Antemortem and Postmortem Methamphetamine Blood Concentrations: Three Case Reports

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We compare antemortem whole-blood to postmortem peripheral blood concentrations of methamphetamine and its metabolite amphetamine in three medical examiner cases. Antemortem specimens, initially screened positive for methamphetamine by ELISA, were subsequently confirmed, together with the postmortem specimens, by GC-MS analysis following solid-phase extraction. Methamphetamine peripheral blood to antemortem blood ratios averaged 1.51 ($\pm$0.049; $n = 3$) and amphetamine peripheral blood to antemortem blood ratios averaged 1.50 ($n = 2$). These data show that postmortem redistribution occurs for both methamphetamine and amphetamine, revealing that postmortem blood concentrations are ~1.5 times greater than antemortem concentrations. Furthermore, as both methamphetamine and amphetamine have previously been shown to have liver/peripheral blood (L/P) ratios of 5–8, it can be proposed that drugs displaying L/P ratios ranging from 5 to 10 may exhibit postmortem concentrations up to twice those concentrations circulating in blood before death.

Introduction

Methamphetamine is a highly addictive central nervous system stimulant that can be injected, snorted, smoked or ingested orally. Although available by prescription for the treatment of attention-deficit disorder (1), the major use (abuse) of methamphetamine remains illicit—generally synthesized in clandestine laboratories. It is metabolized by N-demethylation to amphetamine, which is also a pharmacologically active drug (2).

Single oral doses of methamphetamine have been reported to produce peak plasma concentrations up to 0.02 mg/L with a 12.5 mg dose (3). A 30 mg oral dose resulted in an average peak serum methamphetamine concentration of 0.094 mg/L (range 0.062–0.291 mg/L) (4). Single intravenous doses (0.50 mg/kg) have resulted in an average peak plasma methamphetamine concentration of 0.132 mg/L, with amphetamine at 0.0092 mg/L (5). Half-life of elimination is pH dependent, ranging from 6 to 15 h for methamphetamine and 7 to 34 h for amphetamine (2).

Blood concentrations ranging from 0.15 to 0.56 mg/L have been reported in methamphetamine abusers showing violent and irrational behavior (6) and from 0.05 to 2.6 mg/L in individuals arrested for erratic driving (7). Postmortem blood concentrations have been described to range from 1.4 to 13 mg/L in abusers who died of traumatic injury by violent means (8). Deaths resulting from overdose have been shown with methamphetamine concentrations ranging from 0.09 to 18 mg/L, with an average of 1.0 mg/L (9). When attempting to compare blood and clinical plasma/serum concentrations, it is important to be aware that the blood/plasma ratio for methamphetamine is ~0.6–0.7 (2).

The distribution of methamphetamine and amphetamine in postmortem peripheral blood, central blood and liver has been recently reported (10). Methamphetamine central blood-to-peripheral blood (C/P) ratios were found to average 1.61 ($\pm$0.48), and liver to peripheral blood (L/P) ratios averaged 5.68 ($\pm$2.32). Comparable data were found for amphetamine. These data showed a smaller average C/P ratio than that previously reported by Barnhart *et al.* (11), but established that methamphetamine and amphetamine were most likely prone to some degree of postmortem redistribution (PMR). However, since there was no opportunity for analyses in both antemortem and postmortem specimens from the same individuals, a direct assessment of the degree of PMR could not be determined.

The study reported here examines three cases in which antemortem specimens were collected and postmortem peripheral blood specimens were also available. This study presents an investigation of PMR, and provides better insight on the extent to which methamphetamine and amphetamine concentrations may be expected to increase after death as a result of PMR.

Methods

Cases

Case 1

This 44-year-old man had no reported medical history. On the day of his death, he was with his girlfriend playing video games when he suddenly grabbed his chest and became unresponsive. Bystander cardiopulmonary resuscitation (CPR) was initiated. He was transported by ground ambulance to a nearby hospital with an estimated down time of 35 min. He arrived in the emergency room with CPR in progress with ventricular fibrillation. Despite administration of multiple cardiac medications, including epinephrine, lidocaine and amiodarone, and defibrillation attempts, he died in the emergency room. His girlfriend later admitted that they had been using methamphetamine. The autopsy documented findings of hypertensive and atherosclerotic cardiovascular disease. The heart was enlarged (580 g) with concentric left ventricular hypertrophy. The coronary arteries demonstrated focal, moderate to marked calcific atherosclerotic stenosis of the vessel lumens. Microscopic examination of the heart muscle documented both acute cardiomyocyte necrosis and extensive areas of older fibrosis. Toxicology testing confirmed only methamphetamine. The cause of death was listed as hypertensive and atherosclerotic cardiovascular disease with acute methamphetamine intoxication contributing. Autopsy was performed 30.5 h after death. Antemortem blood specimens were drawn 22 min prior to pronouncement of death.

