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Article

Metabolites of Heroin in Several Different Post-mortem Matrices

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Abstract

In some forensic autopsies blood is not available, and other matrices are sampled for toxicological analysis. The aims of the present study were to examine whether heroin metabolites can be detected in different post-mortem matrices, and investigate whether analyses in other matrices can give useful information about concentrations in peripheral blood. Effects of ethanol on the metabolism and distribution of heroin metabolites were also investigated. We included 45 forensic autopsies where morphine was detected in peripheral blood, concomitantly with 6-acetylmorphine (6-AM) detected in any matrix. Samples were collected from peripheral blood, cardiac blood, pericardial fluid, psoas muscle, lateral vastus muscle, vitreous humor and urine. Opioid analysis included 6-AM, morphine, codeine, and morphine glucuronides. The 6-AM was most often detected in urine ($n = 39$) and vitreous humor ($n = 38$). The median morphine concentration ratio relative to peripheral blood was 1.3 (range 0–3.6) for cardiac blood, 1.4 (range 0.07–5.3) for pericardial fluid, 1.2 (range 0–19.2) for psoas muscle, 1.1 (range 0–1.7) for lateral vastus muscle and 0.4 (range 0.2–3.2) for vitreous humor. The number of 6-AM positive cases was significantly higher ($P = 0.03$) in the ethanol positive group ($n = 6$; 86%) compared to the ethanol negative group ($n = 14$; 37%) in peripheral blood. The distribution of heroin metabolites to the different matrices was not significantly different between the ethanol positive and the ethanol negative group. This study shows that toxicological analyses of several matrices could be useful in heroin-related deaths. Urine and vitreous humor are superior for detection of 6-AM, while concentrations of morphine could be assessed from peripheral or cardiac blood, pericardial fluid, psoas muscle and lateral vastus muscle.

Introduction

In the majority of forensic autopsies, quantitative toxicological analyses are performed in blood. Peripheral blood is considered superior to cardiac blood, as it is usually less affected by post-mortem redistribution (1–4), and because most reference concentrations have been measured in peripheral blood (3). In some autopsies, neither peripheral nor cardiac blood is available, for example due to severe injuries, burns or decomposition. When blood is not available, other matrices are often sampled for toxicological analysis, but the interpretation of the drug concentrations in such cases is challenging. Drug concentrations in other matrices than blood are barely studied, and it is therefore uncertain whether quantitative analyses

can provide useful information. For some drugs, not even qualitative detection in other matrices than blood has been investigated. Matrices that have been suggested for toxicological analyses in complicated cases are among others pericardial fluid, skeletal muscle and vitreous humor (1, 2).

Heroin or morphine intake is reported to be the cause of death in approximately half of the fatal drug overdoses in Norway (5). Morphine is also a metabolite of codeine or ethylmorphine which are commonly used for analgesic and antitussive purposes, respectively. The interpretation of post-mortem morphine concentrations is challenging, as regular use of opioids can lead to extensive tolerance for the effects. Preanalytical variation (3), polydrug use (6, 7) and post-mortem redistribution (8–10) could further complicate the interpretation.

Heroin has an extremely short half-life in blood (less than 5 min), and is immediately converted to the active metabolite 6-acetylmorphine (6-AM), which is further metabolized to morphine with a half-life of about 20–30 min (11). In autopsy cases, detection of 6-AM in blood is regarded as evidence of heroin intake, most likely within the last 1–2 h preceding death (12). In urine, 6-AM can be detected for a longer time period, possibly up to 12 h (13). Morphine itself has a half-life measured in blood of approximately 2–3 h in adults (14), and is glucuronidated to the pharmacologically active morphine-6-glucuronide (M6G) and the inactive morphine-3-glucuronide (M3G) (14–16).

Detection and quantification of heroin metabolites in most other matrices than blood have been sparsely investigated. Some studies have found a fairly good correlation between concentrations in blood and pericardial fluid of various drugs (17–19), but the number of cases is few. Skeletal muscle is a particularly interesting matrix in forensic toxicology, as it is often available even in cases with extensive decomposition or in exhumed or exsanguinated corpses. However, the studies that have investigated morphine concentrations in muscle show conflicting data, and are therefore not conclusive (20–24). Vitreous humor has been investigated in several studies, and this matrix seems particularly useful in the detection of 6-AM, as evidence of heroin use (25–29).

In the majority of heroin-related deaths, other drugs and ethanol are also involved (6, 7, 12). Previous studies have found a possible pharmacokinetic interaction between ethanol and the metabolism of heroin (30, 31). The mechanism underlying a pharmacokinetic interaction is, however, not thoroughly investigated, and it is to the authors' knowledge unknown whether this will affect the distribution of heroin metabolites between the different matrices.

The aims of the current study were to examine whether heroin metabolites could be detected in several different matrices in a large number of post-mortem cases, and to investigate whether quantitative analyses in other matrices than blood can give useful information about concentrations in peripheral blood. A possible effect of ethanol on the distribution of heroin metabolites between different matrices was examined, and the reported pharmacokinetic interactions between ethanol and the metabolism of heroin in peripheral blood were also studied.

Materials and Methods

Materials

The present study included 45 forensic autopsy cases where morphine was detected above the limit of quantification (LOQ) in peripheral blood, and where 6-AM was detected in one or more of the investigated matrices. This study is part of a larger project that analyzed samples collected from a total of 173 forensic autopsies performed in the period June 2013–June 2016.

Sampling and storage

The autopsies were performed 0–6 (median 2) days after death, and samples were collected from peripheral blood, cardiac blood, pericardial fluid, psoas muscle, lateral vastus muscle, vitreous humor and urine. Two samples of peripheral blood were collected; one for the routine analysis, and one for the study analysis. We aimed to collect all the matrices in every case, but in two cases vitreous humor was not available, and in two other cases urine was not available.

