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The relationship between ante-mortem and post-mortem morphine concentrations

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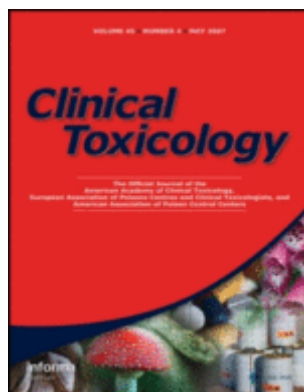
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The relationship between ante-mortem and post-mortem morphine concentrations

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Manuscript ID	LCLT-2018-0585.R2
Manuscript Type:	Clinical Research
Keywords:	Morphine, Post mortem redistribution, Forensic Toxicology, Post mortem examination
Abstract:	<p>Context of the Article: An important forensic problem is whether the presence of a drug such as morphine caused or contributed to a death or was merely incidental. The reliance that can be based on postmortem drug concentrations remains controversial. To investigate this further we obtained antemortem and postmortem samples of individuals admitted to hospital who were receiving morphine and who died in hospital.</p> <p>Methods: Eleven Subjects were recruited. Samples were sent for analysis of free and total morphine concentrations. Results: The median difference (postmortem - antemortem) free morphine concentration was 25.5 (range 0 to +126) microg/L, $P < 0.01$; the mean difference between postmortem and antemortem total morphine concentration was 34.5 (range -225 to 342) microg/L (not significant). Discussion: Our study supports previous investigators who note that there is an inconstant and sometimes tenuous relationship between ante- and postmortem morphine concentrations</p>

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The relationship between antemortem and postmortem morphine concentrations

Abstract

Context of the Article: An important forensic problem is whether the presence of a drug such as morphine caused or contributed to a death or was merely incidental. The reliance that can be based on postmortem drug concentrations remains controversial. To investigate this further we obtained antemortem and postmortem samples of individuals admitted to hospital who were receiving morphine and who died in hospital. Methods: Eleven Subjects were recruited. Samples were sent for analysis of free and total morphine concentrations. Results: The median difference (postmortem - antemortem) free morphine concentration was 25.5 (range 0 to +126) microg/L, $P < 0.01$; the mean difference between postmortem and antemortem total morphine concentration was 34.5 (range -225 to 342) microg/L (not significant). Discussion: Our study supports previous investigators who note that there is an inconstant and sometimes tenuous relationship between ante- and postmortem morphine concentrations

Key Words

Morphine, Postmortem examination, Postmortem redistribution, Forensic Toxicology

Introduction

Morphine is the standard treatment for severe pain. It is well absorbed orally and subcutaneously. It is eliminated mainly in the form of two major metabolites: inactive morphine-3-glucuronide and active morphine-6-glucuronide.[1] The glucuronides are excreted renally, and their elimination rates depend on age, renal function, and the route of administration. [2, 3]

Opiate drugs, including morphine and heroin (diacetylmorphine, diamorphine) are also widely abused. The Office for National Statistics recorded 155 deaths during 1993 in England and Wales in which morphine or heroin or both were certified as the cause of death; by 2016 the number had increased to 1209. [4] The total number of deaths certified from opioids rose from 444 to 1887 during the same period.

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3 An important forensic problem is whether the presence of a drug such as morphine caused or
4 contributed to a death or was merely incidental. Attempts to deduce the timing and likely
5 impact of morphine or heroin ingestion from concentrations of total drug, free drug,
6 metabolites, and their ratios, have proved difficult. [5, 6, 7] The relationship between the
7 measured concentration postmortem and the likely concentration antemortem is not certain;
8 and the degree to which the deceased was tolerant of the effects of the drug can greatly
9 influence individual responses to a given concentration.
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17 The postmortem concentration of many drugs changes with time after death, and differs from
18 one postmortem sampling site to another. [8, 9, 10, 11, 12] Some have argued that 'From our
19 observations, significant postmortem redistribution of morphine and its metabolites seems
20 unlikely.' [13]. Others have shown significant postmortem distribution in animal models [14,
21 15] We sought to assess the relationship between antemortem and postmortem concentrations
22 of morphine in paired samples taken from patients before and after they died.
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29 Methods

30 Ethics

31 The study received a favourable opinion from the National Research Ethics Services (IRAS
32 192718) and was approved by the Health Research Authority.
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38 Population

39 The study took place in a thousand-bedded teaching hospital caring for adults and children.
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43 Sampling

