–2 ml/min. The columns were sequentially washed with 3.0 ml deionized water, 3.0 ml 0.1 M HCl and 3.0 ml methanol and then allowed to dry for 5 min at ≥10 inchHg. The MDMA and MDA were eluted with

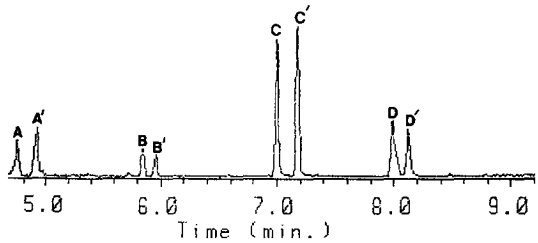

Fig. 1. Total ion chromatogram: R(−) and S(+)-amphetamine (A and A', respectively), R(−)- and S(+)-methamphetamine (B and B', respectively), R(−)- and S(+)-MDA (C and C', respectively) and R(−)- and S(+)-MDMA (D and D', respectively).

3.0 ml ethyl acetate/NH_4OH (98/2) which was prepared fresh daily. The eluates were evaporated to dryness under a gentle stream of nitrogen at room temperature and derivatized as above.

3.5. GC/MS analysis

GC/MS analysis was performed on a Hewlett-Packard 5890 GC equipped with a 12 m×0.2 mm (i.d.)×0.33 μm (film thickness) HP-1 capillary column connected to a Hewlett-Packard 5971-A mass selective detector. Data processing was performed with a HP Chemstation (Version 3.2 software). The GC/MS was operated in the splitless mode for 0.1 min with a helium carrier gas linear velocity of 20 ml/min. Initial oven temperature was 190°C for 4 min with an injection port temperature of 250°C. The temperature was ramped at 20°C/min to a final temperature of 250°C.

The following mass to charge ions were monitored: 123 and 237 (quantitative ion) for amphetamine-d5-LTPC, 92, 120 and 255 (quantitative ion) for methamphetamine-d5-LTPC, 135, 162 (quantitative ion) and 237 for MDA-LTPC and 135, 162 (quantitative ion) and 251 for MDMA-LTPC. The total ion chromatogram for these compounds showing retention times is presented in Fig. 1. The identification of S(+)- and R(−)-MDMA, and S(+)- and R(−)-MDA was based upon the elution order as determined by Fitzgerald et al. [22].

4. Results

Enantiomer and total concentrations of MDMA and MDA in bile, blood, liver, urine and vitreous as determined by liquid/liquid extraction are presented in Table 1. The total blood concentration of MDMA enantiomers of 2.9 mg/l was consistent with the result of the achiral analysis, 2.8 mg/l. Solid phase extraction of blood, liver and urine yielded MDMA and MDA concentrations within 80% to 120% of those of liquid/liquid extraction. For example, MDMA liver isomer values by liquid/liquid versus solid phase extraction were 5.0 and 1.4 mg/l vs. 5.5 and 1.5 mg/l, respectively. Either procedure appears suitable for extraction of

Table 1
MDMA and MDA (enantiomer and total) tissue concentrations in current case

	Bile (mg/l)			Blood (mg/l)			Liver (mg/kg)			Urine (mg/l)			Vitreous (mg/l)		
	R(−)	S(+)	Total	R(−)	S(+)	Total	R(−)	S(+)	Total	R(−)	S(+)	Total	R(−)	S(+)	Total
MDMA	58	15	73	1.6	1.3	2.9	5.0	1.4	6.4	302	227	529	1.2	0.7	1.9
MDA	0.5	1.2	1.7	0.8	0.8	1.6	0.3	0.4	0.7	8	18	26	0.2	0.04	0.244

