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Morphine in postmortem blood: its importance for the diagnosis of deaths associated with opiate addiction

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Summary. This article describes an analytical method for the determination of morphine, the active metabolite of heroin, in post-mortem blood by HPLC with electrochemical detection. An extraction technique allowing the determination of free and total morphine (free morphine + morphine glucuronide) was used. Blood morphine levels in postmortem cases are reported and the ratio of free to total morphine was measured in 52 cases obtained at autopsy. The importance of this ratio is discussed in relation to the circumstances of the death.

Key words: Morphine – Blood – Death – HPLC – Overdose

Zusammenfassung. Bei 52 Heroíntodesfällen wurden im Leichenblut die Konzentrationen an Morphin und Morphinkonjugaten bestimmt. Aus dem Verhältnis der Konzentrationen können Aufschlüsse über die Dauer der Überlebenszeit abgeleitet werden. Zur Analyse des Morphins wird eine HPLC-Methode in Verbindung mit elektrochemischer Detektion vorgestellt.

Schlüsselwörter: Morphin – Blut – Tod – HPLC – Überdosierung

Introduction

In cases of death related to narcotic addiction, individuals are suspected to have consumed an excess dose of opiate, often termed as "overdose".

The forensic pathologist encounters many difficulties in determining with certainty the cause of death on the exclusive basis of the autopsy, especially when no injection site has been found. Only the presence of either generalized stasis, pulmonary or sometimes cerebral oedema, or some signs of asphyxia is compatible with an opiate intoxication. The necessity of a toxicological analysis is then essential. The habits of the subjects and their tolerance levels must be taken into account, since it is difficult to fix a level of morphine which could be considered fatal in every case.

Vycudilik [1] has described the morphine determination in brain segments and its means of determining the survival time. Spiehler and Brown [2] have recently shown that the ratio of free to total morphine in blood could be used to estimate the time elapsed since the injection of heroin.

Shortly after injection, heroin is rapidly deacetylated in blood to 6-acetylmorphine (half-life 9 min) and further hydrolyzed to morphine at a slower rate (half-life 38 min) [3]. The morphine itself is deactivated by glucuronidation. Both heroin and 6-acetylmorphine have very little affinity with opiate receptors and free morphine is certainly responsible for the narcotic effects in the brain via the mu and the kappa receptors [4]. After injection, the concentration of morphine glucuronide increases in relation to a decrease of free morphine in the blood.

In the present study we have determined the ratio of free to total morphine in 52 actual forensic cases collected between 1988 and 1989 and we have investigated these in relation to the circumstances surrounding the death.

To measure the level of morphine in whole blood, it is generally agreed that mass spectrometry should be used, but the preparation of blood for a GC-MS analysis is time consuming and expensive. Several methods using high pressure liquid chromatography (HPLC) with various detectors, e.g. UV and fluorescence, have been reported [5–7].

The major drawbacks of these techniques are their low sensitivity and/or tedious sample preparation. Electrochemical detection seems to provide the required sensitivity for analysis of morphine in plasma or whole blood [7–9]. A modified HPLC produce was also used for the quantitation of morphine in whole blood.

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Method

Chromatography. A single piston pump (Varian 2010) equipped with a reverse phase column (250 mm × 4.6 mm) containing octadecylsilane (Phenomenex) was used in conjunction with a Metrohm 611 electrochemical detector operating at an applied voltage of +750 mV. This electrochemical detector is composed of a low volume flow cell which includes a three electrode detection system connected to an electronic detection unit. The electrodes are a glassy carbon working-electrode, a glassy auxiliary electrode and a silver/silver chloride reference electrode.

Isocratic liquid chromatography at a flow rate of 0.8 ml/min was performed using a mobile phase consisting of 17.5% acetonitrile and 82.5% citrate buffer (0.005 *M* sodium citrate pH 5.0, 0.02 *M* lithium perchlorate). In order to keep the same cation in both the mobile phase and the reference electrode, the 3 *M* KCL internal reference solution was replaced by 3 *M* LiCL.

Samples and extraction. Blood samples were taken at autopsy and frozen (−20°C) until assayed. To one millilitre of blood, 0.5 ml of 10% trichloroacetic acid and 1.5 ml of hydrochloric acid (2 *M* HCl) were added and immediately and throughly vortexed. The liquid supernatant was transferred to screw-top tempered glass tubes (16 by 100 mm).

