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The Significance of Morphine Concentrations in the Cerebrospinal Fluid in Morphine Caused Deaths

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ABSTRACT: Morphine analysis was performed using a variety of immunoassay methods in blood, urine and the cisternal cerebrospinal fluid (CSF) from 23 patients dying opiate-related deaths. Of these, 16 were a result of intravenous morphine or heroin use. The blood and CSF morphine concentrations were determined using both fluorescence polarization immunoassay (FPIA) and radioimmunoassay (RIA), while urine was analyzed by enzyme multiplied immunoassay technique (EMIT). Urine morphine concentrations were greater than 0.300 μg/mL in all but one case. Blood and CSF morphine concentrations were found to be poorly correlated, and it was concluded that one should not be used to predict the other. Following intravenous administration, CSF morphine concentrations of greater than 0.02 μg/mL were however found to be consistent with death from morphine related respiratory depression. As intrathecal or epidural administration of morphine can greatly influence the CSF concentration without inducing respiratory depression, the site of collection of the CSF must be known, as must the route of administration in order to properly interpret CSF morphine concentrations.

KEYWORDS: toxicology, morphine, cerebrospinal fluid, immunoassay

Postmortem blood drug concentrations are used to evaluate the role a drug has played in a death. This approach treats the body as a single compartment however, and assumes that the blood drug concentration is reflective of the concentration at the site of action. When death occurs shortly after a massive bolus ingestion or intravenous injection however, distribution may not be complete and the assumptions required for the single compartment approach may be invalid. In addition, as more is learned about postmortem release of drugs from tissue, and the post mortem distribution and redistribution of drugs between body compartments [*1–3*], further doubts about the validity of the assumption arise. Postmortem changes may account for wide ranges and overlaps of therapeutic, toxic and fatal concentrations reported for many drugs [*4–6*].

Most morphine caused deaths are associated with respiratory or cardiorespiratory depression, functions controlled from the hind brain. We measured morphine concentrations in the surrounding cisternal cerebrospinal fluid (CSF) in cases of death resulting from morphine overdose, in order to determine the significance of these values as predictors of death.

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In addition, we compared two immunoassay methods for screening for morphine in blood, urine and CSF, and used radioimmunoassay (RIA) for quantitative determination in blood and CSF.

Materials and Methods

Collection of Spinal Fluid

CSF samples (10 mL) were drawn specifically in cases where opiate related death was suspected. Control samples from 37 other drug of abuse related deaths were also collected. Spinal fluid was drawn with a syringe from a cisternal puncture prior to internal examination during autopsy. The CSF was transferred immediately to a Vacutainer™ tube containing sodium fluoride (25 mg) and potassium oxalate (20 mg). Samples were refrigerated until analysis. CSF samples were generally not tested until the results of blood and/or urine drug screens were known. In cases in which the urine drug screen was positive for opiates or cocaine, the blood and CSF were tested for a variety of drugs including morphine.

Analytical Methods

The analytical methodologies used were enzyme multiplied immunoassay (EMIT), fluorescence polarization immunoassay (FPIA), radioimmunoassay (RIA), identifications of other drugs in these samples were made using liquid/liquid extraction with gas chromatography/mass spectrometry (GCMS). Each procedure is described as follows, with the exception of GCMS which is described in detail elsewhere [*7–9*].

Enzyme Multiplied Immunoassay Technique (EMIT)

Automated EMIT analysis was performed using a Syva ETS®. Drugs of abuse in urine (d.a.u.) reagent kits for opiates were purchased from Syva® also. Negative and low calibrators from Syva®, were used to set up calibration. The low calibrator concentration for the EMIT assay for opiates was 0.300 μg/mL. The opiate assay is calibrated with morphine, but has high crossreactivity with most opiates including codeine, oxycodone, and morphine-6-glucuronide. Due to cross reactivity with other opiates and morphine and heroin metabolites, quantitative EMIT results represent total opiates as opposed to morphine concentrations.

Fluorescence Polarization Immunoassay (FPIA)

Automated FPIA was performed using an Abbott TDX® instrument. Opiate assays were performed using this method, according to the manufacturers protocol. The method was calibrated using standards with a cut-off of 0.05 μg/mL. The opiate assay cross reacts with most opiate drugs, including morphine-6-glucuronide. As with EMIT, due to cross reactivity with other opiates and morphine and heroin metabolites, quantitative FPIA results can be considered to represent total opiates as opposed to morphine concentrations.

Radioimmunoassay (RIA)

RIA for morphine was performed using a quantitative solid phase procedure (Coat-a-count®, Diagnostic Products Corporation). The antibody in this kit is very specific and no significant cross-reactivity is reported. The assay was calibrated over the range 0.0025

to 0.250 μg/mL, and dilutions were made for higher concentrations. Since the specificity of the antibody is very high, the RIA result will reflect only the free (that is, unconjugated) morphine in the sample.

Results

The spinal fluid remained clear, free from deterioration and generally in good condition for some time after death, by which time the blood had clotted, lysed and begun putrefaction. CSF samples showing the presence of particulate matter or cellular debris were centrifuged prior to analysis.

Table 1 shows the results of 23 cases of opiate related death in which blood and/or urine drug tests revealed the presence of opiates. Of these 23 cases, the cause of death was unknown in two cases, three were codeine/polypharmacy overdoses, one an oxycodone overdose, one a delayed heroin related death, and sixteen were acute heroin or morphine overdoses.

