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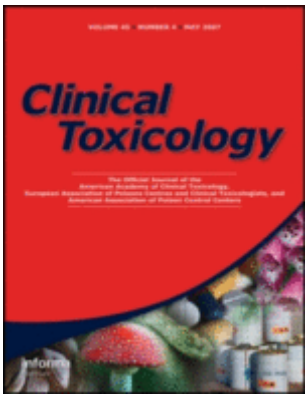

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Key Words

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.

An important forensic problem is whether the presence of a drug such as morphine caused or contributed to a death or was merely incidental. Attempts to deduce the timing and likely impact of morphine or heroin ingestion from concentrations of total drug, free drug, metabolites, and their ratios, have proved difficult. [5, 6, 7] The relationship between the measured concentration postmortem and the likely concentration antemortem is not certain; and the degree to which the deceased was tolerant of the effects of the drug can greatly influence individual responses to a given concentration.

The postmortem concentration of many drugs changes with time after death, and differs from one postmortem sampling site to another. [8, 9, 10, 11, 12] Some have argued that 'From our observations, significant postmortem redistribution of morphine and its metabolites seems unlikely.'[13]. Others have shown significant postmortem distribution in animal models [14, 15] We sought to assess the relationship between antemortem and postmortem concentrations of morphine in paired samples taken from patients before and after they died.

Methods

Ethics

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

Population

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

Sampling

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

Toxicological Analysis

Free morphine quantitation was performed after solid phase extraction by Bond Elut cartridges, using an Agilent® 1200 Series HPLC System fitted with an Agilent® ZORBAX SB-C18, 2.1mm ID x 30mm (3.5µm) column and Agilent® 6460 Triple Quadrupole LC-MS/MS system with an electrospray ionisation (ESI) source in positive ion mode. Total morphine concentration was measured after hydrolysis with β-glucuronidase at 70°C for 3 hours.

The assay is validated as per accepted forensic standards. The serum to whole blood ratio has been shown to be 1.03 (relative standard deviation 3.59%) [16, 17, 18] The limit of detection of the method was 1 µg/L and the limit of quantitation was 5 µg/L. The method was linear from 5–1000 µg/L. The precision was < 7% and bias < 5%. There was no significant ion suppression from common drugs or from compounds produced during the postmortem period.

Statistical analysis

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

Results

Samples were obtained from 11 patients (six women), mean age 65 ± 15 years, range 37–88 years. Antemortem samples were taken a median of 5.75 hours (range 3-10) before death. The postmortem samples were taken a median of 6 days (range 3–12) after death. The demographic data, renal function, doses of opiates, routes of administration, are given in Table 1.

Results for free and total morphine concentration before and after the patient had died are given in Table 2. For all subjects, free morphine concentrations were higher postmortem. The median difference between postmortem and antemortem free morphine concentrations was 25.5µg/L (range 0 to 126), $P < 0.01$. For all but one subject, total morphine concentrations were higher postmortem. The median difference between postmortem and antemortem free morphine concentration was 34.5 µg/L (range -225 to +342) ($P>0.05$).

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 m^2 (range 15 to 90 mL/min/1.73 m^2). 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].

There was no statistically significant difference overall between antemortem and postmortem total morphine concentration for our 11 patients, but the median value +56% with a range -83% to +166%. The increases seen in this study are similarly mirrored in other studies. Hayward et al compared concentrations in two groups of patients, those with trauma and controls who were alive; and a further two groups in whom postmortem samples were obtained. [23]. In both trauma and non-trauma patients, average concentrations were higher in the samples taken from deceased individuals.

As one of us has stated previously, "There is no reliable or obvious connection between concentrations measured in life and subsequent to death. Consequently concentrations measured after death cannot generally be interpreted to yield concentration present before death." [12] Our study reiterates that message.

Limitations

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

Conclusion

It is important in determining cause of death to take into account the clinical features of the case, the inherent uncertainties in estimating antemortem concentrations from postmortem concentrations, and the possible competing causes of death. Our study demonstrates that there is a marked and significant increase in free morphine concentration after death, perhaps as a result of the hydrolysis of morphine glucuronides. While on average total morphine concentration taken from the same subject before and after death did not differ significantly, there was very marked variation among individuals. The postmortem morphine concentration found at autopsy may differ substantially from the antemortem morphine concentration. Therefore, the attribution of cause of death should take into account all the clinical circumstances, and should not be based solely on morphine concentrations determined after death.

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							Free morphine concentration µg/L				Total morphine µg/L			
Case	age	sex	eGFR	morphine dose in 24 hours before death (mg)	Interval from sample to death	Route	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