The samples of peripheral blood (most often the right femoral vein), cardiac blood, psoas muscle and lateral vastus muscle were collected in 25 mL Sterilin tubes (Bibby Sterilin, Staffordshire, UK) containing 200 mg potassium fluoride solution as preservative. The urine samples were also collected in 25 mL Sterilin tubes, but without preservative. The vitreous humor and the pericardial fluid were sampled in 5 mL glass BD Vacutainer evacuated tubes (BD Diagnostics, Plymouth, UK) with 20 mg sodium fluoride and 143 IU of heparin. The vitreous humor was collected from one or both eyes depending of the yield. After arrival in the laboratory, all samples were stored in refrigerators at 4°C until analysis. Collection and preparation of the samples is described in more detail in Øiestad *et al.* (submitted, 2017).

Analytical repertoire and analytical methods

The peripheral blood samples were screened for approximately 100 psychoactive medicinal substances and common drugs of abuse, including alcohols, opioids, benzodiazepines, amphetamines, anticonvulsants, antidepressants, antipsychotics and a selection of new psychoactive substances.

The screening analysis of opioids in peripheral blood was performed using a previously published ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS-MS) method (32). When opioids were detected in peripheral blood, analyses for opioids were also performed in cardiac blood, pericardial fluid, psoas muscle, lateral vastus muscle and vitreous humor. Only cases with morphine detected in peripheral blood were included in this study. The confirmation analyses of 6-AM, morphine, M3G and M6G in blood, pericardial fluid and vitreous humor were performed using an UHPLC–MS-MS method with a LOQ of 0.0033 mg/L and 0.0086 mg/L for 6-AM and morphine, respectively, and 0.014 mg/L for M3G and M6G. In psoas muscle and lateral vastus muscle we used a LOQ of 0.0033 mg/kg and 0.0086 mg/kg for 6-AM and morphine, respectively, and 0.014 mg/kg for M3G and M6G.

Chromatographic and instrumental parameters for analysis of all matrices were performed according to Berg *et al.* (33). Ammonium formate buffer was, however, used instead of bicarbonate buffer. Urine was added internal standard, filtrated using Captiva ND lipids filter plates and run separately as part of our routine work. Samples other than urine were prepared by protein precipitation before filtration on Captiva filter plates. Calibrators and quality control samples (QCs) were prepared by mixing working solutions (100 µL) with aliquots of blank whole blood (100 µL). Samples (100 µL of whole blood, pericardial fluid, muscle or vitreous humor) and blank samples (100 µL blank whole blood) were added 100 µL methanol:water (1:1) to compensate for the volume of the calibration/QC solutions. Internal standard (50 µL was then added, and all sample tubes were shaken on a whirlmixer. Then, 750 µL ice-cold acetonitrile:

methanol (85:15) was added to each tube. The tubes were capped and shaken for 1 min on a multitube vortexer. The samples were then centrifuged for 2 min (4,500 rpm, 4°C) and the organic phase transferred to a Captiva 96 well filterplate (Agilent Technologies, Santa Clara, California, USA). Vacuum was applied and the filtrates were collected in a Captiva collection plate, followed by evaporation at 70°C at a nitrogen flow of 40 psi for approximately 12 min using a TurboVap 96 workstation (Caliper Life Sciences, Waltham, Massachusetts, USA), leaving 200–300 µL of the extract in the wells. The wells were then sealed by a plastic film (Rapid slit seal, BioChromato, Kanagawa-ken, Japan) and transferred to a UHPLC–MS-MS instrument (Xevo TQ-S MS/MS instrument from Waters Corporation, Milford, Massachusetts, USA) for analysis.

Analysis of ethanol in peripheral blood was performed by screening and confirmation by headspace gas chromatography equipped with a flame ionization detector (34).

All samples from each case were analyzed within the same analytical series to minimize analytical variation, except urine, where quantitative results were not considered relevant. Analytical results below LOQ were reported as zero. The duplicate results from the routine analysis of morphine in peripheral blood were also investigated. Only qualitative results for 6-AM in urine were reported in the present material.

Validation of the method

The confirmation method for opioids has previously been validated for whole blood. Intermediate precision given as CV (%) and accuracy given as bias (%) for three concentration levels (6-AM: 0.003 mg/L, 0.010 mg/L, 0.052 mg/L, morphine: 0.009 mg/L, 0.028 mg/L, 0.28 mg/L, M3G and M6G: 0.014 mg/L, 0.046 mg/L, 0.46 mg/L) were ≤13% in all cases ($n = 10$). Matrix effects (ME) and extraction recovery in the different matrices, except urine, were tested at two concentration levels (Tables III and IV) using material from five separate autopsy cases in which no drugs had been detected in screening analysis of peripheral blood. Four different lots of blood bank blood (Oslo University Hospital, Oslo, Norway) were in addition tested at the highest concentration level. ME were evaluated by the post extraction addition approach (35) by comparison of the peak height of blank matrix samples spiked after extraction with the peak height of spiked neat standards prepared in acetonitrile:methanol (85:15, v/v). Extraction recovery was calculated by comparison of the peak heights obtained when the compounds were added before extraction and the internal standard (IS) were added after, with those obtained when both the compounds and IS were added after the extraction step.

Statistical analysis

The data were analyzed using IBM SPSS Statistics 23, Sigma Plot 13.0, and Microsoft Excel. Assessment of the histograms and testing for normality with Kolmogorov–Smirnov test showed that none of the concentrations were normally distributed. Median and range values are therefore reported.

The median concentration ratios of 6-AM, morphine, M3G and M6G in the different matrices relative to peripheral blood were calculated. Spearman's rank correlation was used to investigate the relationship between concentrations of 6-AM and morphine in the different matrices relative to peripheral blood, and between the concentrations of morphine in the two different muscles.