Case 2

This 46-year-old man was the unrestrained rear seat passenger of a pickup truck traveling on an interstate when it veered off the

road and went down a center embankment, rolling over. He was partially ejected. Witnesses found him initially responsive and yelling. He was transported by ground to a nearby location for airlift, but lost his pulse while being loaded onto the helicopter. He arrived at a regional trauma center with resuscitative efforts still underway. Despite resuscitative efforts, death was pronounced almost 2 h following the initial report of the incident. The decedent's medical history included fibromyalgia, chronic fatigue and methamphetamine use. Toxicology testing confirmed methamphetamine and cannabinoids (which were not quantified). The autopsy documented multiple bone fractures and visceral lacerations, and the cause of death was listed as multiple blunt force injuries. Autopsy was performed 22.5 h after death. Antemortem blood specimens were drawn 7 min prior to pronouncement of death.

Case 3

This 37-year-old male had a history of drug and alcohol abuse. On the day of his death, he developed erratic and bizarre behavior after consuming alcohol. While being taken to a regional hospital in a private passenger vehicle, he became unresponsive. On arrival, he had agonal breathing, but no heartbeat. Death was pronounced after resuscitative efforts in the emergency room. Toxicology testing confirmed alcohol (0.02%) and methamphetamine; gastric contents contained 15 mg of methamphetamine. The autopsy documented an empty, small, sealable plastic bag in the gastric contents, indicating ingestion of a "baggie" of methamphetamine. He had no significant trauma or natural disease. Pulmonary edema and congestion were evident (620 g, right; 560 g, left). The cause of death was listed as acute methamphetamine intoxication. Autopsy was performed 5.25 h after death. Antemortem blood specimens were drawn 9 min prior to pronouncement of death.

Postmortem sample collection and storage

Postmortem blood samples were collected by the pathologist during the autopsy and maintained at a refrigeration temperature (4°C) prior to, and after, the analysis. Peripheral blood specimens were drawn from the iliac arteries in the pelvis (sufficiently distant from the heart and other central organs) and stored in 10 mL BD Vacutainer® (Franklin Lakes, NJ) glass tubes containing sodium fluoride (100 mg) and potassium oxalate (20 mg).

Toxicology screening

Toxicological screening was requested and performed on the antemortem whole-blood specimens. The testing regimen consisted of alcohol (GC-FID headspace), and common drugs of abuse by ELISA (cocaine metabolite, opiates, benzodiazepines, fentanyl cannabinoids and amphetamines−methamphetamine) (Immunalysis, Inc., CA). Positive results were confirmed and quantified by subsequent techniques in both the antemortem and postmortem peripheral blood specimens.

Methamphetamine/amphetamine confirmation

Materials

Solvents (dichloromethane, methanol, ethyl acetate, isopropanol and acetone) were EMD Chemicals OmniSolv® grade, purchased from VWR International (Radnor, PA). Pentafluoropropionic anhydride (PFPA) was obtained from Sigma-Aldrich (St. Louis, MO). Ammonium hydroxide (ACS) and glacial acetic acid (ACS) were obtained from VWR International. Zinc sulfate heptahydrate (Certified ACS) was obtained from Fisher Scientific (Pittsburg, PA), and anhydrous sodium acetate (GR ACS Mallinckrodt) was obtained from VWR, Inc. Methamphetamine, amphetamine, methamphetamine-D5 and amphetamine-D5 were obtained from Cerilliant (Austin, TX). Solid-phase extraction (SPE) columns were Trace-B® from SPEWare Corp. (Baldwin Park, CA).

Aqueous working standards containing 1.0 mg/L each of methamphetamine and amphetamine and internal standards containing 1.0 mg/L each of methamphetamine-D5 and amphetamine-D5 were prepared. Linear calibration curves from 0.02 to 2.0 mg/L produced using five calibrators (0.02, 0.05, 0.25, 1.0 and 2.0 mg/L) were made by diluting the working standard. All calibrators were prepared in deionized water. A commercial whole-blood toxicology control containing 0.10 mg/L of methamphetamine and amphetamine obtained from UTAK Laboratories, Inc. (Valencia, CA) (Product #98818), and an in-house whole-blood control containing 0.40 mg/L of methamphetamine and amphetamine (prepared from a second source of drug stock) were run with each batch of calibrators and cases. Additionally, both blank and negative control specimens were extracted with each batch to confirm the lack of interference and/or contamination.