To determine the total amount of morphine (morphine and morphine glucuronide), were the samples then hydrolyzed by incubating at 100°C for 60 min and cooling before proceeding to the next step. For the determination of unhydrolyzed morphine (unconjugated morphine) the tubes were not heated. The contents of the tubes were neutralized with 200 µl 10 *M* NaOH and buffered with 1 ml carbonate buffer (1 *M* Na_2CO_3 pH 9).

The morphine was extracted with 4 ml chloroform/isopropanol (9/1). The tubes were shaken mechanically for 10 min and centrifuged for 5 min.

The upper aqueous layer was aspirated and discarded. The lower organic layer was washed with 1 ml distilled water and then evaporated to dryness. The residue was then dissolved in 100 µl methanol containing 0.5 µg of nalorphine which acts as a chromatographic standard. Samples, blood standards and blanks were extracted simultaneously and concentrations were calculated by comparing the peak heights ratios.

Results and discussion

The described method was used routinely for two years in our institute and the extraction procedure provided material of a quality suitable for HPLC with electrochemical detection. Examples of chromatograms for a blank blood sample, morphine spiked blood sample and post-mortem blood sample are shown in Fig. 1.

Morphine concentrations have been determined in 52 actual forensic cases; the group studied, included 42 men and 10 women and their average age was 28.5 years (range 18–39 years) (Table 1). In all these cases no significant level of codeine was found by our usual screening. The free morphine concentration ranged from 0.05 to 2.05 mg/l with an average value of 0.71 mg/l and a standard deviation of 0.52 mg/l. These values are comparable with those obtained by Stead and Moffat [11] who found the average value of free fatal blood morphine concentration in man to be 0.7 mg/l. Because of lack of information about the purity of the heroin and in the absence of data about the individual tolerance, the relationship between the free morphine concentration and the heroin injected dose cannot be determined. Furthermore it is impossible to specify, at this stage of the study, whether the intake of drug was accidental or had a suicidal purpose. The term "overdose" is relative and depends on actual personal susceptibility. Doses as low as 0.05 mg/l in blood can cause death after a period of abstinence (for example in prison) but survival periods in a comatose state cannot be excluded only on the basis of the free morphine concentration.

If one considers the ratio of free to total morphine, 85% (44 cases) of deaths observed in our study (Table 2) were abrupt and occurred shortly after injection (group 1). Furthermore, this ratio was always greater than 50%, which from consideration of the pharmacokinetics [2] indicates a rapid death in less than three hours. It is also interesting to note that in 30 of these 44 cases (68.2%), the drug material was found near the body. According to the terminology used by Janssen et al. [12], this type of death corresponds to the so called "golden shot". In the 8 remaining cases (15%, group 2), the percentage of free to total morphine was much lower (between 20 to 40%) and a survival period in a comatose state has to be taken into consideration. This group is characterized by low concentrations of free morphine (0.05 to 0.50 mg/l) probably due to elimination during the survival period. In this group no drug addict material was found near the body.

In conclusion, the analytical method described here can be used routinely in forensic cases to measure blood morphine levels. This technique does not require the preparation necessary for a GC-MS analysis and is less expensive.

The wide range of concentrations found in post-mortem blood samples confirms that the term "overdose" is

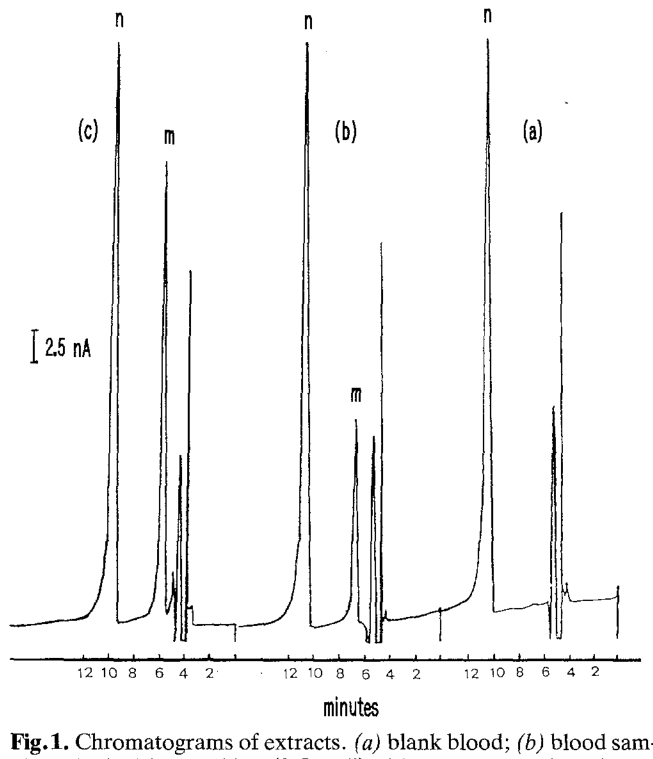

Fig. 1. Chromatograms of extracts. *(a)* blank blood; *(b)* blood sample spiked with morphine (0.5 mg/l); *(c)* post-mortem blood sample; *m:* morphine; *n:* nalorphine

