CASE REPORT

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Distribution of Free and Conjugated Morphine in Body Fluids and Tissues in a Fatal Heroin Overdose: Is Conjugated Morphine Stable in Postmortem Specimens?

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ABSTRACT: The tissue distribution of free and conjugated morphine in a male individual who died after self-injection of heroin and methamphetamine was investigated, and the postmortem stability of morphine in the blood, liver and urine, and that of 6-monoacetylmorphine in the urine was determined. Confirmation and quatitation of morphine, 6-monoacetylmorphine and methamphetamine were performed by gas chromatography/mass spectrometry and gas chromatography, respectively. Blood levels of free and total morphine were very site-dependent with ranges of 462–1350 and 534–1570 ng/mL, respectively. Large amounts of total morphine, 5220, 4200, and 2270 ng/g, had accumulated in the stomach contents, liver, and lung, respectively. The concentration of free morphine in the cerebrospinal fluid was correlated very closely with that in the cerebrum. The proportion of free morphine in various fluids and tissues ranged from 23.0% to 98.8% of total morphine: less than 30% in the stomach contents and urine; 30–60% in the liver, cerebrospinal fluid, lung, and pericardial sac fluid; 61–90% in the spleen, right femoral muscle, myocardium, blood in the left and right ventricles of the heart, and right femoral vein blood; more than 91% in the right kidney and cerebrum. Detectable amounts of 6-monoacetylmorphine, 417 ng/mL and 78 ng/g, existed in the urine and stomach contents, respectively, indicating that this individual might have died within several hours after heroin injection. Methamphetamine concentrations in the blood were also site-dependent within the range 551–1730 ng/mL. In an *in vitro* experiment, free and conjugated morphine were stable in the blood and urine at 4, 18–22, and 37°C for a 10-day study period. In the liver, however, conjugated morphine had been converted almost completely to free morphine at 18–22 and 37°C by the end of the experiment, although it was stable at 4°C. Urine 6-monoacetylmorphine, although degraded slightly at 37°C, was stable at 4 and 18–22°C during the experiment. Thus it appears that non-specific hydrolysis of conjugated morphine to free morphine would not occur in corpses at least for a few days after death. Femoral muscle may be a specimen of choice for roughly predicting the ratio of free to total morphine in blood even when blood specimens are not available, because the femoral muscle is relatively spared of both postmortem diffusion of drugs and bacterial invasion.

KEYWORDS: forensic science, forensic toxicology, morphine, tissue distribution of morphine, postmortem stability of morphine glucuronides, 6-monoacetylmorphine, methamphetamine, gas chromatography

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A thorough understanding of the tissue distribution and postmortem stability of drugs is essential for forensic toxicologists, not only for selecting appropriate specimens for analysis of drugs in corpses, but also for correct interpretation of drug levels in postmortem fluids and tissues, and how seriously individuals have been poisoned by the drugs.

Heroin, after being injected, is rapidly metabolized in blood to 6-monoacetylmorphine with a half-life of approximately 3 min; its conversion is completed within 10–15 min (1,2). 6-Monoacetylmorphine is further deacetylated, presumably in liver and other tissues, to produce morphine at a somewhat slower rate, which is completed within a few hours (2,3). Morphine is then converted mainly to morphine-3-glucuronide, an inactive metabolite, and partly to morphine-6-glucuronide, an active metabolite (4–6). Thus, detection of heroin and its metabolites in postmortem specimens is very helpful for indicating when individuals injected heroin before death. Goldberger et al. (7) reported that rapid deaths were characterized by a higher mean concentration and greater likelihood of detection of 6-monoacetylmorphine in blood and a lower mean concentration of 6-monoacetylmorphine in urine. Staub et al. (8) reported that a mean of 76% of total morphine in blood was present as the free base in individuals who died rapidly after heroin injection, whereas a mean of 31% of the total morphine existed as unconjugated morphine in individuals who survived for a period of time. Spiehler and Brown (9) also reported a mean of 68% unconjugated blood morphine in putative rapid deaths after heroin or morphine use. Despite growing knowledge about changes in the concentration ratios of free to total morphine in blood and urine after heroin or morphine injection (3,10), there is little literature about these ratios in other body fluids and tissues. Moreover, only limited information is available on the postmortem stability of conjugated morphine in tissues, although morphine itself is known to be very stable even in heavily decomposed tissues (9,11,12).