Extraction

Amphetamines were extracted using a solid-phase extraction procedure. A 2.0 mL sample was extracted for all standards, controls and casework. A working internal standard (0.25 mL) was added to all tubes. 5.0 mL of 5% zinc sulfate/methanol solution (50/50) was added to each tube, and the tubes were vortexed and centrifuged at 2,400 g for 10 min. The supernatant was buffered with 4 mL of 0.1 M sodium acetate buffer, pH 6. The SPE columns were conditioned by sequentially adding 2 mL each of ethyl acetate, methanol and acetate buffer (pH 6). The buffered supernatant was added to the SPE columns and allowed to flow through at 2−5 mL/min. Columns were then washed by sequentially adding 2 mL of deionized water and 1.0 mL each of 0.1 M acetic acid, methanol and ethyl acetate. Columns were dried at maximum pressure (40 psi nitrogen) for 60−90 s. Amphetamines were eluted with 2.0 mL elution solvent (dichloromethane/isopropanol/ammonium hydroxide 78/20/2) and allowed to drip through. The extracts were evaporated at room temperature under a stream of nitrogen until just dry. Derivatization was accomplished by adding 50 μL PFPA, capping tightly and vortexing, and allowed to stand at room temperature for 20 min. The extracts were reconstituted with 200 μL of ethyl acetate, mixed by vortexing and then transferred to autosampler vials.

Instrumentation

One microliter splitless injections were made onto an Agilent Technologies 6890 Gas Chromatograph. The GC column was an

HP-1 capillary column (Agilent Technologies 15 m, 0.25 mm diameter, 0.25 µm film thickness) with helium as the carrier gas. The GC oven was programmed to an initial temperature of 50°C, ramped 15°C/min until it reached 250°C and held for 2 min. An Agilent 5973 MSD was used for the selective ion monitoring. The GCMS was controlled by Chemstation software. The total chromatography time per injection was 12 min.

The following ions were monitored and used for measuring the internal standard: m/z 208 for methamphetamine-D5 and m/z 194 for amphetamine-D5. The ions monitored for quantitation were: m/z 204 for methamphetamine and m/z 190 for amphetamine. The ions monitored as qualifiers were: m/z 118,160 for methamphetamine and m/z 91,118 for amphetamine. Other compounds routinely detected and quantified with this method include ephedrine, pseudoephedrine, methylenedioxymethamphetamine, methylenedioxyamphetamine, phentermine and phenylephrine. The limits of detection and quantitation were 0.01 and 0.02 mg/L, respectively, for all compounds.

Accuracy and precision

The accuracy of the method for the quantitation of methamphetamine/amphetamine in blood was established over a 2-year timeframe. It was 103%/100% at 0.10 mg/L and 109%/105% at 0.40 mg/L over 42 analyses. Precision was established over the same period with methamphetamine/amphetamine having coefficients of variation of 2.9%/3.0% and 3.7%/2.6% for concentrations of 0.10 and 0.40 mg/L, respectively, over 42 determinations.

Results and discussion

The causes (manner) of death in cases 1 and 2 were determined to be hypertensive and atherosclerotic cardiovascular disease with acute methamphetamine intoxication (accident) and multiple blunt force injuries (accident), respectively. The third case was concluded to be acute methamphetamine intoxication (accident), with a postmortem concentration within the range previously reported for such cases (9).

Methamphetamine and amphetamine concentrations and ratios for the antemortem and postmortem blood analyses are shown in Table I. All cases showed higher postmortem concentrations—as indicated by ratios where methamphetamine and amphetamine concentrations were ~1.5 times higher in postmortem peripheral blood. Interestingly, the greatest difference was found in case 1, where the longest delay between antemortem blood collection and death was recorded (22 min) together with the greatest delay

Table I

Methamphetamine and Amphetamine Concentrations and Ratios

Case	MeAMP, PB	MeAMP, AM	AMPH, PB	AMPH, AM	MeAMP, PB/AM ratio	AMPH, PB/AM ratio
1	0.34	0.19	ND	ND	1.79	N/A
2	0.44	0.33	0.07	0.05	1.33	1.40
3	13.0	9.3	0.16	0.10	1.40	1.60
				Mean	**1.51**	**1.50**
				S.D.	**0.049**	**N/A**

MeAMP, methamphetamine; AMPH, amphetamine; AM, antemortem blood; PB, peripheral blood; ND, not detected; N/A, not available; Concentrations, mg/L.

before autopsy (30.5 h). When antemortem bloods were collected closer to the time of death and shorter autopsy delays were recorded, the postmortem methamphetamine concentrations showed smaller increases. Even with overdose (case 3), the postmortem methamphetamine concentration showed a minimal increase despite the possibility of incomplete drug distribution due to the acute ingestion of a substantial dose—a "baggie" of methamphetamine in the stomach.

