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Application of pericardial fluid to the analysis of morphine (heroin) and cocaine in forensic toxicology

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Abstract

In this study opiates (morphine and codeine) and cocaine and its related metabolites (benzoylecgonine and cocaethylene) were analyzed in pericardial fluid by GC/MS. This is the first study reporting levels of drugs of abuse in this body fluid. The analytical method used has been previously validated and then applied to 54 drug-related deaths in the Barcelona area (Spain). Median levels were as follows: morphine 589 ng/ml, range 19–8857 ($n = 49$); codeine 26 ng/ml, range 15–343 ($n = 35$); cocaine 78 ng/ml, range 10–220 ($n = 14$), benzoylecgonine 742 ng/ml, range 20–3386 ($n = 15$), and cocaethylene 36 ng/ml, range 9–100 ($n = 13$). In addition, a comparative study of the concentration of opiates and cocaine in pericardial fluid by both semi-quantitative EMIT d.a.u.® and GC/MS (used as reference) was performed. Fairly good correlations for opiates ($r = 0.905$) and cocaine ($r = 0.859$) were found; however, the consistently low results of EMIT in the analysis of cocaine comparing to GC/MS could be caused by matrix effect. In spite of that, it raises the possibility of using the immunoassay as a preliminary technique in forensic toxicology.

Keywords: Pericardial fluid; Drugs of abuse; Opiates; Cocaine; GC/MS; Immunoassay

1. Introduction

The interpretation of toxicological data in cases of drug-related deaths and drug overdoses is not a simple matter. A number of known conditions can make it difficult, such as an adequate database to compare the analytical results, post-mortem degradation (spontaneous or enzymatic), post-mortem redistribution of drugs, and the simultaneous consumption of two or more drugs. Forensic toxicologists have been using different viscera as samples for toxicological analysis, although preferences were further turned to biological fluids, because they are easier to handle and pose less analytical drawbacks than solid organs.

As most drugs are distributed to their site of action by blood, drug concentration measurements in this body fluid provide the best information as to the potential effect on behaviour, clinical symptoms, or vital functions [1]. However, blood samples are not always available due to the cause of death or the post-mortem processes that interfere with the amount and/or the quality of the sample. It has been reported that in some fatal cases of poisoning, in addition to blood and urine, cerebrospinal fluid, vitreous humour, bile, meconium, and other body fluids are useful for toxicological analysis [2–5]. In this respect, pericardial fluid may be an alternative sample to blood for toxicological examinations in drug-related deaths. This body fluid is an ultrafiltrate of plasma with an extremely similar amount of proteins and is located within a tight compartment so that it is almost free of contamination by pathogens [6]. The usual volume of 5–20 ml is enough for analytical purposes.

The objective of the present study was to investigate the occurrence in pericardial fluid of opiates (morphine and codeine) and cocaine and its metabolites (benzoylecgonine and cocaethylene). Cocaethylene is not a true metabolite of cocaine but it appears when cocaine is coadministered with ethyl

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alcohol by means of a transesterification reaction. The analytical method used has been previously validated [7] and applied to 54 drug-related deaths. The second aim of this study was to ascertain the application of a semi-quantitative EMIT® d.a.u.™ assay to pericardial fluid, which might be of interest in forensic toxicology as a preliminary result. This procedure was compared to GC/MS used as reference.

2. Material and methods

2.1. Collection of samples

Pericardial fluid samples (4–6 ml) from 54 drug-related deaths in the Barcelona area were collected in polypropylene tubes by using plastic syringe. Permission from the President of the Catalonian Supreme Court of Justice (ref T.S./G.P. 68196) was obtained in advance. Samples were stored at −20 °C until analysis.

2.2. Preparation of pericardial fluid for opiate analysis

The procedure has been reported elsewhere [7]. Briefly, 50 μl of a mixture of internal standards solution (200 ng of morphine-D3 and codeine-D3 in methanol) was added to 1 ml of pericardial fluid. The pH of the sample was adjusted to approximately 5.2 by adding 1 ml of 1.1 M sodium acetate buffer (pH 5.2). The mixture was vortexed, and then 50 μl of β-glucuronidase (HP2 from Helix Pomatia, Sigma Chemicals, St. Louis, MO) was added and incubated at 37 °C for 12 h in a water bath. Then 350 μl of 1.0 M potassium hydroxide was added to adjust the pH to 9.0, the mixture was centrifuged at 2500 rpm for 5 min, poured into Bond-Elut Certify™ columns (Varian, Harbon City, USA), and gently sucked through.

