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Article

Unexpected Variation of the Codeine/Morphine Ratio Following Fatal Heroin Overdose

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Postmortem samples from 14 cases of suspected heroin overdose were subjected to a preliminary systematic toxicological analysis in order to highlight the presence of unknown exogenous compounds (e.g., drugs of abuse, alcohol) that may have played a role in the mechanism of death. This analysis unveiled histories of poly-drug use in seven of the cases under investigation. Moreover, the concentrations of morphine and codeine in the brain were also investigated, and the results were compared with the data obtained from the blood specimens. The concentration of morphine in blood ranged from 33 to 688 ng/mL, while the concentration of codeine ranged from 0 to 193 ng/mL. However, in the brain, the concentration of morphine was found to be between 85 and 396 ng/g, while the levels of codeine ranged from 11 to 160 ng/g. The codeine/morphine ratio in the blood ranged from 0.043 to 0.619; however, in the brain, the same ratio was found to be between 0.129 and 0.552. In most cases, a significantly higher codeine/morphine ratio was found in the brain, suggesting the accumulation of codeine in brain tissue due its high lipophilicity as compared with morphine.

Introduction

The detection of heroin in the biological fluids of consumers is difficult due to its short half-life (2–6 min after intravenous injection) (1). In the body, it is rapidly hydrolyzed to 6-monoacetylmorphine (6-MAM), which in turn is converted into morphine. Subsequently, morphine is conjugated with glucuronic acid to give mainly morphine-3-β-D-glucuronide and morphine-6-β-D-glucuronide (1, 2).

Consequently, the blood concentration of morphine is used as an important analytical marker to establish the cause of death in cases of suspected heroin overdose (2–4). However, some overdose fatalities show relatively low blood concentrations of morphine, i.e., below or similar to the levels found in long-term heroin consumers or intoxicated users (5). This could be due to a variety of reasons, such as the relationship between the lethal dose and the individual's tolerance (5–8), the complex nature of heroin metabolism, the presence of systemic dysfunction (5, 7) and the concurrent use of other drugs or substances of abuse (1, 6, 9, 10), particularly alcohol (11). Moreover, postmortem redistribution or drug instability can affect the concentration of substances detected in blood after death (12–14).

In our laboratory, postmortem samples are routinely subjected to a systematic toxicological analysis (STA) in order to highlight the presence of unknown exogenous compounds (e.g., drugs of abuse, alcohol) that may have played a role in the mechanism of death. With regard to alcohol, the blood alcohol concentration (BAC; g/dL) is also taken into account in our STA, as suggested by Polettini *et al.* (11), who demonstrated that pharmacokinetic interactions between heroin and alcohol occur when individuals are exposed to high doses of these substances.

In cases in which STA suggests the presence of heroin metabolites, brain specimens are also typically analyzed (2, 15). From these previous analyses, the sites of action of heroin and its main metabolites have been identified, and they are considered to be primarily located in the central nervous system (CNS). It has also been established that the concentrations of heroin metabolites measured in postmortem brain samples are close or equal to the levels responsible for the toxic effects that result in death. Compared with blood and other tissues, the analysis of brain samples has some advantages. For example, the brain is compartmentalized by the blood–brain barrier and is therefore characterized by delayed degradation of toxic substances as well as delayed bacterial transmigration. In addition, it has a lower intrinsic metabolic activity and is less prone to postmortem redistribution. On the other hand, the brain represents a complex matrix and analysis can be complicated.

Most methods for the analysis of brain specimens were published in the 1980s, when large sample quantities were needed due to the lack of sensitivity of the analytical techniques. However, in recent years, there has been an improvement in sample preparation, automation and detection, which has permitted the collection of reference data (13).

