Arch. Pharm. Res. Vol. 16, No. 3, pp. 175-179, 1993

Determination of Methamphetamine and its Metabolite Amphetamine in Biological Fluids from 11 Fatal Cases

Youngchan Yoo, Heesun Chung and Hwakyung Choi

National Institute of Scientific Investigation, Seoul 158-097, Korea

(Received April 20, 1992)

Gas chromatography with flame ionization detector (FID) along with mass spectrometry (GC/MS) were used for the screening and quantification of methamphetamine (MA) and its mjaor metabolite, amphetamine (AM), in blood and urine in eleven fatal cases in which MA abuse was suspected. Postmortem blood MA varied from 0.5-30.2 μg/ml, while AM levels ranged from none detected (6 of 11 cases) to 4.8 μg/ml. Additionally, distribution studies were performed in three of these cases in which tissue samples were available for evaluation. Liver contained the highest concentration of MA among the tissue samples. In eight of the eleven cases, when no other direct cause of death was evident (i.e. 3 cases of traumatic death), either no blood AM was found or the ratio of MA/AM was 3.4 or greater. These data are consistent with acute MA use followed by death due to acute drug intoxication or by the occurrence of hypersensitivity and reverse tolerance seen in cases of chronic drug abuse.

Key words: Methamphetamine, Amphetamine, GC-FID, GC/MS, Postmortem distribution, Reverse tolerance

INTRODUCTION

Since 1985 the incidence of methamphetamine (MA) abuse has been on the rise in Korea. The number of cases found annually has increased from 13 in 1985, to 39 in 1986, 415 in 1987 and 1733 in 1988 (Yoo *et al.*, 1990). Fatalities, as might be expected directly due to MA abuse, have also increased during this period. In this latter context, MA concentration in biological fluids is one of the essential factors in the determination of the cause of death. As from this data the dose taken and the degree of intoxication may be estimated. A number of reports on blood MA concentration in the habitual users and in fatal cases have appeared in Japan (Fukunaga *et al.*, 1987; Une, 1983; Miyazake, 1986; Kojima *et al.*, 1984; Nagata, 1983) and the U.S.A. (Orrenius and Machly, 1970; Reynolds and Weingarten, 1983; Cravey and Reed, 1970; Cravey and Baselt, 1968). However, till this point in time, information of the fatalities has not been reported from Korea.

To establish a range for MA in fatal cases, first, it is necesary to develop a reliable and robust analysis scheme for the determination of MA and its major metabolite, amphetamine (AM) in biological samples. Secondly, MA and AM levels in blood, urine, gastric contents and tissue are examined to view the postmortem distribution of MA in a selected group of fatal cases. Finally, MA concentrations along with case history are utilized to determine the cause of death. These findings were discussed to understand the effect of MA on human behavior.

Correspondence to: Heesun Chung, National Institute of Scientific Investigation, Seoul 158-097, Korea

MATERIALS AND METHODS

Materials

Methamphetamine-HCl, amphetamine-H_2SO_4 and methoxyphenamine-HCl were purchased from Sigma Chemical Co. Trifluoroacetic anhydride (TFAA) was obtained from Aldrich Chemical Co. All other chemicals and solvents were of analytical reagent grade.

Biological specimens

Blood, urine and gastric contents were obtained at autopsy from eleven fatalities each having a history of MA abuse. Tissue was collected in three of these cases. All samples were stored at −40℃ until analysis were performed.

Calibration curve

Two calibration curves for MA and AM over the

range of 0.1-1 and 1-50 μg/ml were determined with two different internal standard solutions (0.01 and 0.1% in MeOH). The ratios of the peak areas of MA and AM to those of internal standard were utilized to calculate the concentration of these analytes in specimens.

Recovery test

One ml of urine was spiked with MA and AM at the concentration of 5, 10, 25 and 50 μg/ml. The extraction method is described below.

Analytical procedure

Routine screering for organic bases, sedatives, opiates, benzodiazepines was performed and their detection was not significant.