The number of 6-AM positive cases was compared between ethanol negative and ethanol positive cases using Fisher's exact test. The ratios between metabolites (morphine/6-AM, M3G/morphine and M6G/morphine) in peripheral blood, as well as the concentration ratio of 6-AM, morphine, M3G and M6G in the different matrices relative to peripheral blood, were compared between the ethanol negative and ethanol positive cases using Mann-Whitney test. P-values < 0.05 were considered statistically significant.

Ethics

The study was approved by the Regional Committee for Medical Research Ethics (reference number 2012/2173) and by the Higher Prosecuting Authority.

Results

The concentrations of 6-AM and morphine in the different matrices, as well as the detection of 6-AM in urine and the degree of decomposition in the individual cases are shown in Table I. The 6-AM was most often detected in urine ($n = 39$) and vitreous humor ($n = 38$). In cases where the morphine concentration was high (approximately 0.20 mg/L or higher) in peripheral blood, 6-AM was also detected in the majority of cases in peripheral blood, cardiac blood and pericardial fluid, but less often in muscle. Morphine was detected in nearly every matrix in most cases, except in cases where the morphine concentration in peripheral blood was in the lowermost concentration range, below 0.030 mg/L ($n = 5$), where morphine was detected in muscles in only two cases.

The median concentrations of 6-AM, morphine, M3G and M6G and their median concentration ratios relative to peripheral blood are shown in Table II. The median ratios of morphine in cardiac blood (1.3), pericardial fluid (1.4), psoas muscle (1.2) and lateral vastus muscle (1.1) relative to peripheral blood were comparable, but the range varied considerably between the different matrices, as shown in Table II.

The concentration ratios of 6-AM and morphine in the different matrices relative to peripheral blood are shown in Figure 1. The 6-AM concentrations in all matrices showed statistically significant ($P < 0.001$) moderate to strong correlations with concentrations of 6-AM in peripheral blood (Spearman's $\rho = 0.76$ for cardiac blood, 0.59 for pericardial fluid, 0.75 for psoas muscle, 0.72 for lateral vastus muscle and 0.83 for vitreous humor). The relationships of morphine concentrations in cardiac blood, pericardial fluid, lateral vastus muscle and vitreous humor relative to concentrations in peripheral blood are displayed in Figure 2. The morphine concentrations in all matrices showed statistically significant ($P < 0.001$) strong correlations with concentrations in peripheral blood (Spearman's $\rho = 0.86$ for cardiac blood, 0.84 for pericardial fluid, 0.88 for psoas muscle, 0.81 for lateral vastus muscle and 0.79 for vitreous humor). The relationship between the morphine concentrations in the two muscles is shown in Figure 3. Comparable morphine concentrations were found in psoas muscle and lateral vastus muscle in the majority of cases as seen in Table I, and the morphine concentrations in the two muscles displayed a statistically significant ($P < 0.001$) strong correlation (Spearman's $\rho = 0.90$). The duplicate concentrations of morphine in peripheral blood from the routine analysis showed a statistically significant ($P < 0.001$), very strong (Spearman's $\rho = 0.99$) correlation (data not shown). Regarding the morphine glucuronides, no clear pattern of distribution was seen, except from a low number of detections in muscles, as seen in Table II.

Table I. Concentrations of 6-acetylmorphine (6-AM) and morphine in peripheral blood (PB), cardiac blood (CB), pericardial fluid (PF), psoas muscle (PM), lateral vastus muscle (VM) and vitreous humor (VH) in the 45 individual cases, organized based on descending concentrations of morphine in peripheral blood. Results of zero are deleted for better readability, n.c. = not collected. Results of 6-AM in urine (U) are reported as d = detected. E = ethanol concentration (g/L) in peripheral blood, D = decomposition graded by the forensic pathologist (0 = no decomposition, 1 = slight decomposition, 2 = moderate decomposition, missing value = decomposition was not graded by the forensic pathologist)