Table 2
Tissue concentrations of all reported drugs in MDMA overdose fatalities

Reference	Blood[a] MDMA	Brain[a] MDMA	Liver[a] MDMA	Urine[a] MDMA	Comments
Dowling et al., [9]	Present	X	X	X	
Dowling et al., [9]	1.1	X	X	X	
Dowling et al., [9]	1.0	X	X	X	EtOH=0.04 g%
Suarez and Riemersma [19]	2	X	X	50	MDA=NMA
Henry et al., [6]	0.36	X	X	X	
Henry et al., [6]	Present	X	X	X	
Henry et al., [6]	Present	X	X	Present	
Henry et al., [6]	0.42	X	X	X	
Henry et al., [6]	0.11	X	X	X	
Henry et al., [6]	1.16 MDA=0.06 Amph=0.1	X	X	X	
Henry et al., [6]	1.26	X	X	X	
Rohrig and Prouty [20]	10.9 (Heart)	13.7	20.2	X	MDA=NMA
Rohrig and Prouty [20]	0.6 Diaz=0.3 Nordiaz=0.7 BE=<0.2	<1.6	1.8	X	
Forrest et al., [21]	2.1 MDA=8.5 Amph=256 MDEA=3.5	X	X	Present Present Present Present	Gastric[a]=96 Gastric[a]=299 Gastric[a]<0.1 Gastric[a]=324

[a]mg/l; X, not done; NMA, no measurable amount.

MDMA and MDA. The total concentrations of MDMA in blood and tissues are consistent with those reported in previous fatal poisoning (Table 2).

R(−)-MDMA was found in higher concentrations than S(+)-MDMA in all specimens analyzed (Table 1). In bile, liver and urine, specimens associated with drug elimination, S(+)-MDA the metabolite of S(+)-MDMA was found in higher concentrations than R(−)-MDA. Although the exact enantiomeric composition of the MDMA administered by the decedent is unknown, it is likely this difference in enantiomer distribution is related to stereoselective biotransformation of S(+)-MDMA. Clandescent synthesis of MDMA is preformed by achiral methods resulting in a racemic product [23].

5. Discussion

As a result of the recent popular interest in the abuse of MDMA, and discoveries in the mid-1980s regarding the toxicity of MDA, investigators have begun to explore the neurochemical effects of MDMA [24]. They have found that much of the neurotoxicity originally attributed to MDMA may actually be a result of its more potent metabolite, MDA [25]. To further complicate the profile of MDMA toxicity, stereochemical and structural parameters also appear to be important determinants of neurotoxicity for MDMA and its analogs. Schmidt demonstrated that both enantiomers of MDMA caused an acute depletion of cortical 5-HT, but only S(+)-MDMA resulted in significant depletions of 5-HT and 5-HT uptake sites 7 days after administration [15]. Johnson et al. observed that the S(+) isomers of both MDMA and MDA were more potent than their R(−) isomers at depleting 5-HT and in depressing tryptophan hydroxylase (TPH), the rate-limiting enzyme in 5-HT synthesis [26]. Hiramatsu et al. have shown that while S(+)-MDMA was more potent then R(−)-MDMA in eliciting some stereotyped behaviors, S(+)-MDA was more potent than both of its parent enantiomers in eliciting these behaviors and produced wet-dog shake behavior [27]. This may indicate that S(+)-MDA has additional effects on other CNS transmitter systems in addition to the serotonergic system.

Fitzgerald et al. reported that S(+)-MDA stereoselectively accumulates in the blood of animals following a racemic dose MDMA [17]. It has since been shown that there is a stereoselective metabolism of S(+)-MDMA to S(+)-MDA resulting in peak plasma concentrations approximately three times that of R(−)-MDA and almost twice as much S(+)-MDA being excreted in the urine as R(−)-MDA [17,18,28].

This communication is the first report of the distribution of the enantiomers of MDMA and its metabolite, MDA in humans. The decedents' blood concentration of S(+)-MDMA was only slightly less than that of R(−)-MDMA (1.3. vs. 1.6 mg/l, respectively); while the S(+)- and R(−)-MDA blood concentrations were identical (0.8 mg/l). Both primary routes of excretion, bile and urine, had greater concentrations of R(−)-MDMA than the S(+) isomer (Table 1). These fluids also contained twice the concentration of S(+)-MDA than the R(−)-isomer (Table 1).

These data indicate that S(+)-MDMA is metabolized and eliminated faster than R(−)-MDMA. The results appear to partially support the data previously reported in rats [17,18,28]. However, it is difficult to draw firm conclusions from this one, isolated case. The route of administration was different in this case from those used in the rat (insufflation vs. i.v./i.p.). In the presented death, the dose was unknown, other drugs were consumed concurrently and the time of last administration prior to death is unknown. Additionally, the decedent was reported to be a chronic polydrug abuser and the effects of chronic administration, tolerance and bioaccumulation on the apparent stereoselective metabolism and neurotoxicity have not been studied. However, the partial support of the animal data presented in this case report, is intriguing and certainly points out the need for further research in this relatively new area of 'stereoselective toxicology'.

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