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G. Skopp · R. Lutz · B. Ganßmann · R. Mattern
R. Aderjan

Postmortem distribution pattern of morphine and morphine glucuronides in heroin overdose

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Abstract The postmortem distribution of morphine and its metabolites was investigated in four cases of heroin overdose to evaluate some of the factors that influence intravasal blood concentrations. Variables included were the chemical stability of morphine conjugates, hemoconcentration, incomplete distribution of the drug and diffusion processes. Blood samples from different sampling sites including the aorta, the infra- and suprarenal portion of the inferior vena cava, the superior vena cava, the femoral and subclavian veins, and the right and left ventricles were examined for morphine, morphine-3-glucuronide and morphine-6-glucuronide, hematocrit and water content. Drug concentrations were determined by HPLC based on the native fluorescence of the analytes. Morphine glucuronides proved to be stable for a time period of 72 h. The water content ranged from 65 to 83% and hematocrit values from 25 to 75%, and were seen as contributory factors to the dramatic differences observed for drug concentrations from different sampling sites. The differences could neither be attributed to incomplete distribution during life-time nor to a diffusion process following the different distribution volumes of morphine and its conjugates. A definite relationship between the ratio of the molar concentrations of morphine and its glucuronides, as assessed in pharmacokinetical studies after morphine dosing, could not be established. For a better understanding more cases and changes over time and tissue concentrations should be analysed.

Key words Morphine · Morphine-3-glucuronide · Morphine-6-glucuronide · Heroin overdose · Postmortem distribution

Introduction

The site-dependence of postmortem blood drug concentrations had been studied for numerous compounds including digoxin [1–3], methamphetamine [4], methadone [5], cimetidine [6], paracetamol and propoxyphene [coproxamol, 7], diphenhydramine and codeine [8], barbiturates [9], cocaine [10], amitriptyline [11], and zopiclone [12]. As many drugs are sequestered within tissues during life and released into the blood in the pre- and postmortem interval, dramatic elevations of drug concentrations were observed in samples taken from nearby visceral sites, and also in postmortem samples taken from peripheral veins. It was presumed that the extent of postmortem release of a drug from tissues is associated with the volume of distribution. The larger the volume of distribution, the larger should be the amount of extravascular drug serving as a depot for release. Tricyclic antidepressants or cardiac glycosides, as examples, exhibited a marked site dependence [3, 13]. In cases of fatal ingestion of amitriptyline, the ratio of parent drug and major active metabolite concentrations was found to be substantially greater compared to therapeutic doses [13].

In heroin-related deaths, the situation will be more complex due to heroin metabolism. After administration, diacetylmorphine can be degraded enzymatically or by hydrolysis in blood, tissues and also in vitro to 6-acetylmorphine [14] and subsequently to morphine [15–17]. The detection of diacetylmorphine is, therefore, usually based on the identification of morphine. The postmortem disposition of morphine was investigated in rats [18, 19] and changes in distribution were observed as early as 5 min after death. Significant increases in morphine levels up to 1900% have been found in the heart, forebrain and liver 96 h after death [19]. In humans, concentrations of total morphine greater than those in blood were found in the lungs [20], liver [21, 22], and bile [22], while concentrations in muscle tissue were similar to those in blood [21].

Glucuronidation of morphine occurs at the C3 and C6 carbons [23] with a lag time of about 6 min. The majority of morphine is converted to morphine-3-glucuronide, while small amounts of the active morphine-6-glucuronide are formed [23]. Compared to morphine, morphine-6-glucuronide binds to delta-opiate receptors with a higher affinity and more selectively to mu than to kappa receptors [24,

G. Skopp (✉) · R. Lutz · B. Ganßmann · R. Mattern · R. Aderjan
Institut für Rechtsmedizin der Ruprecht-Karls-Universität,
Vosstrasse 2, D-69115 Heidelberg, Germany

25]. In pharmacokinetic studies after morphine dosing the ratio of the molar concentrations of morphine-3-glucuronide and morphine-6-glucuronide to the molar concentrations of morphine were found to depend on time and route of administration but not on the dose taken [23]. The time course of the molar concentrations of morphine and metabolites calculated from pharmacokinetic studies [26–29] and in living heroin abusers should be similar.