44 Patients who died in hospital and who had been treated with morphine were identified. They
45 were considered potentially suitable for the study if they had been treated with morphine, and
46 a serum sample had been taken from them within 24 hours before they died and after the last
47 administration of morphine. Consent to use samples for the study was obtained from the
48 patient's relatives. All subjects were transferred to the morgue within 8 hours and usually in
49 less than 2 hours. The deceased were then stored at -4°C until postmortem sampling. Whole
50 unpreserved blood samples were taken from the unligated femoral vein.
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58 Toxicological Analysis

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3 Free morphine quantitation was performed after solid phase extraction by Bond Elut
4 cartridges, using an Agilent® 1200 Series HPLC System fitted with an Agilent® ZORBAX
5 SB-C18, 2.1mm ID x 30mm (3.5µm) column and Agilent® 6460 Triple Quadrupole LC-
6 MS/MS system with an electrospray ionisation (ESI) source in positive ion mode. Total
7 morphine concentration was measured after hydrolysis with β-glucuronidase at 70°C for
8 3 hours.
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15 The assay is validated as per accepted forensic standards. The serum to whole blood ratio has
16 been shown to be 1.03 (relative standard deviation 3.59%) [16, 17, 18] The limit of detection
17 of the method was 1 µg/L and the limit of quantitation was 5 µg/L. The method was linear
18 from 5–1000 µg/L. The precision was < 7% and bias < 5%. There was no significant ion
19 suppression from common drugs or from compounds produced during the postmortem
20 period.
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26 27 Statistical analysis

28 We examined the data using descriptive statistics, and used Wilcoxon's matched pairs
29 signed-rank test to compare antemortem and postmortem concentrations.[19]
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33 34 Results

35 Samples were obtained from 11 patients (six women), mean age 65 ± 15 years, range 37–88
36 years. Antemortem samples were taken a median of 5.75 hours (range 3–10) before death. The
37 postmortem samples were taken a median of 6 days (range 3–12) after death. The demographic
38 data, renal function, doses of opiates, routes of administration, are given in Table 1.
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45 Results for free and total morphine concentration before and after the patient had died are
46 given in Table 2. For all subjects, free morphine concentrations were higher postmortem. The
47 median difference between postmortem and antemortem free morphine concentrations was
48 25.5µg/L (range 0 to 126), P < 0.01. For all but one subject, total morphine concentrations
49 were higher postmortem. The median difference between postmortem and antemortem free
50 morphine concentration was 34.5 µg/L (range -225 to +342) (P>0.05).
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Discussion

Cook *et al* in 2000 published a study of six cases in which they measured antemortem and postmortem concentrations of seven different drugs.[20] They concluded that ‘A large degree of error can arise from attempting to estimate antemortem drug concentrations and the ingested dose from postmortem measurements.’ Tolliver *et al* came to similar conclusions from their study of seven subjects, in which the differences between morphine concentrations measured in antemortem and postmortem samples ranged from -100 µg/L to +1400 µg/L. Their study included subjects who had taken heroin; along with the heterogeneity of the postmortem samples, this hinders interpretation.. [21]. Almost all other studies compare average postmortem concentrations with average therapeutic antemortem concentrations. To our knowledge, the present study is the first to examine paired antemortem and postmortem samples for concentrations of morphine in therapeutic use.

Our subjects were generally older patients with multiple comorbidities including renal impairment: the mean eGFR was 47 mL/min/1.73 m² (range 15 to 90 mL/min/1.73 m²). They had received different doses of morphine prior to their death via a variety of routes. There is no agreed therapeutic range for morphine, as the unconjugated and glucuronides (M6G) morphine can both have clinical effects, and there is such a large variation in the pharmacokinetics and pharmacodynamics of pain relief. The antemortem total morphine concentrations in the samples we examined ranged from 5 µg /L to 611 µg/L.

Our samples were taken sufficiently long after the last administration of morphine that absorption was likely to have been complete. We therefore predicted that, as a consequence of metabolism and elimination, the concentration at the point the patient died would be less than the concentration antemortem. The concentration of free morphine rose significantly from antemortem to postmortem sample (P < 0.01). The median change was 109% (range 0–540%). The finding that the postmortem concentration of free morphine in all but one case exceeded the antemortem concentration was unexpected. One explanation is that free morphine may be generated after death by spontaneous hydrolysis of glucuronides in blood. Skopp and colleagues found that while morphine and its glucuronide metabolites are stable in blood and plasma at 4°C, this is not the case in postmortem whole blood. [22].