	6-AM							Morphine						E	D
	PB mg/L	CB mg/L	PF mg/L	PM mg/kg	VM mg/kg	VH mg/L	U	PB mg/L	CB mg/L	PF mg/L	PM mg/kg	VM mg/kg	VH mg/L	PB g/L	
1	0.066	0.056	0.103	0.004		n.c.	d	1.145	1.530	1.606	0.372	0.204	n.c.		0
2	0.090	0.042	0.088	0.173	0.185	0.547	d	0.959	1.014	1.582	1.110	1.394	0.438	1.6	0
3	0.034	0.031	0.009	0.102	0.100	0.113	d	0.888	0.997	1.047	0.768	0.489	0.148		1
4	0.015	0.015	0.008	0.018	0.035	0.106	n.c	0.859	1.186	0.841	0.583	0.491	0.458		2
5	0.043	0.020	0.028	0.076	0.040	0.183	d	0.541	0.754	0.761	0.918	0.787	0.123		0
6						0.070	d	0.479	0.735	0.673	0.620	0.798	0.110		
7		0.003	0.006			0.048	d	0.474	0.335	0.429	0.548	0.518	0.166		0
8	0.025					n.c	d	0.471	0.042	0.035	0.067		n.c		1
9	0.013	0.005	0.007	0.029	0.034	0.069	d	0.428	0.956	1.049	0.321	0.208	0.074		0
10	0.018	0.015		0.058	0.042	0.086	d	0.359	0.451	0.627	0.450	0.520	0.056	2.1	0
11	0.011	0.006	0.085			0.083	d	0.278	0.504	0.442	0.373	0.265	0.201		0
12	0.007		0.022	0.013	0.021	0.083	d	0.269	0.188	0.326	0.365	0.369	0.055		0
13	0.009	0.005	0.016			0.060	d	0.258	0.887	0.633	0.479	0.260	0.181	0.2	2
14	0.020	0.014	0.014	0.026	0.027	0.102	d	0.256	0.533	0.274	0.258	0.172	0.076		0
15	0.006		0.003			0.044	d	0.252	0.287	0.219	0.219	0.167	0.109		0
16	0.009	0.005	0.003	0.025	0.021	0.057		0.249	0.442	1.050	0.332	0.291	0.068	1.5	0
17						0.050	d	0.249	0.302	0.377	0.301	0.337	0.103	1.3	0
18	0.009		0.008	0.019	0.017	0.114		0.231	0.702	0.852	0.412	0.322	0.108	0.1	0
19	0.008	0.004	0.005			0.069	d	0.230	0.634	0.426	0.169	0.198	0.035		0
20		0.019				0.035	d	0.227	0.368	0.250	0.348	0.310	0.083		0
21	0.008	0.004	0.013			0.058	d	0.226	0.268	0.694	0.192	0.096	0.065		0
22				0.004		0.021	d	0.221	0.246	0.264	0.224	0.030	0.047		0
23	0.008	0.005		0.019	0.021	0.050		0.218	0.307	0.330	0.219	0.250	0.045		0
24						0.011	d	0.218	0.284	0.327	0.301	0.274	0.155		0
25	0.009	0.005	0.009			0.042	d	0.211	0.373	0.347	0.229	0.251	0.037	0.7	0
26	0.020	0.014	0.021	0.025	0.021	0.068	d	0.204	0.260	0.237	0.169	0.249	0.036		0
27			0.009			0.019	d	0.146	0.111	0.132	0.202	0.156	0.058		0
28						0.011	d	0.125	0.150	0.120	0.160	0.164	0.054		0
29			0.009			0.021	d	0.121	0.207	0.182	0.177	0.156	0.090		1
30			0.004			0.023	d	0.106	0.236	0.193	0.104	0.091	0.044		0
31			0.007			0.012	d	0.090	0.067	0.108	0.054	0.058	0.059		0
32						0.018	d	0.089	0.083	0.123	0.124	0.115	0.029		0
33						0.014	d	0.088	0.087	0.091	0.112	0.127	0.033		0
34						0.006	d	0.088	0.045	0.062	0.054	0.030	0.056		1
35							d	0.077	0.056	0.104	0.155	0.082	0.123		1
36		0.004	0.007			0.012	d	0.067	0.176	0.144	0.081	0.085	0.051		0
37						0.005	d	0.039	0.062	0.132		0.039	0.026		0
38						0.013	d	0.033	0.033	0.036	0.052	0.054	0.011		0
39		0.005	0.113			0.014	d	0.031	0.113	0.166	0.047	0.036	0.039		0
40						0.005	n.c	0.030	0.033	0.109	0.056		0.027		1
41							d	0.021	0.058	0.030		0.021	0.030		1
42							d	0.020	0.022	0.013			0.011		0
43							d	0.019	0.054	0.048			0.025		0
44							d	0.019	0.015	0.024			0.021		0
45						0.005		0.010		0.012	0.184		0.030		0

In two cases (4%) only heroin metabolites were detected in peripheral blood. In 43 cases (96%) other drugs than morphine and codeine were detected. Codeine was detected in 35 cases (78%), but was probably derived from acetylcodeine, a common impurity of illegally produced heroin (36, 37) in most cases. The most common other substances detected in peripheral blood were clonazepam and/or its metabolite 7-aminoclonazepam in 31 cases (69%), followed by amphetamine and/or methamphetamine in 17 cases (38%) and tetrahydrocannabinol (cannabis) in 16 cases (36%).

Ethanol was detected in peripheral blood in 7 (16%) of the cases, the median ethanol concentration being 1.3 g/L (range 0.1–2.1 g/L) as seen in Table I. In all of the ethanol positive cases, the metabolites ethylglucuronide and ethylsulphate were detected. The number of 6-AM positive cases in peripheral blood was

Table II. The number of positive cases and median concentrations (conc.) of 6-acetylmorphine (6-AM), morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in peripheral blood, cardiac blood, pericardial fluid, psoas muscle, lateral vastus muscle and vitreous humor. Median concentration ratios relative to peripheral blood are also shown. The median concentrations and concentration ranges are reported as mg/L in peripheral blood, cardiac blood, pericardial fluid and vitreous humor, and mg/kg in psoas muscle and lateral vastus muscle

	Peripheral blood (n = 45)	Cardiac blood (n = 45)	Pericardial fluid (n = 45)	Psoas muscle (n = 45)	Lat. vastus muscle (n = 45)	Vitreous humor (n = 43)
6-AM						
N[a] (%)	20 (44)	20 (44)	24 (53)	14 (31)	12 (27)	38 (88)
Median conc.[b] (range)	0.012 (0.0055–0.090)	0.0059 (0.0033–0.056)	0.0093 (0.0033–0.11)	0.025 (0.0039–0.17)	0.030 (0.017–0.18)	0.046 (0.0047–0.55)
Median ratio (range)		0.6 (0–1.0)	0.7 (0–8.0)	1.3 (0–3.2)	1.2 (0–2.9)	6.4 (3.4–12.4)
Morphine						
N[a] (%)	45 (100)	44 (98)	45 (100)	40 (89)	39 (87)	43 (100)
Median conc.[b] (range)	0.22 (0.0096–1.1)	0.26 (0.015–1.5)	0.25 (0.012–1.6)	0.22 (0.047–1.1)	0.20 (0.021–1.4)	0.06 (0.011–0.46)
Median ratio (range)		1.3 (0–3.6)	1.4 (0.07–5.3)	1.2 (0–19.2)	1.1 (0–1.7)	0.4 (0.2–3.2)
M3G						
N[a] (%)	45 (100)	45 (100)	45 (100)	28 (62)	23 (51)	38 (88)
Median conc.[b] (range)	0.25 (0.036–2.4)	0. 34 (0.020–1.9)	0.60 (0.090–5.1)	0.15 (0.023–0.52)	0.10 (0.038–1.2)	0.12 (0.016–2.0)
Median ratio (range)		1.4 (0.06–21.5)	2.5 (0.07–28.1)	0.21 (0–1.7)	0.09 (0–1.9)	0.55 (0–3.7)
M6G						
N[a] (%)	37 (82)	39 (87)	41 (91)	4 (9)	2 (4)	19 (44)
Median conc.[b] (range)	0.052 (0.015–0.58)	0.072 (0.015–0.63)	0.12 (0.020–1.2)	0.080 (0.061–0.12)	0.20 (0.17–0.22)	0.051 (0.014–0.31)
Median ratio (range)		1.2 (0–10.8)	2.5 (0.1–6.9)	0 (0–1.9)	0 (0–0.45)	0.2 (0–3.4)