In our previous study (16), we developed a gas chromatography/mass spectrometry (GC/MS) method to investigate the brain distribution patterns of heroin metabolites and impurities (morphine and codeine). The method resulted in good recovery of analytes and elimination of interfering species thanks to a sample pretreatment step, which was introduced prior to solid-phase extraction (SPE). After validation, the method was applied to the analysis of samples from six brain areas (hippocampus, frontal lobe, occipital lobe, nuclei, bulb and pons) from two possible cases of heroin-related deaths. No evidence of accumulation of heroin metabolites in specific brain regions was obtained (16). As a consequence, the current study focuses on the use of our validated method for the determination of morphine and codeine levels in nuclei specimens from 14 fatal cases of suspected heroin overdose. The results of this analysis were subsequently compared with metabolite levels in the blood (from STA), which revealed unexpected variations in the codeine/morphine ratios.

Experimental

Cases

Postmortem samples, which were supplied by the Institute of Legal Medicine and Assurance at the University of Palermo,

Table I
An Overview of the Demographic Information and Circumstances Surrounding Death for the Cases Analyzed in This Study

Case no.	Age (years)	Gender	Circumstances of death
1	35	M	Occasional use of illicit drugs reported; injection sites located at autopsy
2	23	M	Found dead in bed, injection sites located at autopsy
3	40	M	Found dead at home, history of drug dependence
4	24	M	Found in bed, no signs of injection, white foam and blood around the mouth and nose
5	42	M	Found dead at home, history of drug addiction, injection sites located at autopsy
6	45	M	Found dead in a street in close proximity to a syringe
7	36	M	Found dead in a street, injection sites located at autopsy
8	41	M	Found dead in a car
9	35	M	Received methadone as treatment for heroin dependence
10	45	M	Found dead in a toilet in close proximity to a syringe
11	44	M	Found dead at home with a syringe in the arm
12	31	M	Found dead in a toilet with a syringe in the arm
13	42	M	Found dead at home, history of drug dependence
14	35	M	Found dead in a toilet in close proximity to a syringe

were collected from 14 deceased patients (Table I). The subjects were men aged between 23 and 45 years, and circumstantial evidence suggested that the deaths in each case were heroin related.

All autopsies performed at the Institute of Legal Medicine and Assurance at the University of Palermo were reviewed, and the autopsy documentation, including the charts, final reports and circumstances surrounding each death, was recorded.

Tissue samples from each organ were routinely collected from the subjects during the autopsy and stained with hematoxylin and eosin for histological assessment. During the overall examination of each subject, the cause of death could not be conclusively determined. In most cases, arteriosclerosis and myocardial hypertrophy were excluded, and toxicological analysis was subsequently used to determine the cause of death.

Preliminary screening of the samples was carried out, followed by STA, including volatile organic compound (VOC) analysis and quantitative GC determination of the heroin metabolites.

Chemicals and reagents

Morphine, codeine and nalorphine (internal standard, IS) were purchased from S.A.L.A.R.S. (Italy). Methanol of analytical grade, 5-sulfosalicylic acid dihydrate, trichloroacetic acid and zinc sulfate were obtained from Sigma–Aldrich (Germany). Toluene, sodium tetraborate/hydrochloric acid (pH 9) buffer solution and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) were purchased from Fluka (Switzerland), and dichloromethane and isopropyl alcohol were obtained from Prolabo (Italy). Ammonium sulfate was supplied by Carlo Erba (Italy), as well as glacial acetic acid. Water (18.2 $m\Omega\ cm^{-1}$) was obtained using a Milli-Q purification system (Millipore, France). All reagents were of analytical grade and stored according to the manufacturer's instructions.

Systematic and toxicological analysis

As a part of the STA protocol, sample screening and SPE were followed by GC–MS analysis. These methods are typically applied to blood, urine and bile specimens, and allow the detection and quantitative determination of the main metabolites of heroin, i.e., morphine and, where detectable, 6-MAM. In addition to these metabolites, impurities and 'cutting agents' (inexpensive chemicals used to dilute illicit drugs), which tend to be present in 'street heroin', were also detected; for example, acetylcodeine and its metabolite codeine, paracetamol and caffeine were commonly found. To establish the 'total' amount of morphine (and the 'total' amount of codeine) in the urine and bile samples, a chemical hydrolysis procedure was adopted prior to SPE.