In four detailed case studies of fatal heroin overdose we demonstrate the complexity of morphine, morphine-3-glucuronide and morphine-6-glucuronide concentrations in postmortem blood samples from different sampling sites.

Materials and methods

Blood samples were collected at autopsy rom suspected cases of heroin overdose. Prior to sampling, a urine or a liquor sample was screened for abused drugs by immunoassay according to the manufacturer's instruction (Abuscreen Online, Hofmann-La Roche, Grenzach-Whylen, Germany). After the chest and the abdominal cavities were opened, the vessels were ligated or cross-clamped prior to blood sampling by needle puncture. Blood was obtained from the thoracic aorta, the inferior vena cava (suprarenal, infrarenal), the superior vena cava, the subclavian and femoral veins, and from the right and left ventricles of the heart. Additionally, vitreous humor and liquor were collected. All samples were centrifuged (10 min, 3500 rev./min), serum was removed and frozen (–20° C), and thawed just prior to extraction and analysis.

All reagents were of HPLC or analytical grade. Morphine hydrochloride trihydrate (fw 375.8, CAS 6055-06-07) was purchased from Merck (Darmstadt, Germany), morphine-3-β–D-glucuronide (fw 461.5, CAS 20290-09-9), morphine-6-β-D-glucuronide dihydrate (fw 497.5, CAS 20290-10-2) from Sigma (München, Germany). Triethylammonium phosphate buffer (TEAP, 1M) was obtained from Fluka (Buchs, Switzerland) and diluted in a ratio of 1 : 40 with double distilled water before use.

Extraction

Solid phase extraction was carried out with Bond Elut (C8, 50 mg) extraction columns (Varian, Harbor City, Calif.) and 500 µL of ammonium bicarbonate buffer (1 m*M*, pH 9.2) was added to 100 µL of the serum sample prior to extraction. The diluted sample was transferred to the pre-conditioned extraction column (3 mL distilled water, 3 mL buffer pH 9.2) and drawn down in a Vac-Elut system (Varian, Harbor City, Calif.). The column was washed with 1 mL of buffer pH 9.2 and dried (40 min, in a vacuum). The adsorbed drugs were eluted with 2 × 200 µL methanol and the combined fractions were evaporated to dryness under nitrogen. The residue was reconstituted with 100 µL methanol and 80 µL was injected into the HPLC system.

HPLC

HPLC analysis was performed with a Hewlett Packard 1050 series LC pump, a Shimadzu fluorescence detector and a Spectra Physics SP 4290 integrator. Samples were eluted from an Et 250 Nucleosil 100 5 C18 AB reverse phase column (Macherey & Nagel, Düren, Germany) with diluted TEAP as the mobile phase. For detection, the excitation wavelength was 220 nm, and emission was recorded at 340 nm.

Linearity, reproducibility, recovery and stability of the analytes

Standard calibration curves were obtained in double runs using 7.5–3750 ng/mL serum blank of the standard substances (morphine, morphine-3-glucuronide and morphine-6-glucuronide). The detection limits were 7.5 ng/mL for morphine-3-glucuronide and 25 ng/mL for morphine and morphine-6-glucuronide each (n = 5, mean + 3 SD). The calibration curve was found to be linear for morphine-3-glucuronide from 7.5 to 3750 ng/mL (r = 0.996), for morphine and morphine-6-glucuronide from 37.5 to 3750 ng/mL (r = 0.996, r = 0.998 resp.), using 100 µL serum. Intraday and interday coefficients of variation ranged from 4.4% to 6.1% for morphine-3-glucuronide, from 2.9% to 5.7% for morphine and from 3.7% to 7.2% for morphine-6-glucuronide. The recovery from spiked samples (n = 5) was determined to be 77%, 64% and 70% for morphine, morphine-3-glucuronide and morphine-6-glucuronide respectively. The possible conversion of the glucuronides to morphine during the extraction procedure was checked by adding 100 ng of morphine-3-glucuronide or morphine-6-glucuronide to 1 mL drug-free serum followed by extraction. Additionally, the spiked samples were stored for a time period of 72 h prior to extraction.