Columns were previously conditioned with 2 ml of methanol and 2 ml of deionized (Milli Q) water and prevented from running dry. After applying the samples, columns were successively washed with 2 ml of deionized water, 1 ml of 0.1 M acetate buffer pH 4.0 and 2 ml of methanol. Finally, analytes were eluted with 2 ml of a freshly prepared mixture of chloroform:isopropanol (80:20, v/v) containing 2% ammonia. The eluates were collected and evaporated to dryness under a gentle nitrogen stream at 40 °C in a water bath. Residues were kept in a vacuum oven for 30 min at 50 °C and then derivatised with 100 μl of *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA). The mixture was vortexed for 10 s and then heated at 60 °C for 5 min in a heating block. After being cooled at oom temperature, 20 μl of *N*-methyl-bis-trifluoroacetamide (MBTFA) were added and vortexed for 10 s. The mixture was heated again at 60 °C for 10 min in the heating block, cooled at room temperature and 1 μl aliquots of the derivatised extract were injected into the GC/MS system (split ratio 10:1). This consisted of a Hewlett-Packard 5890A Series II gas chromatograph equipped with an HP-Ultra 1 capillary column (cross-linked methylsilicone, 0.2 mm × 25 m) and coupled to an HP 5971A mass-selective detector. The injector port and detector temperatures were operated at 280 °C. The oven temperature was increased from 100 to 290 °C, at 20 °C/min, with a final hold time of 5 min. The mass spectrometer was operated in electron impact mode and three diagnostic ions for each compound were monitored (SIM): *m/z* 429, 414 and 401 for morphine-bis-*O*-TMS; *m/z* 371, 313, and 178 for codeine-bis-*O*-TMS. Three characteristic ions for each deuterated analogue were monitored at *m/z* + 3, respectively.

2.3. Preparation of pericardial fluid for cocaine analysis

The procedure has been reported elsewhere [7]. Briefly, 50 μl of a mixture of internal standards solution (200 ng of benzoylecgonine-D3 and cocaine-D3, and 100 ng of cocaethylene-d_8 in methanol) was added to 1 ml of pericardial fluid. The pH of the sample was adjusted to 7.0 by adding 1 ml of 0.1 M pH 7.0 sodium phosphate buffer. The mixture was vortexed, centrifuged at 2500 rpm for 5 min, poured into Bond-Elut Certify™ columns, and gently sucked through.

Columns were previously conditioned with 2 ml of methanol and 2 ml of 0.1 M sodium phosphate buffer pH 7.0 and prevented from running dry. After applying the samples, columns were successively washed with 3 ml of deionized water, 3 ml of 0.1 M HCl, and 9 ml of methanol. Finally, analytes were eluted with 2 ml of a freshly prepared mixture of chloroform:isopropanol (80:20, v/v) containing 2% ammonia. The eluates were collected and evaporated to dryness under a gentle nitrogen stream at 40 °C in a water bath. Residues were kept in a vacuum oven for 30 min at 50 °C and then derivatised with 70 μl of pentafluoropropionic anhydride (PFPA) and 30 μl of hexafluoroisopropanol (HFIP). The mixture was vortexed for 10 s and then heated at 70 °C for 10 min in a heating block, then cooled at room temperature, and evaporated to dryness under a gentle nitrogen stream at 40 °C. The residue was reconstituted with 50 μl of ethyl acetate and 1 μl aliquots of the derivatized extract were injected into the same GC–MS system mentioned above. In this case, an HP-ultra 2 capillary column (5% phenylmethylsilicone gum, 0.2 mm × 12.5 m) was used. The oven temperature was increased from 100 to 280 °C (20 °C/min), with a final hold time of 4 min. Three diagnostic ions for each compound were monitored (SIM): *m/z* 439, 334, and 318 for benzoylecgonine-HFIP; *m/z* 303, 272, and 182 for cocaine, and *m/z* 317, 272, and 196 for cocaethylene. Two characteristic ions for each deuterated analogue were monitored (*m/z* 442 and 321 for benzoylecgonine-D3-HFIP, *m/z* 306 and 185 for cocaine-D3; and *m/z* 325 and 204 for cocaethylene-d_8).

2.4. Analysis of pericardial fluid for opiates and cocaine by immunoassay

A semi-quantitative determination of opiates and cocaine was carried out in pericardial fluid by using EMIT® d.a.u.™ assays (Dade Behring) in a Vitalab Viva analyzer. The calibration curve for the assay was built with four points from the calibrator solutions supplied by the manufacturer. The points selected were as follows: 0 (blank, water); 150 ng/ml (calibrator A – 300 ng/ml – diluted with water at 1:1, v/v); 300 ng/ml (calibrator A); and 1333 ng/ml (morphine) or 1000 ng/ml (benzoylecgonine) obtained by diluting the calibrator B (morphine 4000 ng/ml;

benzoylecgonine 2000 ng/ml) with water at 1:2 and 1:1 (v/v), respectively.