Head space-gas chromatography/flame ionization detector (HS-GC/FID) analysis was performed on blood samples to determine the presence of VOCs, with particular reference to ethanol. Where available, femoral blood samples were used, as these samples tend to exhibit less pronounced postmortem diffusion phenomena, which may affect the accuracy of the measurements.

Screening

When urine samples were available, sample screening was initially performed (Table II) by applying a small volume of the specimen onto a commercial device (RapidTest d.a.u.®10, Siemens). This very simple test provides a qualitative screen for the presence of four classes of compounds, which are related to the following:

(i) Methamphetamine (cutoff: 1,000 ng/mL)
(ii) Morphine (cutoff: 300 ng/mL)
(iii) Cocaine and benzoylecgonine (cutoff: 300 ng/mL)
(iv) 11-Nor-Δ9-tetrahydrocannabinol (cutoff: 50 ng/mL)
(v) Phencyclidine (cutoff: 25 ng/mL)
(vi) Oxazepam (cutoff: 300 ng/mL)
(vii) Secobarbital (cutoff: 300 ng/mL)
(viii) Methadone (cutoff: 300 ng/mL)
(ix) Nortriptyline (cutoff: 1000 ng/mL)
(x) D-Amphetamine (cutoff: 1000 ng/mL)

Generic investigation for the detection of basic compounds

All the available biological fluids were analyzed by GC–MS from a qualitative point of view, i.e., molecules were identified via known retention times and mass spectra. If a drug of abuse and/or its metabolites and/or other exogenous compounds were detected, a further GC–MS analysis was performed for quantitative purposes. Sample preparation (e.g., initial volume of matrix) and instrument settings were targeted to the compounds of interest, and working standard samples, i.e., blank biological fluids spiked with standard solutions of the most common drugs of abuse and their metabolites (total amount: 1 μg), were analyzed together with the unknown specimens. The quantification procedure was typically applied to methadone and its main metabolites, EMDP (2-ethyl-5-methyl-3,3-diphenyl-pyrroline) and EDDP (2-ethyl-1,5-dimethyl-3,3-diphenyl-pyrrolidine), using proadifen as an IS; to amphetamines, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA), using 2-phenyl-ethyl amine as an IS; to cocaine and benzoylecgonine, using scopolamine as an IS; and to codeine, morphine, 6-MAM and acetylcodeine, using nalorphine as an IS.

Aliquots of blood, urine or bile (between 250 μL and 2 mL depending on the nature of the sample and its availability), with 50 μL IS (in methanol; concentration: 20 μg/mL), were diluted with sodium tetraborate/hydrochloric acid (pH 9) buffer solution to a volume of 2 or 4 mL. The resulting mixtures were

Table II
Results of the STA

Case	Screening	STA	Blood (ng/mL)	Urine (ng/mL)	Bile (ng/mL)	Blood alcohol concentration (g/dL)
1	MOR	Morphine	614	266	1,842	n.p.
	COC	Codeine	129	n.p.	n.p.	–
		Cocaine	20	4,156	–	–
		BZE	2,544	117,772	–	–
2	MOR	Morphine	33	25,947	22,211	n.p.
	COC					
		Codeine	10	3,974	255	–
		Cocaine	20	419	756	–
		BZE	10	124	n.p.	–
3	MOR	Morphine	97	58	200	150
		Codeine	60	n.p.	n.p.	–
		Desmethyl diazepam	1,497	20	n.p.	–
4	MOR	Morphine	315	7,602	91,235	n.p.
	COC	Codeine	73	646	n.p.	–
		BZE	n.p.	479	n.p.	–
5	MOR	Morphine	338	1,089 (ng/g)	–	n.p.
		Codeine	64	Kidney	–	–
6	MOR	Morphine	688	99	3,765	60
	COC	Codeine	150	n.p.	44	–
	THC	Cocaine	n.p.	54	n.p.	–
		BZE	n.p.	8,000	n.p.	–
7	MOR	Morphine	615	161	–	200
	THC	Codeine	193	22	–	–
8	MOR	Morphine	533	35,935	43,786	n.p.
	COC	Codeine	23	1,744	122	–
		Cocaine	75	37,798	–	–
		BZE	1,847	45,5896	–	–
9	MOR	Morphine	77	12,505	–	50
	MET	Codeine	6	472	–	–
		Methadone	890	1,395	–	–
		EMDP	621	2,470	–	–
		EDDP	719	1,810	–	–
10	MOR	Morphine	228	8,48	571	n.p.
		Codeine	25	n.p.	n.p.	–
11	MOR	Morphine	313	740	6,638	n.p.
		Codeine	72	n.p.	n.p.	–
12	MOR	Morphine	441	1,986	5,559	n.p.
		Codeine	20	75	n.p.	–
		6-MAM	n.q.	–	–	–
13	MOR	Morphine	130	860	620	220
		Codeine	Trace	50	n.p.	–
14	MOR	Morphine	171	140	860	130
		Codeine	Trace	20	300	–
		6-MAM	n.p.	n.q.	–	–