In this study, we investigated: 1) the tissue distribution of free and conjugated morphine in a case of fatal heroin overdose, and 2) the stability of free and conjugated morphine, and 6-monoacetylmorphine in postmortem blood, liver and/or urine.

Case History

The victim was a 42-year-old man, who was found dead in a prone position in bed at a hotel. At autopsy, the postmortem interval was estimated to be 8–9 h. Old and new injection marks were observed in the left cubital fossa. Petechiae were observed on the conjunctiva of the left eye, laryngeal mucosa, epicardium, and mucosa of the renal pelves to a slight or moderate degree. The lungs were edematous; the left weighed 370 g and the right 550 g. There were no remarkable changes other than congestion in other organs.

Toxicological Findings

Screening for Drugs-of-Abuse with Triage™ and a Gas Chromatograph/Mass Spectrometer

Triage screening was performed on deproteinized urine and blood (13). The urine was positive for opiates and amphetamines; the blood was positive for opiates.

GC/MS screening for urine was performed as follows: To 1-mL urine were added 2-mL saturated acetate buffer (pH 9.0) and 6-mL chloroform: isopropanol (3:1). The mixture was shaken using a mechanical shaker for 20 min and centrifuged. Five milliliters of the lower organic phase was transferred to a glass tube containing 1 mL 0.4 N HCl and the mixture was vortexed for 1 min using a vortex mixer. Then 0.95 mL of the upper aqueous phase was transferred to a glass tube containing 2-mL saturated acetate buffer. The mixture was extracted with 6-mL chloroform:isopropanol (3:1) using the vortex mixer for 1 min. The lower organic phase was then evaporated to dryness. The residue was derivatized with 0.1 mL ethyl acetate and 0.1 mL trifluoroacetic anhydride at room temperature for 1 h. The residue was again taken to dryness and then dissolved in 50-μL ethyl acetate. A 5-μL aliquot of the mixture was injected onto a 2 m by 0.26 cm ID glass column packed with 2% OV-1 on 60–80 mesh Chromosorb W AW DMCS, on a GC/MS consisting of a Shimadzu GC-9A (Kyoto, Japan) and a Shimadzu QP 1000 (Kyoto, Japan). The temperature of the injection port and separator was 280°C. The column temperature was programed as follows: The initial temperature of 150°C was maintained for 2 min and then increased to 280°C at a rate of 10°C/min. The final temperature was maintained for 10 min. The electron impact ionization energy and accelerating voltage were 70 eV and 3 kV, respectively. The carrier gas was helium, with a flow rate of 40 mL/min. Mass spectral analysis was performed in the scan mode for masses from 50 to 500 atomic mass units. The GC/MS analysis of the urine revealed the presence of morphine, 6-monoacetylmorphine, amphetamine, and methamphetamine. No heroin was detected.

GC Quantitation of Free and Total Morphine, and 6-Monoacetylmorphine

One milliliter of fluids and 1 g of tissue.homogenates (tissue: H_2O = 1:3) were processed as described above for free morphine (A). These specimens were also processed by a method described by Spiehler and Brown (9) for apparent free morphine (B) composed of A and morphine originating from 6-monoacetylmorphine, and for total morphine including morphine glucuronides. As an internal standard, 1 or 10 μg levallorphan was used for quantitation of morphine in each specimen. Each extract, which was derivatized with trifluoroacetic anhydride, was injected into a Shimadzu GC-14B (Kyoto, Japan) equipped with a 15 m by 0.53 mm ID TC-1 capillary column and flame thermoionization detector. The temperatures of the injection port and column were identical to those for the GC/MS. The temperature of the detector was maintained at 280°C. The carrier gas was nitrogen, with a flow pressure of 15 kPa. The concentrations of 6-monoacetylmorphine were determined using the formula: (B − A) × M.W. of 6-monoacetylmorphine/M.W. of morphine. As shown in Table 1, morphine levels in the blood were greatly site-dependent. Large amounts of total morphine had accumulated in the stomach contents, liver, and lung. The concentration of free morphine in the cerebrospinal fluid was correlated very closely with that in the cerebrum. Free morphine in various fluids and tissues ranged from 23.0% to 98.8% of total morphine: less than 30% in the stomach contents and urine; 30–60% in the liver, cerebrospinal fluid, lung, and pericardial sac fluid; 61–90% in the spleen, right femoral muscle, myocardium, blood in the left and right ventricles of the heart, and right femoral vein blood; more than 91% in the right kidney and cerebrum. Detectable amounts of 6-monoacetylmorphine, 417 ng/mL and 78 ng/g, existed in the urine and stomach contents, respectively.