One ml of blood, urine or gastric contents and/or 1 gm of tissue homogenates was used for the the analysis. All tissue samples were homogenized in a glass homogenizer after addition of 5 ml of saturated salt solution. One ml of 6N-HCl and 10 ml of ether were added to homogenates. After shaking, the upper layer was discarded. The aqueous layer was washed twice with 10 ml of ether. It was adjusted to pH 9-10 with sodium hydroxide solution and 100 μl of 0.1% or 0.01% methoxyphenamine (internal standard) in MeOH was added. It was extracted with 5 ml of ethyl acetate, three times. The combined organic extracts were evaporated under vacuum after adding a few drops of acetic acid to prevent evaporation of amines. 50 μl of ethyl acetate and trifluoroacetic anhydride (TFAA) were added and heated at 65℃ for 15 min. The mixture was evaporated to dryness under nitrogen. The residue was dissolved in 100 μl of ethyl acetate. The specimen was initially analyzed on a Varian 4600 GC equipped with 15 m DB-5 megabore column. GC condition was as follows: column; DB-5, 0.53 mm×15 m, column temperature; programmed from 120℃ to 170℃ at 5℃/min and 170℃ to 260℃ at 20℃/min initial time 1 min, final time 10 min; injection port temperature; 270℃, detector temperature; 280℃, carrier gas; He.

Structural identification was confirmed on a Finnigan MAT ITD 800 GC/MS equipped with 0.32 mm×25 m SE-54 capillary column. MS conditions were as follows: ionization energy; 70 eV, transfer line temperature; 270℃, EM voltage; 1400v.

RESULTS

Analysis of methamphetamine/amphetamine

AM and MA were determined and confirmed by GC/MS as trifluoroacetyl derivatives measured on SE-54 (Fig. 1). Calibration curves of MA and AM were linear from 0.1 to 1 to 50 μg/ml. In higher concentration range, their linearities were y=0.0995x−0.1362 (r=0.9982) for AM and y=0.1003x−0.0454 for MA (r=0.9993), while in lower concentration AM showed y=2.4743x−0.2858 (r=0.9356) and MA revealed y=1.1654x−0.0585 (r=0.9992).

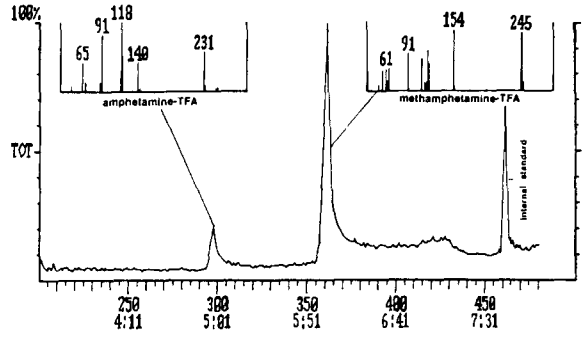

Fig. 1. Total ion chromatogram and mass spectra of methamphetamine-TFA and amphetamine-TFA in abuser's urine.

Table I. Recovery of methamphetamine and amphetamine by GC/FID from spiked urine

Amount added (μg)	Recovery (%) (mean± SD)		RSD (%)	
	AM	MA	AM	MA
5	101.9± 1.3	94.6± 1.9	1.3	2.0
10	97.2± 4.8	93.9± 1.1	4.9	1.2
25	99.9± 4.9	96.3± 1.6	4.9	1.7
50	96.6± 1.7	98.9± 2.0	1.8	2.0

n=3

As low as, 0.05 μg/ml of both MA and AM was detected in this method. The recoveries of MA and AM from spiked urines are given in Table I. The recoveries of MA and AM over the concentration range studied varied from 93.9± 1.1% and 96.6± 1.7% respectively. Coefficient of variation was found comparable for MA and AM with the range of 1.2-5.0%.

The concentration of methamphetamine/amphetamine in the specimens of all cases.

Table II is a tabulation of the concentration of MA and AM in blood, urine, the history and the cuase of death in eleven cases. In these eleven cases the range of MA in blood was between 0.5-30.2 μg/ml while AM varied from 0-4.8 μg/ml. The concentration of MA in urine ranged from 34.5-143.6 μg/ml whereas the content of AM varied from 0-18.9 μg/ml.