For the screening test, only positive results (>cutoff) are reported. For the blood alcohol concentration, n.p., not present, i.e., at a concentration <0.5 g/L. For GC–MS analysis (blood, urine and bile); n.p., not present, i.e., below the limit of detection (LOD); n.q., not quantified due to a lack of standard or sample; trace, above the LOD but below the lower limit of quantitation; –, data not available.

MOR, morphine; COC, cocaine; BZE, benzodiazepine; THC, tetrahydrocannabinol; MET, methadone; EMDP, 2-ethyl-5-methyl-3,3-diphenyl-pyrroline; EDDP, 2-ethyl-1,5-dimethyl-3,3-diphenyl-pyrrolidine; 6-MAM, 6-monoacetylmorphine.

centrifuged (3,500 rpm, 10 min) and the resulting supernatant loaded (1–2 mL/min) onto Bond Elut Certify extraction cartridges (mixed-mode cartridges packed with non-polar C8 and a strong anion exchange SAX sorbent; Agilent), which were previously conditioned with methanol (2 mL) and water (2 mL). Each cartridge was then washed with water (2 mL) and 1 M acetic acid (3 mL), and desiccated for 5 min before washing with 0.5 mL methanol. The cartridge was then dried *in vacuo* for 1 min, and the analytes subsequently eluted twice with a freshly prepared mixture of dichloromethane, isopropanol and 30% ammonium hydroxide (8/2/0.2, *v*/*v*/*v*; 1 mL). The solvent was evaporated, and the resulting residue was dissolved in methanol (50 μL). After drying, the sample was derivatized via the addition of a mixture of MSTFA in toluene (1/4, v/v; 50 μL) and heating at 70°C for 30 min. GC–MS analysis was then performed.

Generic investigation for the detection of acidic compounds

Aliquots of blood, urine or bile (between 250 μL and 2 mL depending on the nature of the sample and its availability), with 50 μL IS (hepta-barbital, 50 μg mL^{-1} in methanol), were diluted with sodium tetraborate/hydrochloric acid (pH 9) buffer solution to a volume of 2 or 4 mL. The subsequent mixtures were subjected to SPE, as described above; however, in this case, the analytes were eluted with a freshly prepared mixture of acetone–chloroform (1:1, v/v; 4 mL). The solvent was evaporated, and the residue analyzed by GC–MS after being dissolved in methanol (50 μL) and then derivatized with 20 μL MethElut reagent in methanol (Thermo Scientific, Rockford, IL, USA).

Hydrolysis of morphine and codeine glucuronides in urine and bile samples

Aliquots of urine or bile (from 200 μL to 1 mL depending on the availability of the sample) were diluted with water to a volume of 2 mL. To this, 6 N hydrochloric acid (1 mL) and the appropriate premixed IS (20 μg/mL) were added. Each sample was then heated to 100°C in a water bath under pressure for 30 min. After cooling, 30% ammonium hydroxide (550 μL) was added. After several minutes, 2 mL pH 9 buffer was also added, and the pH was adjusted to 8.0–8.5 with 30% ammonium hydroxide. The samples were then subjected to SPE and analyzed as described above.