It is now well documented that postmortem drug concentrations in blood may not always reflect antemortem drug concentrations due to the movement of the drugs after death. The mechanisms involved in PMR, however, are both complicated and poorly understood. Nevertheless, postmortem drug concentrations in postmortem blood may follow some generally accepted trends that aid interpretation. Generally speaking, the characteristics of the drug itself can be used to predict if a drug is subject to PMR—large changes in blood drug concentrations are predicted for basic, lipophilic drugs with a high volume of distribution (>3 L/kg). When PMR occurs, blood specimens drawn from the central body cavity and heart will generally have higher drug concentrations postmortem than specimens drawn from peripheral areas, most commonly the femoral region. The diffusion of drugs from organ tissue into the blood may explain the observed phenomenon (12). To compensate for PMR, it is frequently recommended that postmortem blood specimens are being collected from at least two areas of the body at autopsy; a peripheral area and a central area (often the heart), so that a comparison can be made.

Prouty and Anderson (13) first provided detailed information about blood drug concentrations attained from different sites for over 50 drugs. Then Dalpe-Scott *et al.* (14) presented a tabular list of the drug concentrations from both cardiac and peripheral blood samples expressed as a ratio of cardiac-to-peripheral blood (C/P) for over 100 drugs. The C/P ratio became the accepted benchmark with the accepted guideline that "high ratios" were associated with "potential for redistribution" (14). Based upon previous work, the C/P ratio model suggests that both methamphetamine and amphetamine have some propensity for PMR—ratios averaging 1.6 to 2.1 (10, 11).

Limitations of the C/P model, however, have been documented. While drug properties such as volume of distribution, protein binding and pKa are thought to contribute to PMR, a relationship between C/P and drug properties has not been established (15). In addition, there has been little agreement as to what ratio actually defines that a compound is prone to PMR or not (16). Reports of a C/P ratio >1.0 have been published for salicylate (17), tramadol (18) and carisoprodol (16), which are not prone to redistribution. Arterio-venous differences, anatomic variability within individuals and statistical chance may result in a C/P ratio >1.0 in drugs that do not redistribute. In addition, resuscitation attempts may result in a C/P ratio <1.0 (19). Inaccurate ratios may also be obtained as an artifact of sampling when the cardiac blood volume is depleted by the collection of blood from connected blood vessels, from trauma, or in cases of acute overdose where the drug has not undergone complete absorption and/or distribution. Additionally, although postmortem drug redistribution normally results from the diffusion of drugs from organ tissues into blood, and this process is most significant for blood collected from the central cavity, the possibility of some degree of PMR occurring in the peripheral blood cannot

be discounted ([20]). Consequently, the established C/P ratios can be inconclusive and even misleading with respect to the interpretation of PMR.

The liver (L) to peripheral blood (P) ratio has recently been proposed as an alternative and more reliable marker for PMR, with L/P ratios exceeding 20–30 indicative of a propensity for significant PMR, and ratios <5 indicative of little to no propensity toward PMR ([16], [21–23]). The L/P ratios for methamphetamine and amphetamine have been established to be ~5–8 ([10]), suggesting a minimal potential for PMR. Since these compounds are basic and lipophilic, with volumes of distribution (Vd) of 3–7 L/kg ([2]), this is also consistent with reports that such drugs—Vd > 3 L/kg—may be prone to some PMR.

Information from these three new case reports is supportive of both C/P ratio and L/P ratio data, and shows that PMR occurs for methamphetamine and amphetamine. Moreover, it purports that the postmortem blood concentrations may be ~1.5 times greater than blood levels circulating in the body at the time of death. These data also fit with the notion that L/P ratios <5 exhibit little to no PMR. With slightly higher ratios (~5–8), the expected PMR would be minimal or modest, consistent with postmortem blood methamphetamine and amphetamine increases of ~1.5 times (or less) above antemortem concentrations. Furthermore, it can be proposed that drugs with L/P ratios ranging between 5 and 10 may exhibit postmortem concentrations up to twice those concentrations circulating in blood before death. Hence, larger L/P ratios (ranging between 10 and 20) will then be consistent with more substantial differences between postmortem and antemortem concentrations—conceivably between 2 and 3 times—and even higher ratios producing even greater PMR. It is hoped that further development of this hypothesis will eventually lead to some predictive ability to assess the degree of postmortem drug concentration increase (or degree of PMR) of many other drugs based on their characteristic L/P ratios. This capability will provide an obvious advantage over the current description of many drugs, which simply states that PMR "may occur."

Acknowledgments

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