Water content

Blood samples of about 0.5 g (sw) were dessicated at 115° C until a constant weight was observed (ca. 50 h). The water content (w) was calculated from the weight decrease (dw) according to w = (dw/sw) × 100 (%).

Hematocrit

The hematocrit (%) was measured by a standard centrifugation method [30].

Case history and autopsy findings

Case 1

A 23-year-old man (height 175 cm, weight 58 kg) with a half-year history of heroin abuse was found dead on a sofa in a supine position. A syringe was found near the body, and 0.75 g of an illicit, good quality sample of heroin were missing. At autopsy no significant pre-existing natural diseases were found, but nine recent needle puncture marks were seen in the right elbow. There was evidence of aspiration of some gastric material. Additional findings were an interstitial lung edema, a dilation of the right atrium and an abnormal accumulation of blood of the internal organs. The autopsy was performed 44 h after discovery of the body and at most 57 h after death.

Case 2

A 27-year-old man was found dead sitting on a toilet, the upper part of the body bending forward. The man was a known abuser of illegal drugs, but the syringe found in the cabin was apparently unused. The lavatory was regularly opened at 6.00 a.m. and the deceased was found at 7.45 a.m. About 3 h later, the body was stored in a cool box until autopsy was carried out after another 15 h. The body measured 175 cm and weighed 68 kg and there was strongly livid colouration of the face. The autopsy revealed a fresh needle puncture mark on the left arm, and a marked aspiration of gastric contents, but pre-existing diseases were not found. The ethanol concentration in blood from the femoral vein was 140 mg/dL.

Case 3

A 33-year-old man (height 176 cm, weight 76 kg) was found dead by his wife lying in a supine position in the toilet of a restaurant 8 min after leaving the table. The man had previously consumed a bottle of vodka, and was known to have abused heroin for about 2 years. At the autopsy, 36.5 h after death, more than 100 fresh and

old needle puncture marks were found on both arms, insteps and on the neck. Other findings were an interstitial lung edema, a moderate brain edema, an abnormal accumulation of blood in the internal organs and a dilation of the right atrium and the right ventricle of the heart. Aspiration of gastric material was also found which could be attributed to the resuscitation attempts. The ethanol concentration in blood from the femoral vein was 109 mg/dL.

Case 4

A 25-year-old man returned to his appartment in the evening heavily under the influence of alcohol. In the morning he was seen lying on the sofa and 16 h later he was found dead by his fellow occupant still lying there in a supine position. There was a red-brown coloured foam at the mouth. The autopsy was performed 33 h after discovery of the body (height 182 cm, weight 86 kg). A dilation of the right atrium and the right chamber of the heart and a marked interstitial lung edema were found, but a needle puncture mark was not found. A sample of blood from the femoral vein contained 25 mg ethanol/dL and 0.017 mg benzoylecgonine/L, but the most striking toxicological finding was the detection of morphine in the gastric contents probably due to oral/nasal intake of heroin.