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3 There was no statistically significant difference overall between antemortem and postmortem
4 total morphine concentration for our 11 patients, but the median value +56% with a range -
5 83% to +166%. The increases seen in this study are similarly mirrored in other studies.

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8 Hayward et al compared concentrations in two groups of patients, those with trauma and
9 controls who were alive; and a further two groups in whom postmortem samples were
10 obtained. [23]. In both trauma and non-trauma patients, average concentrations were higher
11 in the samples taken from deceased individuals.
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17 As one of us has stated previously, “There is no reliable or obvious connection between
18 concentrations measured in life and subsequent to death. Consequently concentrations
19 measured after death cannot generally be interpreted to yield concentration present before
20 death.” [12] Our study reiterates that message.
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23

24 25 26 Limitations

27 Ours is a comparatively small study of the relationship between morphine concentrations
28 measured before and after death. Antemortem samples were taken some time after the last
29 administration of morphine, but we cannot be sure in dying patients that they were all taken
30 after the maximum concentration had been reached. We have not made pharmacokinetic
31 calculations to extrapolate from the antemortem concentration at the time of sample to the
32 concentration at the time the patient died; the applicable pharmacokinetic parameters are not
33 known for the population we studied. Samples were taken at postmortem, and the time from
34 death to postmortem sampling was variable. The postmortem samples were taken from the
35 femoral vein, and we had no comparative data from other sampling sites. We cannot therefore
36 present the classic measures of postmortem redistribution such as central-peripheral
37 ratio.[12] These, however, are proxy measures for the change that we examined directly. The
38 antemortem samples were all serum samples, while the postmortem samples were clotted
39 whole blood. However the overall difference between the two samples is probably small, as
40 drug concentrations are measured in the chemically extracted “liquid” component. Total
41 morphine concentration was measured by hydrolysis, so that it was not possible to measure
42 individual glucuronide concentrations. These may have yielded more information on the
43 changes we observed.
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58 59 60 Conclusion

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3 It is important in determining cause of death to take into account the clinical features of the
4 case, the inherent uncertainties in estimating antemortem concentrations from postmortem
5 concentrations, and the possible competing causes of death. Our study demonstrates that there
6 is a marked and significant increase in free morphine concentration after death, perhaps as a
7 result of the hydrolysis of morphine glucuronides. While on average total morphine
8 concentration taken from the same subject before and after death did not differ significantly,
9 there was very marked variation among individuals. The postmortem morphine concentration
10 found at autopsy may differ substantially from the antemortem morphine concentration.
11 Therefore, the attribution of cause of death should take into account all the clinical
12 circumstances, and should not be based solely on morphine concentrations determined after
13 death.
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Case	age	sex	eGFR	morphine dose in 24 hours before death (mg)	Interval from sample to death	Route	Free morphine concentration µg/L				Total morphine µg/L			
							AM	PM	Difference PM - AM	% change after death	AM	PM	Difference PM - AM	% change after death
1	88	f	49	48		sc	78	172	94	121	133	261	128	96
2	68	f	30	2.5	12	iv	5	29	24	480	18	41	23	128
3	72	f	19	5	18	po	5	5	0	0	14	12	-2	-14
4	73	m	49	24		sc	43	169	126	293	71	189	118	166
5	59	f	52	15	15	po	32	38	6	19	268	43	-225	-84
6	84	m	18	10	4	po	10	12	2	20	16	19	3	19
7	72	m	80	48		sc	27	111	84	311	238	171	-67	-28
8	56	f	90	140	12	po	40	41	1	3	240	221	-19	-7.9
9	49	m	29	48		sc	54	107	53	98	61	108	47	77
10	57	f	90	24		sc	5	32	27	540	5	36	31	620
11	37	m	15	72		sc	NM	NM	--	--	611	953	342	56
Median	68		49.0	24.0	12.0		29.5	39.5	25.5	109	71.0	108	23.0	32
Mean	65		47.4	39.7	12.2		29.9	71.6	41.7	188	152	186	34.5	23

Table 1 Patient characteristics and results for free and total antemortem and postmortem morphine concentrations

AM = antemortem, PM = postmortem, NM = not measured, m = male, f = female, sc = subcutaneous, po = oral, iv = intravenous