Table 1. Morphine concentrations in post-mortem blood ($n = 52$)

Sex	Age (years)	Group	Morphine (mg/l)		Free to total morphine (%)
			free	total	
M	32	1	1.25	1.95	64
M	20	1	0.85	1.25	68
F	23	1	1.25	2.15	58
F	21	1	1.50	1.60	94
F	24	1	1.30	1.35	96
M	29	2	0.35	1.15	30
M	32	1	1.45	2.45	59
F	28	1	0.90	1.20	75
M	34	1	1.15	1.60	72
M	37	1	0.85	0.85	100
M	22	1	0.50	0.60	83
M	34	1	1.45	1.90	76
F	26	1	0.60	0.60	100
M	36	1	0.40	0.40	100
M	26	1	0.40	0.75	53
M	32	1	0.55	0.80	69
M	22	1	0.75	1.50	50
M	34	2	0.80	2.25	36
M	23	1	0.30	0.40	75
M	23	1	1.40	1.50	93
M	31	1	0.90	1.20	75
M	18	2	0.30	0.75	40
M	24	1	0.70	1.35	52
M	35	1	1.00	1.35	74
F	26	1	0.30	0.55	55
M	23	2	0.50	2.30	22
F	34	1	0.25	0.25	100
M	31	1	0.30	0.30	100
M	29	1	0.90	1.55	58
M	28	1	0.60	0.65	92
F	24	1	1.15	1.45	79
M	35	1	0.60	0.70	86
M	26	1	0.65	0.90	72
M	26	1	0.30	0.40	75
M	21	1	0.35	0.50	70
M	20	1	0.50	0.60	83
F	25	1	0.20	0.25	80
M	29	1	0.05	0.05	100
M	36	1	0.20	0.25	80
M	30	1	0.05	0.05	100
M	31	1	2.05	2.25	91
M	32	1	0.50	1.00	50
F	22	2	0.05	0.20	25
M	29	1	0.65	0.80	81
M	32	1	1.90	2.45	78
M	29	1	2.10	3.90	54
M	39	2	0.50	1.40	36
M	33	1	0.65	1.20	54
M	28	1	0.45	0.65	69
M	23	2	0.10	0.50	20
M	39	1	0.20	0.35	57
M	36	2	0.10	0.25	40

Table 2. Statistical data of forensic cases ($n = 52$)

	Free morphine (mg/l)	Free to total morphine (%)
1. Rapid death ($n = 44$)		
Mean value	0.78	76.1
Range	0.05–2.05	50–100
Standard deviation	0.53	16.2
Material found near the body ($n = 30$)		
Mean value	0.85	74.8
Range	0.20–2.05	50–100
Standard deviation	0.55	16.1
Material not found ($n = 14$)		
Mean value	0.63	79.1
Range	0.05–1.45	52–100
Standard deviation	0.45	16.7
2. Death after a survival period ($n = 8$)		
Mean value	0.34	31.1
Range	0.05–0.50	20–40
Standard deviation	0.26	8.0
3. Overall ($n = 52$)		
Mean value	0.71	69.2
Range	0.05–2.05	20–100
Standard deviation	0.52	22.4

relative and does not sufficiently characterize death associated with heroin addiction. In determining the ratio of free to total morphine we have shown that in 85% of the cases, the death should be attributed to a so called "golden shot". In the remaining cases, the death is not so rapid and a survival period in a comatose state has to be taken into consideration.

At this stage of the study, it is impossible to determine whether the intake of heroin was accidental or had a suicidal purpose, in the absence of data about the individual tolerance and the number of daily injections. Since the addicts can consume heroin several times per day and, therefore, their blood contains varying levels of morphine conjugates at the time of fatal heroin injection. However the determination of total morphine, in parallel with free morphine concentration, is of relevant value in forensic cases. Moreover it is a very powerful tool to determine the circumstances of the deaths, where heroin has played a decisive role.

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