Results

The drug concentrations in serum samples from different sites taken at autopsy are set out in Table 1 together with

Table 1 The concentrations (ng/mL) of morphine (mor), morphine-6-glucuronide (m6g) and morphine-3-glucuronide (m3g) in serum from different sampling sites, liquor and vitreous humor in cases 1–4, the ratio of the molar concentrations (m6g/mor, m3g/mor), water content (%) and hematocrit (%) of the corresponding blood sample

	mor	m6g	m3g	m6g/mor	m3g/mor	Water content	Hematocrit
Case 1							
Aorta	98	78	597	0.60	4.96	75.47	70
IVC sr	165	201	654	0.92	3.23	75.49	60
IVC ir	115	154	600	1.01	4.25	76.85	68
SVC	283	103	596	0.27	1.71	77.37	60
R sub v	126	76	430	0.46	2.78	81.27	42
L sub v	186	93	639	0.38	2.80	74.26	70
R fem v	117	72	478	0.46	3.33	77.96	65
L fem v	128	93	534	0.55	3.40	77.45	60
R ventr.	157	160	733	0.77	3.80	77.20	60
L ventr.	333	96	604	0.22	1.48	73.91	76
Liquor	51	n.f.	61		0.97	n.d.	n.d.
Case 2							
Aorta	154	n.f.	764		4.04	76.07	45
IVC sr	185	462	866	1.89	3.81	77.25	38
IVC ir	611	568	1078	0.70	1.44	79.36	25
SVC	230	299	674	0.98	2.39	74.60	55
L sub v	379	m.i.	m.i.			70.85	70
R fem v	129	53	554	0.31	3.50	71.16	75
L fem v	73	60	220	0.62	2.54	69.65	70
R ventr.	107	368	m.i.	2.60		76.41	45
L ventr.	293	448	m.i.	1.15		83.83	20
Liquor	188	85	402	0.34	1.74	n.d.	n.d.
Vit humor	318	n.f.	776		1.99	n.d.	n.d.
Case 3							
Aorta	235	152	1339	0.53	4.64	78.40	54
IVC ir	451	205	1544	0.34	2.79	74.18	65
SVC	306	162	1424	0.40	3.79	76.84	55
L sub v	606	66	1141	0.08	1.53	74.06	60
L fem v	312	50	775	0.12	2.02	76.63	50
R ventr.	169	141	1133	0.63	5.46	79.46	25
L ventr.	472	n.f.	149		0.26	71.06	55
Liquor	221	n.f.	247		0.91	n.d.	n.d.
Case 4							
IVC sr	321	245	952	0.58	2.41	72.38	52.5
IVC	205	183	721	0.67	2.86	72.66	65
SVC	232	143	692	0.50	2.43	65.00	65
L sub v	167	96	700	0.43	3.41	72.09	75
R fem v	178	104	631	0.44	2.89	73.10	60
L fem v	137	95	715	0.52	4.25	72.32	60
R ventr.	328	225	970	0.52	2.41	76.04	60
L ventr.	192	73	532	0.31	2.26	72.95	75

vitr.: vitreous, IVC sr: inferior vena cava, suprarenal, IVC ir: inferior vena cava, infrarenal, SVC: superior vena cava, R: right, L: left, sub v: subclavian vein, fem v: femoral vein, ventr.: ventricle, n.f.: not found, n.d.: not determined, m.i.: peak influenced by biological matrix

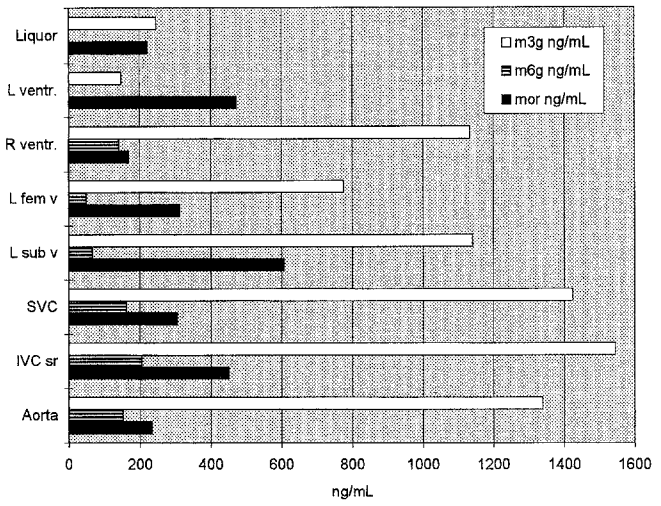

Fig. 1 Morphine and morphine glucuronides concentrations (ng/mL) from different sampling sites in case 3