Determination of VOCs by HS-GC

HS-GC analyses were carried out on an Ultra Thermo Electron Trace GC (Thermo Fisher, Waltham, MA, USA) with a split–splitless injection system and an HS 2000 Thermo Electron autosampler, coupled with an FID. The system was managed by the Thermo Electron Chrom-Card 2.3 software. The GC machine was equipped with a VF-624 ms capillary column (30 m × 0.25 mm ID, thickness: 0.25 μm). The GC-FID system was operated under the following conditions: 50–150°C, 10°C/min; final isotherm: 3 min; temperature: inlet 150°C, detector: 250°C; split flow: 30 mL/min; split ratio: 15; carrier constant flow: 1.3 mL/min (helium); detector gas flow: 35 mL/min (hydrogen), 350 mL/min (air); makeup gas (helium): 30 mL/min; signal range: 1; HS autosampler syringe temperature: 60°C; HS autosampler incubation temperature: 80°C with alternate stirring; HS autosampler incubation time: 30 min; and HS autosampler injection volume: 0.5 mL. Samples were prepared by mixing 0.5 mL water with 0.5 mL blood and a small quantity of sodium fluoride to avoid enzymatic activation, as well as the appropriate IS in propanol (0.5 mL, 0.4 μL mL^{-1}).

Brain tissue sample preparation and deproteinization

Each sample was homogenized by blending or ball milling (depending on the quantity of the material available) and deproteinized via an ultrasonic bath using the following protocol: 500 mg brain tissue and 50 μL IS were diluted with 4 mL water and 2 mL

pH 9 buffer solution. The resulting mixture was sonicated for 15 min at room temperature. After centrifugation (4,000 rpm, 5 min), the clear supernatant was separated and extracted via SPE.

Extraction procedure

Homogenized and deproteinized encephalic samples were centrifuged (4,000 rpm, 5 min) and extracted using Bond Elut-LRC Certify solid-phase extraction cartridges (Varian, Palo Alto, CA, USA) with a Varian vacuum manifold (Varian, CA, USA). Cartridges were first conditioned with 2 mL methanol and 2 mL (pH 9) buffer solution. The supernatants resulting from the centrifugation were loaded on to the cartridges and permitted to absorb through gravity. The cartridges were then washed with 2 mL water, 3 mL of 1 M hydrochloric acid and 0.5 mL methanol. The analytes were subsequently eluted with 2 × 1 mL elution solvent (dichloromethane/isopropyl alcohol/ammonium hydroxide, 8/2/0.2, v/v/v).

Chromatography

The extracts were evaporated to dryness under a stream of nitrogen at 40°C and derivatized with a mixture MSTFA in toluene (1/4, v/v; 50 μL) at 70°C for 30 min. GC–MS analysis was performed on a GC 6890 Plus with a mass selective detector and 6890 autosampler. Data were analyzed with the MSD ChemStation D.03.00 software (Agilent Technologies). Chromatographic separation was carried out on a DB-5MS inert capillary column (30 m × 0.25 mm ID, thickness 0.25 μm; J & W Scientific, Folson, CA, USA).

The GC–MS system was operated under the following conditions: injection temperature: 280°C (splitless mode; 0.25 min splitless time); interface transfer line: 280°C; ion source: 230°C; initial column temperature: 70°C. The temperature was subsequently increased to 180°C at a rate of 40°C/min, then to 300°C at a rate of 10°C/min and held at this temperature for 5 min. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. MS analysis was performed in SCAN (50/550 m/z) and SIM mode with a quadrupole mass detector operated in electron ionization mode, with a beam energy of 70 eV. The injection volume was 1 μL.