In eight of these cases there was no other traumatic or pathological cause of death. All these cases must be considered as MA fatalities in light of no other significant toxicology findings although these deaths may be consigned to heart failure in four cases, undetermined in 3 cases and a documented overdose in one case. In the remaining three cases a clear trau-

Table II. Postmortem methamphetamine and amphetamine concentrations in blood and urine, the cause of death and the history of eleven fatal cases

Case No.	Blood (μg/ml)			Urine(μg/ml)			Cause of death	History
	MA	AM	MA/AM	MA	AM	MA/AM		
1	0.6	n.d	–	34.5	n.d	–	sudden heart failure	found dead on the road
2	7.7	n.d	–	n.s	n.s	–	drug overdose	violent behavior hospitalized and death
3	30.2	n.d	–	n.s	n.s	–	unknown	unconsiousness after taking drug hositalized and died
4	1.4	0.1	14.0	n.s	n.s	–	unknown	abnormal excitation and death
5	16.4	4.8	3.4	134.8	18.9	7.1	sudden heart failure	found dead at the motel
6	3.2	n.d	–	45.3	n.d	–	unknown	arrested, violent behavior unconsiousness at 15:00 and death at 18:00
7	3.2	Tr	–	143.6	8.5	16.9	cardiac arrest	violent behavior and death
8	1.2	n.d	–	78.8	n.d	–	sudden heart failure	injection himself at 5:00 violent behavior and death on the way to the hospital at 6:10
9	1.4	1.0	1.4	56.2	3.3	17.0	falling	commit suicide in the state of hallucination
10	1.0	0.3	3.3	37.5	3.0	12.5	hemorrhage in brain	fighting, killed by a hammer
11	0.5	n.d	–	n.s	n.s	–	exsanguination	a stab wound on the thigh

MA; methamphetamine, AM; amphetamine, n.s; not submitted, Tr; trace, n.d; not detected

Table III. Methamphetamine concentrations in blood, urine (μg/ml) and tissue of three fatal cases (μg/g)

Case No	Blood	Urine	Gastric c.*	Liver	Brain	Heart	Lung	Spleen
6	3.2	45.3	17.0	5.5	n.a	n.a	2.6	n.a
7	3.2	143.5	15.2	2.9	0.4	0.6	1.5	0.1
10	1.0	37.5	3.2	3.9	2.2	0.1	0.5	0.4

matic cause of death could be dtermined, i.e. falling from a height in one case, cerebral cranial head trauma (beating with a hammer) in a second case, and exsanguination due to stab wound in third case.

From the obtained history it was determined that in the majority of cases the individual behaved violently and demonstrated an abnormal amount of excitability prior to death.

The methamphetamine concentration in tissues of 3 cases

A study of postmortem distribution of MA studied in three cases of the fatalities evaluated (Table III). Among liver, brain, heart, lung and spleen, liver demonstrated the highest content of MA. In case 6 and 7 the value in gastric contents was much higher than that in case 10. It was assumed that in these cases each individual ingested a large amount of the drug just prior to be arrested.

DISCUSSION

Various methods have been used for the determination of MA and its metabolite, AM, from body fluids. The most common methods for the detection of MA have been based on derivatization of the amine followed by GC-FID determination (Bailey and Shaw, 1989; Yamamoto *et al.*, 1981; Inoue *et al.*, 1983). The advent of capillary column led to the analysis of stimulants without derivatization, especially in the nitrogen/phosphorus detector (NPD) mode (Suzuki *et al.*, 1989; Christophersen *et al.*, 1988; Fitzgerald *et al.*, 1988).

In current work, when MA and AM were derivatized by TFAA, 0.05 μg/ml of AM and MA in the FID mode were detected. Calibration works of range from 0.1 to 1 and 1 to 50 μg were linear. Precision data over range of 5-50 μg/ml showed reproducibility. Variability of less than 5% was obtained. Because derivatization of MA with TFAA is another way for the verification of samples, it was possible to eliminate any false positive results in the current work.