the water content and the hematocrit values. Morphine glucuronides were not cleaved to morphine during the extraction procedure. There was no indication of hydrolysis when spiked samples were stored for a time period of 72 h prior to analysis. The concentrations of morphine, morphine-3-glucuronide and morphine-6-glucuronide decreased in the following manner: morphine-6-glucuronide < morphine < morphine-3-glucuronide for almost all samples investigated, but exhibited a marked site-dependent concentration range.

The highest concentrations of glucuronides were determined from the infrarenal portion of the inferior vena cava in cases 2 and 3 (Fig. 1). In both cases death had occurred within a short time (< 1.75 h) after administration of heroin. In cases 1 and 4, the highest glucuronide concentrations were determined in serum samples from the suprarenal portion of the inferior vena cava. However, case 4 may be an exception in that heroin had probably been snorted while swallowing a considerable portion of the drug. Heroin is very rapidly deacetylated in the stomach and the morphine formed is subject to enteral absorption and a marked first-pass metabolism [27].

A relationship between drug concentration and a particular sampling site could not be found for the lowest glucuronide concentrations and the extremes of the morphine concentrations (Table 1).

The ratios of the molar concentrations of morphine-6-glucuronide and morphine-3-glucuronide to morphine are outlined in Table 1 and Fig. 2. For example, in case 1 the ratios of morphine-6-glucuronide and of morphine-3-glucuronide to morphine ranged from 0.22 to 1.01 and from 1.48 to 4.96 in serum samples from different sampling sites. The largest range of the morphine-6-glucuronide/morphine ratio was observed in case 3 where several-fold