Method validation

The specificity, accuracy, precision and linearity as well as the limit of detection (LOD) and limit of quantitation (LOQ) were evaluated by analyzing working standard solutions prepared with morphine and codeine at different concentrations (10, 25, 50, 100, 250, 500 and 1000 ng) and nalorphine as an IS. In particular, working standard solutions (0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 μg mL^{-1}, 100 μL) were prepared in separate tubes and evaporated to dryness. A blank brain sample was then added (500 mg) and after centrifugation 4,000 rpm, 1 min), 4 mL water, 50 μL IS (4 μg/mL) and 2 mL (pH 9) buffer solution were added.

For quantitative analysis, the following ions were assessed: m/z 371 for codeine, m/z 429 for morphine and m/z 455 for nalorphine. The specificity was assessed by extracting control (blank) brain samples in each validation run. The lack of interfering peaks at the same analyte retention times conferred acceptable selectivity.

The linearity of the response of the GC–MS analysis was assessed for codeine and morphine by plotting drug/IS peak area ratios versus the total amount of drug in the standard solutions, with intervals of 10–1000 ng. The calibration curves gave good correlation coefficients ($R^2 > 0.9994$) for both analytes over the whole range.

Accuracy was expressed as the percent recovery (%REC) evaluated by analyzing, in triplicate, six standard morphine solutions, ranging from 25 to 1,000 ng_{tot}, and seven standard codeine solutions, ranging from 10 to 1,000 ng_{tot}. The averaged results were found to be satisfactory.

The same standard solutions were analyzed, in triplicate, over 3 days (I, II and III) in order to evaluate the precision of the method. The obtained data demonstrated adequate reproducibility.

The LOD and LOQ were also evaluated and were found to 10 and 25 ng for morphine, respectively, and 5 and 10 ng for codeine, respectively.

Results and Discussion

The STA results for the 14 assessed cases are reported in Table II. As is evident, in seven of the cases, other drugs of abuse, such as cocaine, benzodiazepines or methadone, were detected in addition to heroin metabolites. In six cases, significant concentrations of ethanol were also found to be present; however, it was difficult to establish a correlation between the BAC and the morphine concentration, probably because of the statistically low number of cases considered. However, since ethanol is an enzyme inhibitor, it is fair to assume that it may affect heroin metabolism (11).

These data have to be taken into account when determining the cause of death in these cases. In cases of heroin overdose, it is useful to also evaluate the distribution of heroin metabolites in the tissues, i.e., in the liver, kidney, lung and brain. In this study, we analyzed brain tissue, as it is well known that heroin acts upon the brain, and information about its brain levels following an overdose could be used to interpret the cause of death as well as provide additional information with regard to the tolerance of the individual subject.

The brain is a complex matrix, making analysis complicated. In our previous work (16), we optimized an extraction procedure and validated an analytical method to determine morphine and codeine levels in brain tissue. The concentration of these analytes in different areas of the brain was also evaluated in our previous work, and the results suggested that there was a homogeneous distribution. To probe further the distribution of heroin metabolites in brain tissue, we applied our method to the analysis of brain samples (nuclei) from 14 fatal cases of suspected heroin overdose. Moreover, in the current study, the concentrations of the analytes in the brain were compared with the levels detected in blood.

Table III shows the analytical results for all 14 cases. As has been previously reported (2–5), high variability was found with regard to the morphine levels among the 14 blood samples. Similarly, the codeine concentrations varied from undetectable (below the LOD) to 193.1 ng/mL. No relationship between the levels of the two analytes in each individual sample was found (Figure 1). This is indeed reasonable, as the concentration of

codeine is linked to the impurities found in 'street heroin' and because more than one metabolic pathway is implicated in heroin metabolism.

With regard to the brain tissue samples, a wide distribution of both the concentration of morphine and the concentration of codeine was noted, which is in agreement with the literature (15). In fact, a wide range of blood–brain concentration ratios has been previously reported; for example, Kintz *et al.* (9) investigated the blood–brain ratios of heroin in three heroin users and found them to be 13, 1.5 and 0.24, with tissue concentrations ranging from 0.005 to 0.089 mg kg^{-1} of wet brain (10). In our study, the blood level of morphine was similar to the level in the brain in the majority of cases (Figure 1C), which agrees with what has been previously reported (2).