GC Quantitation of Methamphetamine

Determination of methamphetamine in the body fluids and tissues was performed by a GC method we have described previously (13). Methamphetamine concentrations in the blood were also site-dependent within the range 551–1730 ng/mL. In other specimens, 610 to 9760 ng/mL or ng/g methamphetamine was detected (Table 2). Although amphetamine concentrations were not determined, its level in the urine was roughly estimated to be 1/10 of the urine methamphetamine level. Only trace amounts of amphetamine existed in other specimens.

In Vitro Experiment

The blood, urine, and liver obtained from the present autopsy case were used. A portion of each specimen in a glass bottle with a cap was stored at 4, 18–22, and 37°C, and duplicate analyses of

TABLE 1—*The concentrations of morphine and 6-monoacetylmorphine in various fluids and tissues of an individual who died after self-injection of heroin and methamphetamine.*

Specimen	Morphine (ng/mL or ng/g)			6-MAM* (ng/mL or ng/g)
	Free	Total	% Free	
Blood				
Left ventricle of the heart	800	1170	68.4	ND
Right ventricle of the heart	462	534	86.5	ND
Right femoral vein	1350	1570	86.0	ND
Pericardial sac fluid	757	1500	50.5	ND
Cerebrospinal fluid	364	960	37.9	ND
Urine	435	1680	25.9	417
Cerebrum	336	340	98.8	ND
Heart muscle	668	848	78.8	ND
Lung				
Right middle lobe	1140	2270	50.2	ND
Liver				
Deep tissue of right lobe	1440	4200	34.3	ND
Spleen	700	956	73.2	ND
Right kidney	1790	1900	94.2	ND
Right femoral muscle	736	992	74.2	ND
Stomach contents	1200	5220	23.0	78

*6-Monoacetylmorphine.

TABLE 2—*The concentrations of methamphetamine in various fluids and tissues of an individual who died after self-injection of heroin and methamphetamine.*

Specimen	Methamphetamine (ng/mL or ng/g)
Blood	
Left ventricle of the heart	875
Right ventricle of the heart	551
Right femoral vein	1730
Pericardial sac fluid	750
Cerebrospinal fluid	610
Urine	9550
Cerebrum	2060
Heart muscle	2970
Lung	
Right middle lobe	1730
Liver	
Deep tissue of right lobe	8280
Spleen	5280
Right kidney	6880
Right femoral muscle	1450
Stomach contents	9760

each specimen were performed for free and total morphine for up to 10 days. The urine specimens were also analyzed for 6-monoacetylmorphine. The pH of each specimen was monitored.

Results

No change in the concentration of either free or conjugated morphine was observed in the blood and urine at any temperature for a 10-day study period with the reproducibility of the analytical method used (C.V. = approximately 10%). In the liver, however, conjugated morphine had been converted almost completely to free morphine at 18–22 and 37°C by the end of the experiment, although no hydrolysis of conjugated morphine was observed at 4°C (Fig. 1). No changes in the levels of liver total morphine were observed during the experiment. Urine levels of 6-monoacetylmorphine scarcely changed at 4 and 18–22°C during the 10-day period, and decreased very slightly at 37°C on and after day 5 (Fig. 2).

The pH of the blood was 6.4 at the start of the experiment and increased to 6.6, 7.0 and 7.3 at 4, 18–22, and 37°C, respectively, 10 days later. In the liver, the initial pH value of 6.0 changed greatly depending on the temperature; little change was observed at 4°C during the 10-day study. No changes in urine pH were observed at any of the temperatures selected during the experiment (Fig. 3).