[a]Number of positive cases.
[b]Of positive cases.

significantly higher (P = 0.03) in the ethanol positive group (n = 6; 86%) compared to the ethanol negative group (n = 14; 37%). The metabolic ratios of morphine/6-AM did not differ between the ethanol positive and the ethanol negative group (P = 0.5). The metabolic ratios of M3G/morphine and M6G/morphine were both significantly lower (P = 0.003 for M3G/morphine and P = 0.001 for M6G/morphine) in the ethanol positive group (median 0.39 for M3G/morphine and 0.0 for M6G/morphine) than in the ethanol negative group (median 2.0 for M3G/morphine and 0.37 for M6G/morphine), all data being from peripheral blood.

The distribution did not differ between the ethanol positive and ethanol negative cases, as the concentrations ratios of 6-AM, morphine and the morphine glucuronides in the different matrices relative to peripheral blood were not significantly different between the two groups (data not shown). Results were corrected for multiple testing according to the Bonferroni method (38).

Matrix effects and recovery

Matrix effects (ME) were investigated for all matrices except urine as shown in Table III. ME were found to be 93–115% for 6-AM, 81–126% for morphine, 45–86% for M3G and 49–94% for M6G. When calculated against each compound's respective deuterated internal standard, or for 6-AM carbon-13 labeled internal standard, the ME varied between 90% and 107%.

The extraction recoveries were investigated for all matrices except urine as shown in Table IV. The extraction recoveries for 6-AM and morphine were 59–72% for blood (peripheral blood, cardiac blood, blood bank blood), pericardial fluid and vitreous humor, and 21–36% for the muscles. For M3G and M6G the recoveries were 31–54% for blood, and 58–71% for pericardial fluid and vitreous humor. A difference was seen between the glucuronides in muscle, with M3G having recoveries between 52% and 62%, whereas M6G had recoveries between 20% and 46%. For the muscle samples, recoveries for all compounds, except M3G, were quite variable, and the use of a corresponding stable isotope labeled internal standard for each compound is therefore mandatory.

Discussion

In the present material, the most suitable matrices for qualitative detection of 6-AM were urine and vitreous humor, followed by pericardial fluid, cardiac blood and peripheral blood. In muscle, 6-AM was often not detected. Due to the instability of 6-AM in blood (39), qualitative detection of 6-AM is usually more important than the concentration. This is opposed to morphine, for which postmortem blood concentrations often are important when interpreting toxicological results. The concentrations of morphine in cardiac blood, pericardial fluid, psoas muscle and lateral vastus muscle were roughly within the same concentration range as in peripheral blood in most cases, whereas the concentrations of morphine in vitreous humor were generally lower than in peripheral blood. For the morphine glucuronides, no clear pattern of distribution was found, except for a low number of detections in muscles.

Cardiac blood

The number of 6-AM positive cases was similar in cardiac blood and peripheral blood (n = 20, 44%), but some cases had 6-AM detected in peripheral blood and not in cardiac blood and vice versa.

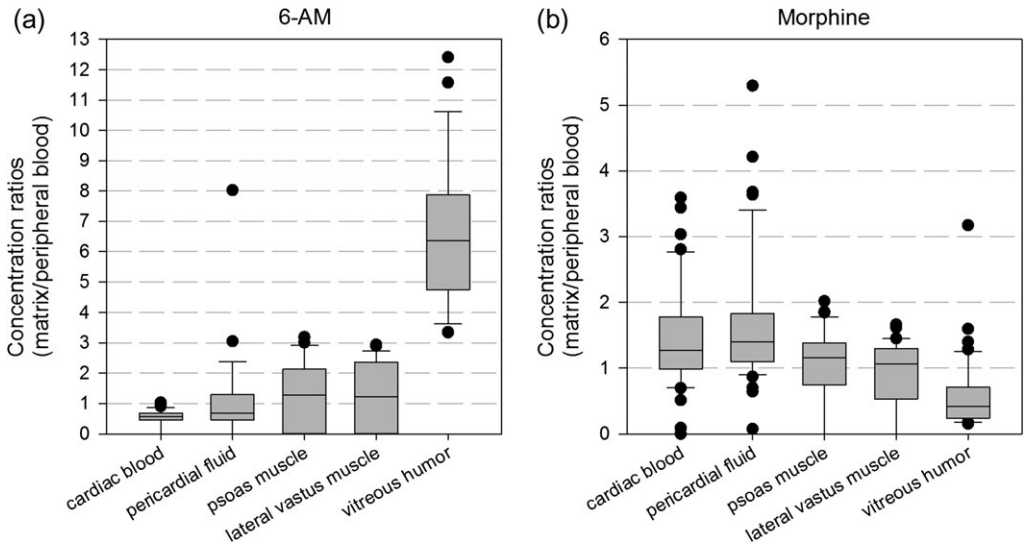

Figure 1. Concentration ratios of (a) 6-acetylmorphine (6-AM) and (b) morphine in cardiac blood (mg/L), pericardial fluid (mg/L), psoas muscle (mg/kg), lateral vastus muscle (mg/kg) and vitreous humor (mg/L) relative to peripheral blood (mg/L). The horizontal line in each box represents the median concentration ratio. The box lengths represent the 25–75 percentiles, whereas the whiskers represent the 10–90 percentiles. Outliers are shown by black dots. One outlier with a morphine concentration ratio of 19 in psoas muscle/peripheral blood was excluded from the figure.