The most interesting results in our study come from the comparison of the codeine/morphine ratios in the blood and brain (Table III and Figure 1). Indeed, the ratios were found to be unexpectedly higher in the brain samples than in the blood in the majority of cases, and this result was demonstrated to be statistically significant by a *t* test, even though the number of cases was limited ($P < 0.05$; $t = 2.6736$). However, for this very reason, it is not possible to draw any general conclusions. The higher ratio in the brain may be due to the high lipophilicity of codeine (log P 1.39) compared with morphine (log *P* 0.87) (17), which may subsequently lead to a low concentration of morphine in the brain when compared with codeine. In only three cases (Nos. 2, 3 and 6), the concentration of codeine in the blood was higher than it was in the brain. Unfortunately, after analyzing the history of the subjects and the circumstances surrounding their deaths, we were unable to find an explanation for this. However, the use of other drugs of abuse may interfere with the accumulation and metabolism of heroin derivatives, leading to unexpected results.

Table III
Concentration of Morphine and Codeine in Blood (ng/mL) and Brain (ng/g) Samples and the Codeine/Morphine Ratios for Each Case

Case no.	Blood			Brain		
	Morphine	Codeine	Codeine/morphine	Morphine	Codeine	Codeine/morphine
1	614	129	0.210	320	120	0.375
2	33	10	0.303	85	11	0.129
3	97	60	0.619	211	51	0.242
4	315	73	0.232	190	69	0.363
5	338	64	0.189	391	101	0.258
6	688	150	0.218	396	76	0.192
7	615	193	0.313	140	50	0.357
8	533	23	0.043	271	132	0.487
9	77	6	0.078	100	31	0.310
10	228	25	0.110	230	100	0.435
11	313	72	0.230	290	160	0.552
12	441	20	0.045	321	120	0.374
13	130	<LOD	–	87	31	0.356
14	171	<LOD	–	149	28	0.188