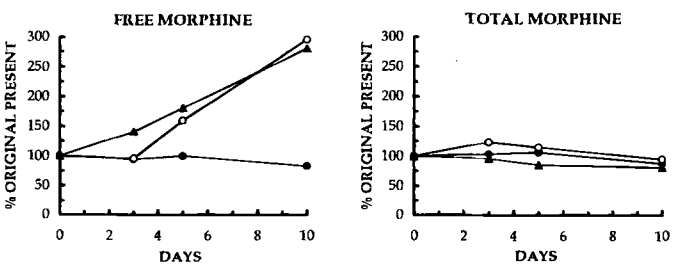

FIG. 1—*Postmortem stability of free and conjugated morphine in a liver sample obtained from a heroin addict. A portion of the liver sample was put into a glass bottle with a cap, and left at 4°C (●), 18–22°C (○), or 37°C (▲). Each point represents the mean of two measurements.*

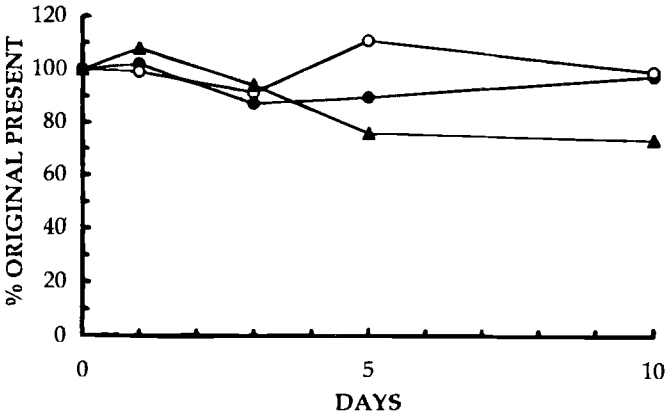

FIG. 2—*Postmortem stability of 6-monoacetylmorphine in a urine sample obtained from a heroin addict. An aliquot of the urine sample was put into a glass bottle with a cap, and left at 4°C (●), 18–22°C (○), or 37°C (▲). Each point represents the mean of two measurements.*

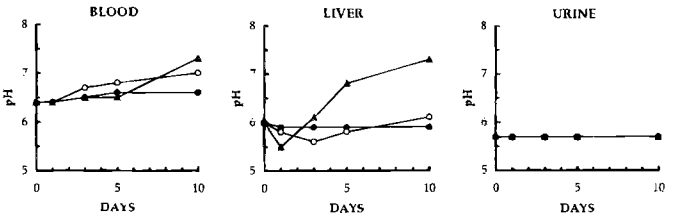

FIG. 3—*Postmortem changes in pH values of blood, liver, and urine samples obtained from a heroin addict. A portion of each sample was put into a glass bottle with a cap, and left at 4°C (●), 18–22°C (○), or 37°C (▲).*

Discussion

Concentration ratios of free to total morphine in the blood and urine of individuals who have died after heroin or morphine injection have been investigated extensively by many forensic toxicologists, and have been found to be helpful for judging when individuals have self-injected these narcotics (3,8–10). For example, Staub et al. (8) reported that free morphine in blood represented 50–100% of total blood morphine (mean: 76.1%) in 44 individuals who died rapidly after heroin injection, whereas free morphine represented 20–40% of the total morphine (mean: 31.1%) in eight individuals who survived for a period of time. Spiehler and Brown (9) also reported that 56 putative rapid deaths after heroin or morphine use gave a mean of 68% unconjugated blood morphine with a range of 26–100%. Little, however, is known about free/total morphine ratios in other body fluids and tissues.

In the present case, large differences in morphine levels were evident among blood samples taken from different sites. The morphine level in blood taken from the left ventricle of the heart might have been affected postmortem by the pulmonary vein blood, which contained a large amount of total morphine, resulting in a much higher concentration of morphine in the left ventricular than in the right ventricular blood, and in a lowered ratio of free to total morphine in the blood of the left ventricle (14). An elevated level of morphine in the right femoral vein blood was surprising, because femoral vein blood has generally been thought to be spared from postmortem diffusion of drugs (15,16). Logan and Smirnow (17) reported that postmortem free morphine levels in femoral vein blood were significantly lower than those in blood in the left

ventricle of the heart when the morphine levels in the left ventricular blood were greater than 300 ng/mL. In the present case, the morphine level in the blood taken from the right ventricle of the heart may mirror its level in the blood at the time of death most precisely among the blood samples examined. Although the cause of the unexpected elevation of the morphine level in the femoral vein blood was not clear because of no evidence of injection of heroin and methamphetamine at any site other than the left cubital fossa, one possible explanation may be that the femoral vein blood was mixed postmortem with the inferior vena cava blood which contained a high level of morphine originating from the liver, kidney, and stomach contents. The site dependence of blood morphine paralleled well that of co-existing methamphetamine. Thus, the methamphetamine level in the right ventricular heart blood as well as the morphine level may represent most precisely the level in blood at the time of death.