The reason for this is not clear. The median morphine concentration ratio in cardiac blood relative to peripheral blood was 1.3 (range 0–3.6), which is roughly in accordance with previous studies (8, 14, 23). The heart, and thus the cardiac blood is located close to organs like the lungs and gastrointestinal tract, which could have high drug concentrations and therefore serve as a reservoir for many drugs (4). However, as morphine has a relatively small volume of distribution, this redistribution is thought to be of less significance (1, 3, 40). Case number 8 had a morphine concentration in cardiac blood that was lower than 10% of the concentration in peripheral blood, as seen in Table I. The reason for this is not obvious, but contamination, for example from a recent heroin injection close to the sampling site in the femoral region, cannot be excluded.

Pericardial fluid

We detected 6-AM in 24 (53%) cases in pericardial fluid compared to 20 (44%) cases in peripheral blood, and the concentrations of 6-AM displayed a statistically significant ($P < 0.001$) moderate correlation (Spearman's $\rho = 0.59$) to the concentrations in peripheral blood. To our knowledge, detection of 6-AM has not previously been investigated in pericardial fluid.

Morphine concentrations in pericardial fluid have been investigated in an earlier study that reported 49 morphine positive cases with a mean morphine concentration of 1.022 (±1.35) mg/L, roughly twice as high as in blood, and a median concentration of 0.589 (range 0.019–8.9) mg/L (19). In the present study, the median morphine concentration in pericardial fluid was lower, 0.25 (range 0.012–1.6) mg/L, with the median pericardial fluid/peripheral blood ratio being 1.4 (range 0.07–5.3). When looking at the individual cases, the results indicate that the morphine concentrations in pericardial fluid were mainly within the same concentration range as in peripheral blood. However, there are a few divergent results which complicate the interpretation. As shown in Table I, case number 8 had a very low morphine concentration in pericardial fluid compared to peripheral blood. However, this case also had a much lower morphine concentration in cardiac blood than in peripheral blood, described above. The opposite was also found, as a few cases (e.g., cases numbers 16 and 39) had morphine concentrations in pericardial fluid that were up to five times higher than in peripheral blood. The reason for these divergent concentrations is not clear, but it could be related to differences in distribution of morphine to pericardial fluid caused by unequal time spans between heroin intake and death, as well as unequal post-mortem redistribution in blood and pericardial fluid.

The average volume of pericardial fluid is 15–35 mL (41) which should be sufficient for extensive toxicological analysis. Our results indicate that analyses of pericardial fluid could provide important information regarding the detection of 6-AM as well as qualitative, and quantitative, analyses of morphine in cases where blood is not available.

Muscle

The results show that 6-AM was only detected in muscles in around 30% of our cases, opposed to morphine which was detected in muscles in almost 90% of the cases. In a recent post-mortem study of rabbits, 6-AM was detected in muscle in three animals that were autopsied 24 h after death (42). The authors suggest that muscle is a preferred matrix for heroin verification when vitreous humor is not available. However, the rabbits were sacrificed 1 h after heroin injection, in contrast to our cases, where death could have occurred much later after the heroin intake. The majority of our autopsies were performed more than 24 h after death, and a larger post-mortem degradation of 6-AM could also have occurred. To the authors' knowledge, detection of 6-AM in muscle has not previously been studied in humans.

The morphine concentrations showed a statistically significant ($P < 0.001$) and strong correlation between muscle and peripheral blood (Spearman's $\rho = 0.88$ for psoas muscle and $\rho = 0.81$ for lateral vastus muscle). The results from the present study differ from those of Hargrove *et al.* (20) that found no clear correlation between morphine concentrations in skeletal muscle and peripheral blood in 18 autopsy cases where the deceased had been prescribed morphine. However, the study did demonstrate that morphine was