In specimens from elven cases, the range of MA concentration in blood was from 0.5-30.2 μg/ml. Cases 2, 3 and 5 demonstrated high blood MA concentrations of 7.7, 30.2 and 16.4 μg/ml respectively. In these

cases with no other intereceeding pathological or traumatic cause of death, the cause of death was determined to be directly due to an acute MA intoxication. This is further supported by the evidence of a high ratio of MA/AM in each case (3.4 or greater in each case). These cases are consistent with other reports in which acute intoxication fatalities have been reported with levels exceeding 5.0 μg/ml (Fukunaga *et al.*, 1987). However, the lethal MA concentration in blood has varied in works by other investigators. Not all have found high levels of MA in fatal cases. Fukunaga *et al.* (1987) have reported the concentration of MA in blood in a range of 0.7-6.4 μg/ml in nine cases of MA poisoning. Cravey and Reed (1968) determined levels of 0.8 μg/ml in four cases of death by oral ingestion of the drug. Cravey and Baselt (1968) also reported on a case of a youth who following ingestion of a large amount of drug, demonstrated a 40 μg/ml concentration in blood, developed hyperpyrexia and died 5.5 hours later. Bailey and Shaw (1989) reported that the concentration range of 0.02-3.05 μg/ml for MA detected in cases related to both homicidal and accidental overdose. Orrenius and Machly (1970) also has reported on fatal cases with levels in blood MA lower than 0.5 μg/ml. These findings suggest that fatal poisoning by MA may occur in abusers who have demonstrated low blood levels of MA.

In case 1, 4, 6, 7 and 8 involving moderate to low levels of MA, the violent behaviors and abnormal excitment observed were apparently due to the intoxication derived from MA. This is especially noted to be so in cases 1 and 8, where according to the deceased's history, they had not used any MA for 2 or 3 months prior to their last used even though they had demonstrated a chronic abuse history. In these cases, the concept of hyparsensitivity and reverse tolerance may account for the fatality in the context of reduced blood levels. Fukunaga *et al.* (1987) have reported that small doses of MA may lead to a hypersensitivity response in the heart and circulatory collapse following the production of reverse tolerance. This finding is further supported by findings from cases 1, 5 and 8 in which heart failure was noted as the cause of death.

In each of the first eight cases noted on Table II, it is quite clear that MA must be considered as the cause of death in these cases, whether or not the blood concentration is high or low, because of lacking in any traumatic, pathological finding or additional drug toxicity.

Although, blood MA concentration was relatively low in case 9 and lower in case 10 and 11, the causes of death were not directly due to MA, as other traumatic causes of death interceded in each of these cases (Table II).

Postmortem MA distribution in three cases revealed that the highest concentration of the drug in the liver among tissues (Table III). This trend is consistent with findings in Kojima *et al.*'s (1984) and Nagata's (1983) work.

It is clear that large amounts of MA can lead to toxicity and fatality in humans. However it is difficult ot establish the minimum lethal concentration of MA in humans, because hypersensitivity and reverse tolerance can also occur in the chronic abuser. In current work, we have demonstrated a group of eight cases in which death was directly due to MA toxicity or indirectly as a result of hypersensitivity and reverse tolerance. Despite .the fact that in 3 additional cases the presence of drug alone does not account for cause of death, it is still of value to be able to interpret the effect of MA when a fatality has occurred. To grasp the overall effect of MA on human fatalities, we must consider the significancke of MA concentration, as well as, the history and behaviors of the drug user/abuser.

CONCLUSION

The analysis of MA and AM in biological fluid and tissue was performed using GC-FID involving TFA derivatized analystes. The analysses were linear through 50 μg/ml, recovery was 93.9± 1.1-101.9± 1.3% over the range studied. Method was sensitive down to 0.05 μg/ml.