As reported by Spiehler et al. (18), the cerebral level of free morphine may easily be predicted from its level in the cerebrospinal fluid (CSF). However, the pattern of accumulation of conjugated morphine in the two specimens was totally different; a large amount of conjugated morphine accumulated in the CSF, but not in the cerebrum. This indicates that conjugated morphine permeates the blood-CSF barrier relatively easily, but not the blood-brain or brain-CSF barrier. Gouke et al. (19) also demonstrated the accumulation of morphine glucuronides in the CSF of cancer patients who had been treated with parenteral morphine. The concentrations of free morphine in the cerebrum and CSF may be useful for judging whether blood specimens examined have been affected by postmortem diffusion of tissue morphine, because free morphine in the cerebrum and CSF roughly paralleled that in blood from the right ventricle of the heart which may have been spared from postmortem redistribution of morphine from the surrounding tissues.

The ratio of free to total morphine was greatly dependent on the specimens that were examined. The kidney, which is thought to be one of the most suitable specimens for morphine analysis (4), showed a much greater affinity for free morphine than for conjugated morphine. This may be due to rapid excretion of morphine glucuronides from the kidney into urine. Femoral muscle may be a good specimen for predicting roughly the ratio of free to total morphine in blood even when blood specimens are not available, because the femoral muscle is relatively spared from both postmortem diffusion of drugs and bacterial invasion. Garriott (20) also reported the usefulness of skeletal muscle as a specimen for forensic drug analysis. Stomach contents as well as urine might be specimens of choice for detecting 6-monoacetylmorphine and morphine in deaths involving heroin. Pericardial sac fluid, in which a relatively large amount of total morphine was accumulated, might be another useful specimen for detecting morphine in corpses.

Stevens (12) reported that free morphine was very stable even in putrefying human liver. Spiehler and Brown (9) demonstrated that the ratio of free to total morphine in human blood treated with sodium fluoride was stable at room temperature for up to two years, indicating that morphine glucuronides are very stable in preserved postmortem blood. However, there is little information about postmortem stability of conjugated morphine in postmortem fluids and tissues without a preservative. In our *in vitro* experiment using the blood, liver, and urine samples obtained from the present heroin death, it was confirmed that morphine itself is very stable in postmortem specimens at temperatures of 4–37°C. Conjugated morphine, however, was hydrolyzed almost completely to free morphine in the liver at 18–37°C during the 10-day study period, although it was very stable in the organ stored at 4°C. In the blood and urine, morphine glucuronides were not converted to free morphine at any of the temperatures selected. Residual liver glucuronidase activity or some bacterial enzymes might be mainly responsible for conversion of morphine glucuronides to free morphine; spontaneous hydrolysis seems to make little contribution, because the conversion was independent of pH. Although the present data are limited, the findings indicate that morphine glucuronides may be stable in human corpses at ambient temperature for a few days postmortem. Additionally, 6-monoacetylmorphine seems to be relatively stable in urine. In the present case with a postmortem interval of 8–9 h, no hydrolysis of morphine glucuronides and 6-monoacetylmorphine would have been occurred postmortem.

The decedent might have been in a state of highly developed tolerance to methamphetamine, because he had abused the drug for at least 15 years. The blood methamphetamine level of 551 ng/mL might have been only negligibly toxic for him (21); methamphetamine might have counteracted the lethal effects of heroin to some degree, resulting in slightly prolonged survival. Unfortunately, it was unclear whether or not he had had a long history of heroin abuse. However, he might have died due to heroin poisoning within several hours after heroin injection, because high levels of morphine with larger ratios of free/total morphine were detected in the blood and tissues, and detectable amounts of 6-monoacetylmorphine existed in the urine and stomach contents.

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