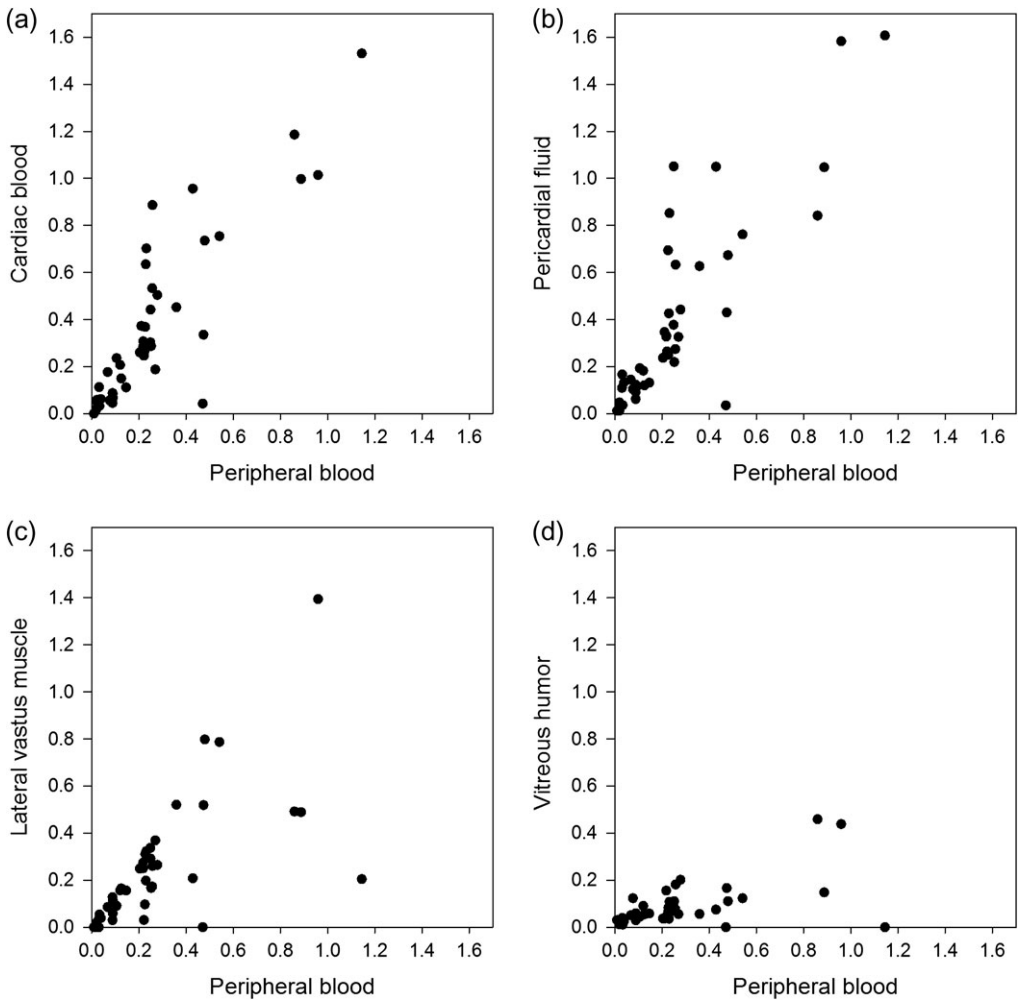

Figure 2. The morphine concentrations in (a) cardiac blood (mg/L), (b) pericardial fluid (mg/L), (c) lateral vastus muscle (mg/kg) and (d) vitreous humor (mg/L) related to concentrations in peripheral blood (mg/L).

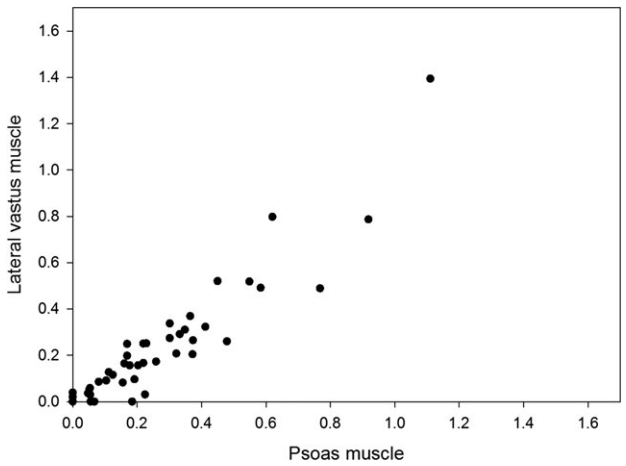

Figure 3. The morphine concentrations in lateral vastus muscle (mg/kg) relative to concentrations in psoas muscle (mg/kg).

detected in muscle in all cases where it was detected in blood (20). Garriott *et al.* (24) reported an average muscle/blood morphine ratio of 1.4 (n = 9). Furthermore, a recent study by Staeheli *et al.* (23) found morphine concentration ratios in thigh muscle/peripheral blood ranging from 1.2 to 3.9 in 12 cases, and suggested thigh muscle as a possible alternative when peripheral blood is not available. The results from our study were roughly in accordance with the results from Garriott *et al.* and Staeheli *et al.*, with a median morphine concentration ratio of psoas muscle/peripheral blood of 1.2 (range 0–19.2) and lateral vastus muscle/peripheral blood of 1.1 (range 0–1.7). We did, however, find some cases with much lower ratios than Staeheli *et al.* (e.g., case number 1 as seen in Table I). The inhomogeneity of the muscle matrix, leading to difficulties in providing a representative aliquot for analysis (1) could explain the divergent results.

As the psoas muscle is closer to the abdominal organs, it is theoretically more prone to post-mortem changes than the lateral vastus muscle. However, in the majority of cases, the morphine concentrations in psoas muscle and lateral vastus muscle were within the same concentration range, as seen in Table I and Figure 3.

Taken together, the results indicate that skeletal muscle might be a useful matrix for detection, and even quantitative analysis of morphine in post-mortem cases. For detection of 6-AM, muscle seems less useful. The present results also indicate that psoas muscle and lateral vastus muscle have comparable concentrations of morphine in most cases.