Postmortem blood MA concentration in eleven fatalities ranged from 0.5-30.2 μg/ml, while blood AM ranged from 0-4.8 μg/ml; in urine MA concentrations ranged from 34.5-143.6 μg/ml and AM varied from 0-18.9 μg/ml. Distribution studies of MA in tissue revealed that the liver demonstrated the highest postmortem concentration.

In eight of the elven fatalites death was due directly to MA toxicity or indirectly due to hypersensitivity and reverse tolerence following an acute MA exposure. In these cases a MA/AM ratio was greater than 3.4 and there were no other pathological processes, traumatic injuries or drug toxicities.

ACHNOWLEDGEMENTS

The authors are grateful to Dr. Bradford Hepler, the chief toxicologist at Wayne County Medical Examiner's Office, U.S.A for his advice.

REFERENCES CITED

Bailey, D. N. and Shaw, R. F., Cocaine and methamphetamine-related deaths in San Diego County (1987); Homicides and accidental overdoses. *J. Forensic Sci.*, 34, 407-422 (1989)

Christophersen, A., Dahlin, E. and Pettersen, G., Gas chromatographic quantification of underivatized am-

phetamine in whole blood. *J. Choromatogr,* 432, 290-296 (1988).

Cravey, R. H. and Baselt, R. H. Methamphetamine poisoning. *J. Forensic Sci. Soc.,* 8, 118-120 (1968).

Cravey, R. H. and Reed, D., Intraveneous amphetamine poisoning; report of three cases. *J. Forensic Sci. Soc.,* 10, 109-112 (1970).

Fitzgerald, R. L., Ramos, J. M., Bogema, S. C. and Poklis, A., Resolution of methamphetamine stereoisomers in urine drug testing; Urinary excretion of R-(−)-methamphetamine following use of Nasal inhalers. *J. Anal. Toxicol.,* 12, 255-259 (1988).

Fukunaga, T., Mizoi, Y., Adachi, J. Tatsuno, Y., Fujiwara, S. and Ueno, Y., Methamphetamine concentration in blood, urine and organs of fatal cases after abuse. *Jpn. J. Legal Med.,* 41, 328-334 (1987).

Inoue, T., Suzuki, S. and Niwaguchi, T., Stability of perfluoroacyl derivatives of methamphetamine and its metabolites. *Eisei Kagaku.,* 29, 412-417 (1983).

Kojima, T., Une, I., Yashiki, M., Noda, J., Sakai, K. and Yamamoto, K., A fatal methamphetamine poisoning associated with hyperprexia. *Forensic Sci. Int.,* 24, 87-93 (1984).

Miyazake, T., Relationship between metamphetamine and amphetamine concentrations in the blood and symptoms in metamphetamine abusers. *Jpn. J. Legal Med.,* 40, 132-139 (1986).

Nagata, T., Signification of methamphetamine concentration in body fluids and tissues. *Jpn. J. Legal. Med.,* 37, 513-518 (1983).

Orrenius, S. and Machly, A. C., Lethal methamphetamine intoxication a report of three cases. *J. Legal Med.,* 67, 184-189 (1970).

Reynolds, P. C. and Weingarten, H., Presented at the quarterly meeting of the Califomia association of toxicologistsk, Yosemite National Prark, California, Nov. 5 (1983).

Suzuki, S., Inoue, T., Hori, H. and Inayame, S., Analysis of methamphetamine in hair, nail, sweat, and saliva by mass fragmentography. *J. Anal. Toxicol.,* 13, 176-178 (1989).

Une, I., A study on the metabolism of methamphetamine in its abusers. *Jpn. J. Legal Med.,* 37, 814-822 (1983).

Yamamoto, T., Terade, M., Yoshida, T., Yoshimura, S. and Kuroiwa, Y., Determination of sympathomimetic amine by gas chromatography equipped with flame thermoionic detector and irs application to forensic chemistry, *Eisei Kagaku.,* 27, 326-330 (1981).

Yoo, Y. C., Chung, H. S., Lee, H. S., Choi, H. K., Yang, J. Y. and Kim, C. S., Statistical survey of methamphetamine abuse in Korea. *Annual report of N.I.S.I.,* 22, 138-147 (1990).