3. Results and discussion

This is the first paper reporting levels of some drugs of abuse in pericardial fluid and may begin the database for further comparison purposes. To our best knowledge, only one study has previously addressed the measurement of drugs in pericardial fluid [8], although it focussed on acidic and neutral drugs and several pesticides, but not on drugs of abuse.

In this study, 54 cases of drug-related death were included. Combinations of at least two or more drugs were found in 92.6% of cases, one of them being morphine. This figure is slightly higher than the 82% reported by Darke et al. [9] and the 85% reported by Gerostamoulos et al. [10]. Morphine appeared together with cocaine in 35% of the cases, with ethanol in 28%, and with benzodiazepines in 65%. According to the ''Plan Nacional sobre las Drogas'' [11], 88% of the 489 drug-related deaths reported throughout Spain in 1999 were opiate-related deaths, and 63.3% cocaine-related deaths. In our study we found 94.4% and 37.0%, respectively. The ''Plan Nacional sobre las Drogas'' reports that males comprised 86.7% of drug-related deaths cases, and that the mean age was 32.8 years. These figures are very similar to the 90.7% and 29.9 years found in our study.

The concentrations of opiates (morphine and codeine) and cocaine (and metabolites) in pericardial fluid are shown in Table 1. If mean levels of these drugs are compared to those found in blood (data retrieved from forensic casework files and not shown) it is observed that morphine and cocaine concentrations in pericardial fluid are roughly two-fold those found in blood. However, the mean value for benzoylecgonine in pericardial fluid is about 50% higher than that found in blood. In contrast, the mean concentration of codeine is almost identical in both body fluids. It appears that both cocaine and benzoylecgonine are likely to be accumulated in pericardial fluid where they remain longer than in blood.

Table 2 shows the comparative analysis of the concentration of opiates and cocaine in pericardial fluid measured by both semi-quantitative EMIT d.a.u.™ and GC/MS (used as reference). This study was undertaken to ascertain the potential usefulness of the immunoassay for the rapid estimation of drugs of abuse levels in pericardial fluid as a preliminary analysis. As compared to GC/MS, the EMIT assay found slightly lower concentrations of opiates in pericardial fluid, although the differences failed to be statistically significant ($p = 0.107$). A strong correlation was found between both procedures ($r = 0.905$, $p < 0.001$, $n = 42$) and the regression equation obtained was the following:

$$[\text{Opiates (morphine plus codeine)}]\ \text{by GC/MS} = 0.733 \times [\text{opiates by EMIT}] + 456.61$$

More divergent results were found in the case of cocaine, as the mean concentration detected by EMIT was about 2.5 times lower than that found by GC/MS. Nevertheless, a fairly good correlation was obtained between both procedures ($r = 0.859$, $p < 0.001$, $n = 12$). The regression equation obtained was:

$$[\text{Cocaine and metabolites}]\ \text{by GC/MS} = 1.54 \times [\text{cocaine by EMIT}] + 480.04$$

At first glance, the EMIT assay should have obtained higher concentrations of both types of drugs of abuse, as the antibody may cross-react with other minor metabolites (in addition to the main compound) that were not determined by GC/MS. Besides, EMIT usually gives rise to more misleading (false positive) results due to matrix-effect and other types of interferences that do not occur with GC/MS. The consistently low results of EMIT in the analysis of cocaine comparing to GC/MS could be caused by matrix effect. The matrix interference of pericardial fluid on EMIT was checked by spiking blank pericardial fluid with known amounts of morphine and cocaine (0, 150, 500, and 1000 ng/ml). The following results were obtained, respectively, for each drug: linearity 0.995 and 0.893; variability 0.6% and 0.7%; recovery 108% and 116%. Since EMIT assay is designed for use only with human urine, other substances may interfere with the test and give false results. When pericardial fluid instead of urine was used for cocaine assay, the different in nature of these body fluids could result in the suppression of the EMIT results. One of the possible interference could be caused by the presence of higher concentration of sodium chloride in the pericardial fluid than urine as this compound is known to mask the cocaine assay using EMIT. This assumption is

Table 1
Levels of opiates and cocaine (and metabolites) in pericardial fluid (ng/ml)

	Mean ± S.D.	Range	Median	*N*
Morphine	1022 ± 1351	19–8857	589	49
Codeine	45 ± 57	15–343	26	35
Cocaine	89 ± 72	10–220	78	14
Benzoylecgonine	994 ± 931	20–3386	742	15
Cocaethylene	42 ± 31	9–100	36	13

Table 2
Comparative analysis of opiates and cocaine in pericardial fluid (ng/ml) analyzed by semi-quantitative EMIT d.a.u.™ and GC/MS