Table III. Matrix effects (ME) for 6-acetylmorphine (6-AM), morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in peripheral blood, cardiac blood, blood bank blood, pericardial fluid, psoas muscle, lateral vastus muscle and vitreous humor. The calibrator concentrations (conc.) are reported as mg/L in peripheral blood, cardiac blood, blood bank blood, pericardial fluid and vitreous humor, and mg/kg in psoas muscle and lateral vastus muscle

Compound	Calibrator conc.	Peripheral blood		Cardiac blood		Blood bank blood		Pericardial fluid		Psoas muscle		Lateral vastus muscle		Vitreous humor	
		ME (%)	ME corr[a] (%)	ME (%)	ME corr[a] (%)	ME (%)	ME corr[a] (%)	ME (%)	ME corr[a] (%)	ME (%)	ME corr[a] (%)	ME (%)	ME corr[a] (%)	ME (%)	ME corr[a] (%)
6-AM	0.010	108	100	114	106			103	100	100	106	97	98	102	100
	0.33	113	100	115	99	93	100	110	101	95	98	96	97	100	98
Morphine	0.029	101	107	98	107			94	97	82	91	81	90	94	99
	0.57	118	98	126	100	101	104	117	101	106	102	104	101	101	96
M3G	0.047	59	103	48	104			52	103	48	106	45	105	65	101
	0.94	66	96	64	96	86	104	59	99	61	100	65	100	73	96
M6G	0.047	63	100	51	98			51	98	49	99	49	100	65	96
	0.94	84	95	84	95	94	101	77	97	76	97	78	97	86	94

[a]ME corrected with internal standard (IS).

Table IV. Extraction recoveries for 6-acetylmorphine (6-AM), morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in peripheral blood, cardiac blood, blood bank blood, pericardial fluid, psoas muscle, lateral vastus muscle and vitreous humor. The calibrator concentrations (conc.) are reported as mg/L in peripheral blood, cardiac blood, blood bank blood, pericardial fluid and vitreous humor, and mg/kg in psoas muscle and lateral vastus muscle

Compound	Calibrator conc.	Extraction recovery (%)						
		Peripheral blood	Cardiac blood	Blood bank blood	Pericardial fluid	Psoas muscle	Lateral vastus muscle	Vitreous humor
6-AM	0.010	66	61		69	32	36	68
	0.33	60	64	69	65	27	32	65
Morphine	0.029	61	63		67	26	26	67
	0.57	59	63	64	67	21	28	72
M3G	0.047	35	35		59	52	59	66
	0.94	33	36	54	59	56	62	71
M6G	0.047	33	37		61	43	46	68
	0.94	31	35	40	58	20	27	68

Vitreous humor

The number of 6-AM positive cases was almost twice as high in vitreous humor as in blood. This is in accordance with previous studies; Rees *et al.* (25) investigated 70 cases involving heroin/morphine and found that 59% of the heroin intake cases could not be verified without analyzing vitreous humor compared to blood. Approximately the same findings have also been shown by others (26). The median concentration ratio of 6-AM in vitreous humor/peripheral blood in the present material was 6.4 (range 3.4–12.4), which is also roughly in agreement with previous studies (25, 26). A recent pharmacokinetic study in living pigs (43) found that the concentration of 6-AM in vitreous humor exceeded that in blood approximately 2 h after injection of heroin, and the detection time of 6-AM in vitreous humor was estimated to more than 20 h and thus much longer than in blood. The reason for the high concentration of 6-AM in vitreous humor is not fully understood, but the lipophilicity of heroin and 6-AM facilitating transport across membranes, as well as less esterase activity in vitreous humor compared to blood could possibly explain the findings (25, 44).

Pragst *et al.* (27) found lower concentrations of 6-AM in vitreous humor than in urine in most cases ($n = 29$), but in two cases 6-AM was detected in vitreous humor but not in urine. In the present study, 6-AM was detected in four cases in vitreous humor but not in urine, while in five cases 6-AM was detected in urine but not in vitreous humor. Further studies are warranted to compare the time span of detection of 6-AM in vitreous humor and urine.

For morphine, the findings were opposite to that of 6-AM, with generally lower morphine concentration in vitreous humor compared to peripheral blood. The median concentration ratio vitreous humor/peripheral blood was 0.4 (range 0.2–3.2), which is in agreement with several previous studies (25–27). However, it has been suggested that the morphine concentration ratios of vitreous humor/peripheral blood vary significantly depending on whether death occurs rapidly (low ratio) or more protracted (high ratios), with a considerable overlap between the two groups, and that a concentration of morphine in vitreous humor therefore cannot be used to estimate the blood concentration (25).

The small available volume of vitreous humor has limited its use in toxicological analyses. With new analytical methods requiring smaller volumes, vitreous humor could become more important, especially regarding the detection of 6-AM.

Interaction with ethanol

Pharmacokinetic interactions between ethanol and the metabolism of heroin have previously been reported, with ethanol inhibiting the metabolism of 6-AM to morphine as well as the glucuronidation of

morphine (30, 31). The results from the present study were roughly in accordance with previously published results. However, 6-AM was detected in blood in 6 out of 7 ethanol positive cases in the present material. Detection of 6-AM in blood indicates a rapid death after intake of heroin, and this could possibly explain our findings of lower morphine-glucuronide/morphine ratios in the ethanol positive cases. Ethanol did not seem to affect the distribution of heroin metabolites to the different matrices, and to the authors' knowledge this has not previously been investigated. However, the number of ethanol positive cases in the present material was quite low ($n = 7$).

Conclusion

This study shows that analyses in other matrices than peripheral blood might provide important information in heroin-related deaths, and collection of other matrices can be recommended when blood is not available. Quantitative analysis of morphine in cardiac blood, pericardial fluid, psoas muscle and lateral vastus muscle can give an indication of the post-mortem peripheral blood concentration, especially if concentrations from two or more matrices are viewed together. However, caution must be taken when interpreting such results, as some diverging concentrations were found in all matrices. The interpretation of morphine concentrations in vitreous humor appeared to be complicated, but vitreous humor and/or urine should be collected in all cases if available, as these matrices were superior in detecting 6-AM and thus verifying heroin intake.

To be able to compare concentrations of heroin metabolites in different matrices with the concentrations in peripheral blood, we had to include cases where all matrices were available, thereby excluding cases where blood was not available. Further studies are needed to explore how concentrations of heroin metabolites in the different matrices are affected by severe injuries, burns or decomposition.

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