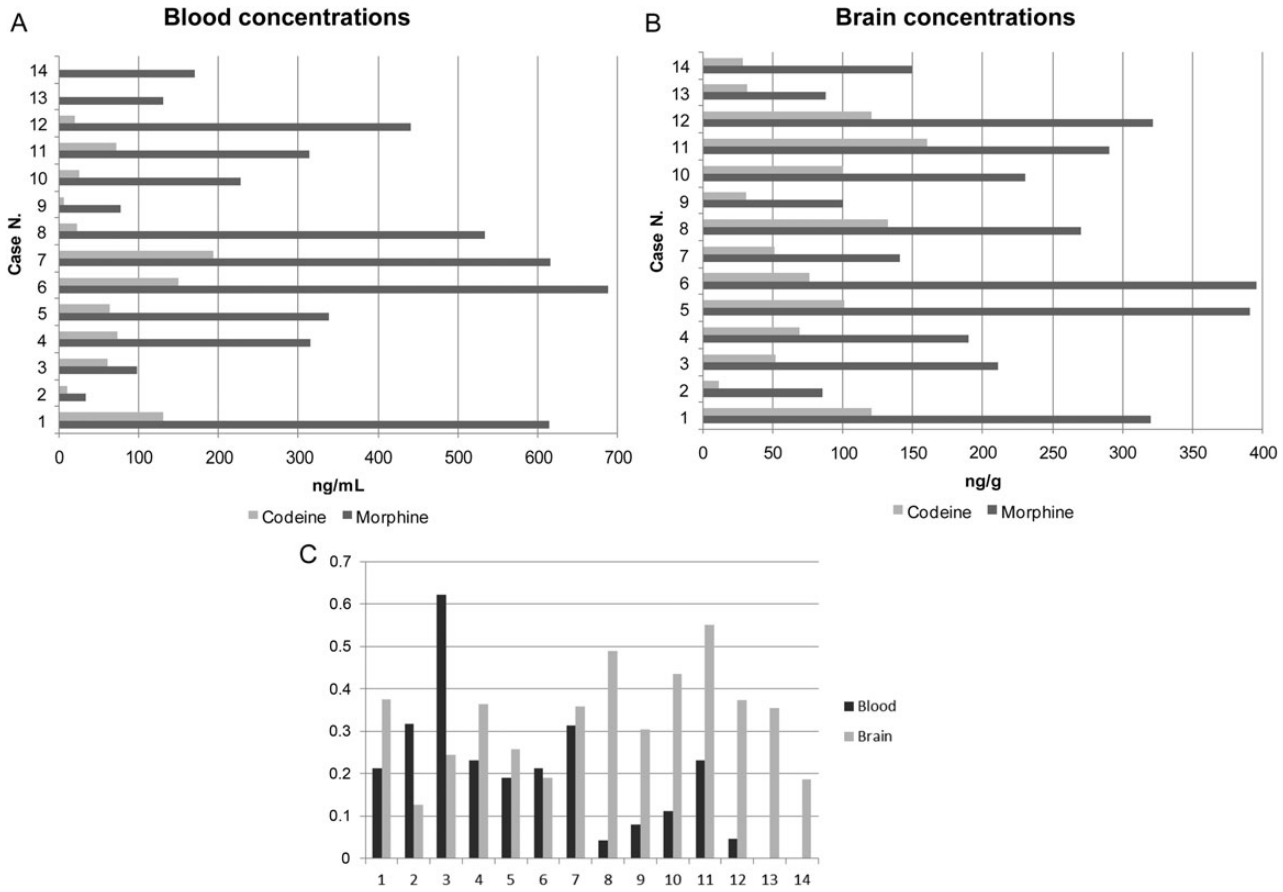

Figure 1. An overview of the analyte concentrations in the 14 blood (a) and brain tissue (b) samples. A comparison of the codeine/morphine ratios in blood and brain samples (c) is also shown.

Conclusions

We applied our validated method (16) to the determination of morphine and codeine levels in brain (nuclei) tissue samples from 14 fatal cases of suspected heroin overdose. The resulting data were compared with the data obtained from blood samples, which are usually used to establish the cause of death.

Regardless of the tissue analyzed, the cause of death could not be conclusively determined from the isolated toxicological measurements, although some firm inferences could be drawn. It is an accepted belief that postmortem blood samples cannot be used alone to determine the cause of death or significant impairment. Knowledge of the individual's clinical history and the autopsy findings must also be taken into account.

In our study, 14 cases were considered (Table I), and STA was carried out on all the available biological fluids, which revealed the concurrent use of other drugs of abuse, such as cocaine, methadone and benzodiazepines as well as the presence of cutting agents, such as dextromethorphan and paracetamol. In all cases, morphine was detected, indicating that the deaths may have been heroin related. The concentrations of morphine and codeine (the presence of codeine being related to impure 'street heroin') were determined in blood and brain samples, and in the majority of cases, the concentration of codeine was found to be higher in the brain than in the blood. In particular, in cases 13 and 14, codeine was found only in the brain and was not detected in the blood samples.

It is difficult to interpret acute narcotism-related deaths on the basis of chemical and toxicological data alone; however, in this study, we attempted to elucidate the ratios between morphine and codeine, and we found that the codeine-to-morphine ratio was higher in the brain than in the blood in the majority of cases (Figure 1C). A possible explanation for this phenomenon may be the greater ability of codeine to reach the CNS due to its higher lipophilicity (17).

In conclusion, we have found that codeine can accumulate in the brain tissue of suspected heroin users; thus, its detection within the brain could help determine the cause of death in suspected overdose cases. On the basis of the results obtained in this preliminary work, we are continuing to verify our assumptions by determining and evaluating the concentrations of morphine and codeine in the blood and brain tissues of a larger sample of heroin overdose fatalities.

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