Drug	Parameter	EMIT	GC/MS[a]	Ratio
Opiates	Mean ± S.D.	888 ± 1734	1106 ± 1406	1.12 ± 1.26
	Median	556	677	0.70
	N	46	46	42
	Range	11–11770	19–8968	0.10–6.29
Cocaine	Mean ± S.D.	189 ± 473	1114 ± 975	0.35 ± 0.31
	Median	46	798	0.34
	N	23	15	12
	Range	0–1462	30–3535	0.00–0.80

In the case of opiates, the paired *t*-test failed to show significant differences between the semi-quantitative EMIT and GC/MS (963.2 ± 1797.4 vs. 1162.5 ± 1456.2, respectively, $p = 0.107$; $n = 42$). By contrast, in the case of cocaine statistically significant differences were found as semi-quantitative EMIT obtained lower levels than GC/MS (532.6 ± 558.9 vs. 1300.8 ± 1001.2, respectively, $p = 0.001$; $n = 12$).

[a] Includes morphine and codeine (opiates), and cocaine, benzoylecgonine and cocaethylene (cocaine).

supported by the electrolyte levels of blank pericardial fluid (163 mEq/l of sodium and 113 of chloride), in contrast to blank urine (37 mEq/l and 50, respectively).

The lower specificity of the antibody against benzoylecgonine for cocaine (Behring, instructions for users) might also explain the discrepancies; however when benzoylecgonine levels in pericardial fluid measured by GC/MS are compared with the semi-quantitative EMIT for cocaine (whose antibody is raised against benzoylecgonine), the differences still remain. No satisfactory explanation for the discrepancies can be drawn at this time. Anyway, EMIT seems to be of usefulness only as a preliminary assay and, although a rather good estimation of GC/MS values could be inferred from the regression equations calculated (mostly in the case of opiates), a GC/MS confirmation is mandatory as it is a current rule in forensic toxicology. Further investigations addressing this issue should be done to gain insight on the EMIT–GC/MS correlations and for potential applications.

In conclusion, this is the first paper reporting levels of drugs of abuse (morphine and cocaine) in pericardial fluid. The number of samples studied ($n = 49$ and 14, respectively) provides reference values, such as mean, median levels and range, that might be used for comparison purposes. However, further studies are warranted to support the role of pericardial fluid in forensic toxicology.

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References

[1] C.N. Chiang, R.L. Hawks, Implications of drug levels in body fluids: basic concepts, NIDA Res. Monogr. 73 (1986) 62–83.
[2] E.M. Koves, Use of high-performance liquid chromatography-diode array detection in forensic toxicology, J. Chromatogr. A 692 (1995) 103–119.
[3] K.L. Crump, I.M. McIntyre, O.H. Drummer, Simultaneous determination of morphine and codeine in blood and bile using dual ultraviolet and fluorescence high-performance liquid chromatography, J. Anal. Toxicol. 18 (1994) 208–212.
[4] B.K. Logan, R. Luthi, The significance of morphine concentrations in the cerebrospinal fluid in morphine caused deaths, J. Forensic Sci. 39 (1994) 699–706.
[5] A.M. Bermejo, I. Ramos, P. Fernández, M. López-Rivadulla, A. Cruz, M. Chiarotti, N. Fucci, R. Marsilli, Morphine determination by gas chromatography/mass spectroscopy in human vitreous humor and comparison with radioimmunoassay, J. Anal. Toxicol. 16 (1992) 372–374.
[6] A.T. Gibson, M.B. Segal, A study of the composition of pericardial fluid, with special reference to the probable mechanism of fluid formation, J. Physiol. (Lond.) 277 (1978) 367–377.
[7] M.T. Contreras, Aplicación del líquido pericárdico al análisis de drogas de abuso en Toxicología forense, Doctoral thesis, Servicio de Publicaciones, Universidad de Granada, 2002.
[8] F. Moriya, Y. Hashimoto, Pericardial fluid as an alternative specimen to blood for postmortem toxicological analyses, Leg. Med. (Tokyo) 1 (1999) 86–94.
[9] S. Darke, I. Topp, H. Kaye, W. Hall, Heroin use in New South Wales Australia 1996–2000: 5 year monitoring of trends in price, purity, availability and use from the Illicit Drug Reporting System (IDRS), Addiction 97 (2002) 179–186.
[10] J. Gerostamoulos, V. Staikos, O.H. Drummer, Heroin-related deaths in Victoria: a review of cases for 1997 and 1998, Drug Alcohol Depend. 61 (2001) 123–127.
[11] Observatorio Español sobre Drogas, Informe no. 4, Plan Nacional sobre drogas, Ministerio del Interior, Madrid, 2001.