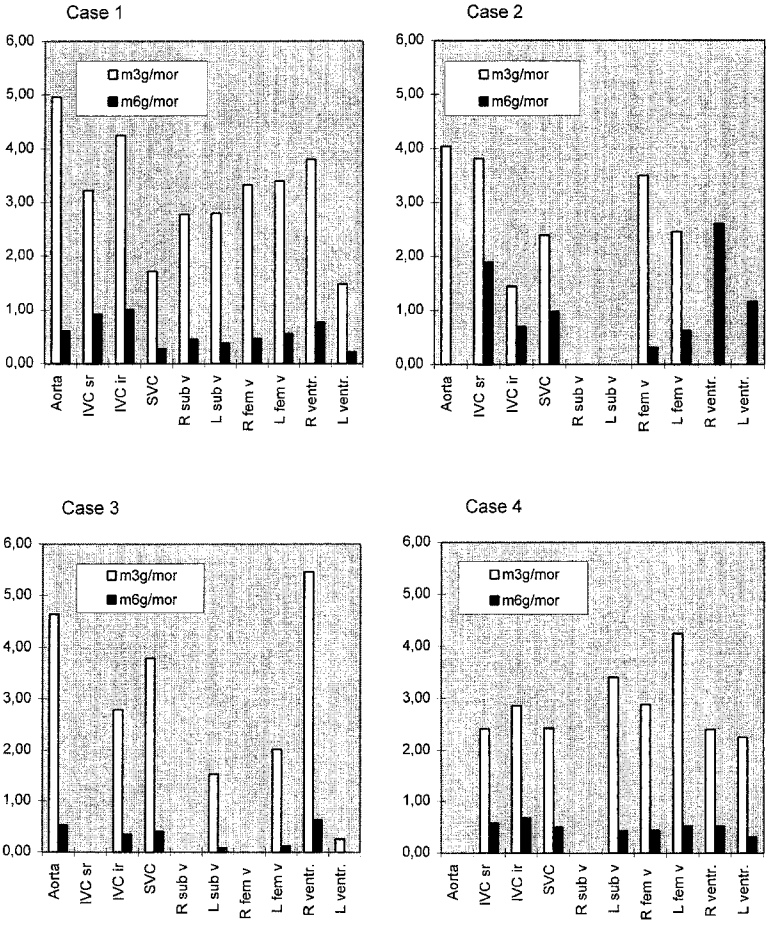

Fig. 2 The ratio of the molar concentrations of morphine-6-glucuronide and morphine-3-glucuronide to morphine as determined from different sampling sites (case 1–4)

differences of the molar concentration ratios between serum samples from different sites were found. The site-to-site ratios of morphine-3-glucuronide and of morphine-6-glucuronide to morphine were narrower in case 4, where lower concentrations of morphine and morphine-6-glucuronide were determined from samples of the subclavian and the femoral veins and higher concentrations in serum from the inferior and the superior vena cava. The water content of the blood samples from different sampling sites ranged from 65% to 83%, while the hematocrit values ranged from 25% to 75% (Table 1).

Discussion

The interpretation of drug levels in postmortem blood samples poses one of the most serious challenges to the forensic toxicologist. In deaths involving heroin, the use of drug metabolism data for the estimation of survival time was investigated by several working groups, based on the concentrations of total and free morphine or 6-acetylmorphine in blood, brain regions or in tissue [20, 31–33], but not on the direct determination of morphine and its glucuronides.

The question that often arises is whether erroneous interpretation of a morphine blood concentration can occur if a low concentration was found and a clear cause of death was not ascertained at autopsy. Although the morphine concentrations in this study were not very critical to the diagnosis of heroin-related death, the determination of the morphine-6-glucuronide concentration may be helpful in other cases.

The data presented are discussed with regard to incomplete distribution, properties of the blood sample, physicochemical parameters and distribution volume of the analytes and their diffusion behaviour.

In the heroin-related deaths investigated in this study several-fold differences in the morphine, morphine-3- and morphine-6-glucuronide concentrations were found between blood samples from different sampling sites. The large differences observed cannot be attributed to incomplete drug distribution before death, except for case 3 with a maximum survival time of 8 min. The phenomenon of dependence on blood sampling site is not restricted to the ante- or postmortem interval since it has been reported for several compounds after dosing in humans and in animals [34]. However, the arterial-venous differences were insufficient to account for those determined in postmortem blood samples from different sampling sites, and they may only be contributory factors in deaths with a very short survival time such as case 3.

Another factor influencing postmortem distribution is the blood sample itself which is a variable mixture of plasma and erythrocytes. After death, blood gravitates and distends the toneless capillaries and small veins of the lower areas of the body. The vessels become leaky resulting in plasma loss and hemoconcentration [35]. From a clinical study of an epidemic of heroin intoxications in which 149 patients were involved, the hematocrit values recorded on admission ranged from 31% to 58% [36]. The present data spread over a wider range and showed poor correlation with water content. Morphine has been proven to be evenly distributed between plasma and erythrocytes or whole blood [18, 37]. If morphine glucuronides as highly polar conjugates are preferably distributed in plasma, a higher morphine-6-glucuronide or morphine-3-glucuronide/morphine ratio should result in a lower hemotocrit value. But even if case 3 is excluded, a clear tendency between the ratios and the hematocrit values could not be established from the present data. The water content of the samples ranged from 65% to 83%, and a low value was often associated with a low ratio of the molar concentration of glucuronides and morphine, supporting this hypothesis which is currently under investigation. The differences in morphine glucuronide concentrations can partly be explained by the variable water content of the blood samples.

A large site-to-site variation in drug concentrations was often found to be associated with a high distribution volume. The apparent volume of distribution of morphine is reported to be 3–5 L/kg and approximately 0.28 L/kg for morphine glucuronides [23]. Therefore, morphine concentrations should exhibit a larger concentration range when compared to glucuronide concentrations. With the exception of case 3, where the distribution of morphine can be assumed to be incomplete prior to death, the molar concentrations of morphine-3-glucuronide and morphine-6-glucuronide varied two- to threefold at most, while for case 2, the molar morphine concentrations were 107 nmol/L in a sample from the femoral vein and 1142 nmol/L in a sample from the infrarenal portion of the inferior vena cava. From force-field and quantum mechanics Carrupt et al. [25] concluded that morphine glucuronides can exist in two conformational forms, the folded conformers being more lipophilic. Therefore, a certain site-to-site variation could be associated with the ambiguous nature of morphine-glucuronides.

There is a more attractive explanation for the phenomenon of postmortem redistribution. The antemortem distribution pattern of a drug substance and its storage in tissues depends on physicochemical parameters such as molecular size, degree of ionization and lipophilicity, pharmacokinetic properties such as distribution volume, plasma protein binding and terminal half-life and on the mode of administration. The rate of distribution to the tissues is determined by the blood flow perfusing the organs and the ease by which the drug molecules cross the capillaries and penetrate the cells. For most drugs, blood flow is the rate-limiting step in distribution. At the moment of death, this dynamic process ceases and turns to stasis where passive diffusion of the drug substance along a concentration gradient is expected. Ongoing autolysis will result in the breakdown of the cell membranes and macromolecules which maintain the concentration gradient and are responsible for drug binding.

From experiments on the permeation behaviour of morphine and 3- or 6-glucuronides across a vascular wall over a time period of at least 72 h passive diffusion of morphine-3- and morphine-6-glucuronide occurred more rapidly

compared to morphine. The time lag depended on the initial substance concentration [38]. Therefore, a definite relationship between the ratio of the molar concentration of morphine-3-glucuronide or morphine-6-glucuronide to morphine, comparable to data from pharmacokinetic studies, could not be established from postmortem samples. A higher ratio will not necessarily be consistent with decreased drug action or a longer survival time [38].

Provided that the concentrations of morphine and morphine glucuronides are smaller or similar to those in blood and the antemortem distribution is complete, peripheral blood drug concentrations should fall within a relatively narrow range. Drug concentrations in subclavian and femoral venous serum samples were relatively evenly distributed in cases 1 and 4 but not in case 2. For case 2 the higher morphine concentration in the blood sample from the left subclavian vein may be attributed to the position and to anatomical differences, such as the thickness and structure of veins from the supracardiac and infracardiac regions of the body. Logan and Smirnov [39] studied 32 deaths involving morphine and concluded that significantly higher site-dependent differences occurred only when the morphine concentration in ventricular blood exceeds 300 ng/mL. But their study differed from the present study in that a radioimmunoassay procedure with little cross-reactivity for morphine glucuronides was used and blood samples were drawn from the left ventricle only. Glucuronide concentrations are considerably higher than morphine concentrations, therefore, the results may not be as reliable as those obtained from a direct determination of the analytes [40]. In the present study, there was no consistent pattern in the differences between left and right ventricle samples. It may be assumed that there is a lower risk of postmortem release of morphine and morphine glucuronides from the myocardium into ventricular blood according to a comparable finding for codeine [41].

Currently, more cases are being analysed for a better understanding of the postmortem redistribution of morphine and its glucuronides. The data from this study are limited and general conclusions are tenuous but demonstrate that site-dependence of drug concentrations is not restricted to substances with a high distribution volume. There is a risk of assessing survival time based on the time course of the ratio of parent drug to metabolite found in pharmacokinetical studies. It is desirable to analyse peripheral instead of "visceral" blood, and vessels should be ligated prior to collection of a specimen. For a proper interpretation the sampling site and the circumstances surrounding the death, especially the mode of drug administration, should be known.

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