Morphine was detected in the CSF and the urine in all cases. Anecdotal experience and reports [*10*] have shown that occasionally overdose levels of morphine can be found in the blood, when the morphine level in the urine is low or less than the assay cut-off. These cases are consistently ones in which the subject is found with a needle in place, or in the immediate vicinity, suggesting that death followed very shortly after administration. Subject 1 in our cases showed a urine morphine concentration of less than 0.300 μg/mL, and also had the lowest CSF morphine concentration, suggesting death followed rapidly after administration and prior to complete distribution of the drug. Circumstances at the scene in this case were consistent with death concurrent with administration.

CSF morphine concentrations even in acute overdoses were generally less than 0.300 μg/mL, which was our standard cut-off concentration for urine EMIT testing. EMIT testing under these conditions was therefore of little practical use in detecting the morphine present in the CSF, with only three of the sixteen morphine overdose cases giving positive (>0.300 μg/mL) results.

In general, there was good agreement between the FPIA and EMIT testing with respect to whether the CSF opiate concentration was above or below 0.300 μg/mL. Inspection of the actual instrument readings shows that EMIT would have been as effective as FPIA in establishing the presence or absence of opiates in the CSF if the EMIT assay had been calibrated at a lower concentration.

In light of the apparently low CSF/blood partitioning for morphine, FPIA was conducted using a much lower cut-off (0.050 μg/mL). Under these conditions, FPIA was effective at detecting morphine in CSF in all cases where it was also present in the blood. Neither in the opiate related deaths, nor in any of the 37 control samples was morphine found in the CSF and not in the blood. In addition to morphine, other compounds particularly codeine, oxycodone, and 6-monoacetyl morphine all gave positive results with FPIA. Additional 37 cases tested there were no apparent non-opiate false positives using this cut-off concentration.

RIA for opiates was performed on the blood and CSF, using a cut-off of 0.005 μg/mL. The RIA antibody is reportedly much more specific than the EMIT or FPIA antibodies, and this is borne out in these results. However, due to the less specific nature of the FPIA antibodies, in particular their cross reactivity with morphine glucuronides, FPIA was a more sensitive screening test for total morphine in CSF than was RIA.

Considering the sixteen cases of acute morphine overdose, it was found that the mean CSF morphine concentration was 0.062 μg/mL (SD = 0.028, range 0.023 to 0.14, n = 16). The blood morphine concentrations ranged from 0.021 to 0.294 μg/mL, and the CSF/blood ratios ranged from 0.18 to 1.82 with a mean of 0.56 (SD = 0.47, n = 16). Wahba et al. [*11*] have recently reported ratios of CSF/blood ratios for morphine in

TABLE 1—*Analytical and case information from analysis of opiates in urine by EMIT, in CSF by EMIT, FPIA, and RIA, and in blood by RIA.*

Urine opiates (EMIT[a]) (μg/mL)	CSF opiates (EMIT[a]) (μg/mL)	CSF opiates (FPIA) (μg/mL)	CSF morphine (RIA) (μg/mL)	Blood morphine (RIA) (μg/mL)	CSF/blood morphine ratio	Cause of death
1. <0.300	<0.300	...	0.023	0.130	0.18	heroin OD
2. pos.	<0.300	...	0.030	0.021	1.43	heroin OD
3. pos.	<0.300	...	0.034	0.101	0.34	heroin OD
4. pos.	<0.300	0.104	0.048	0.156	0.31	cocaine/heroin OD
5. pos.	<0.300	0.093	0.049	0.294	0.17	heroin OD
6. pos.	<0.300	0.095	0.049	0.105	0.47	heroin OD
7. pos.	<0.300	...	0.052	0.179	0.29	heroin OD
8. pos.	<0.300	...	0.054	0.166	0.33	morphine OD
9. pos.	<0.300	...	0.055	0.120	0.46	heroin OD
10. pos.	pos.	...	0.060	0.320	0.19	heroin OD
11. pos.	<0.300	0.117	0.062	0.189	0.33	heroin OD
12. pos.	<0.300	0.201	0.074	0.150	0.49	heroin OD
13. ...	pos.	...	0.082	0.045	1.82	heroin OD
14. pos.	<0.300	0.130	0.085	0.170	0.50	heroin OD
15. pos.	<0.300	...	0.092	0.130	0.71	heroin OD
16. pos.	pos.	0.152	0.140	0.920	0.63	heroin OD
17. ...	<0.300	0.051	0.007	0.064	—[a]	heroin OD (delayed)
18. pos.	pos.	0.312[c]	0.011	0.027	0.41	multiple drug OD
19. pos.	pos.	2.320[c]	0.009	0.017	0.53	multiple drug OD
20. pos.	pos.	1.900[c]	0.055	0.100	0.55	multiple drug OD/CO
21. pos.	<0.300	0.073[b]	neg.[d]	neg.[d]	...	oxycodone OD
22. ...	<0.300	0.056	0.006	...	...	unknown
23. pos.	<0.300	...	...	...	...	unknown

[a]Delayed death, admission blood, post mortem CSF.
[b]oxycodone.
[c]codeine.
[d]less than 0.0025 μg/mL.
... not performed.

postmortem samples from heroin users, using the same quantitative RIA technique (Coat-a-Count, Diagnostic Products). They found a range of ratios from 0.18 to 1.740, with a mean of 0.56 (SD = 0.47, n = 9), values in excellent agreement with our findings here.

Discussion

Morphine is a polar hydrophilic compound and after oral, intravenous or intramuscular administration, will distribute into all water containing tissues. The CSF is however an enclosed fluid, and entry of all drugs including morphine is controlled by diffusion and secretion of the drug across the endothelial blood/CSF barrier (B1CB), predominantly in the brain [*12,13*]. The nature of these barriers is not well understood, although drug transport is a function primarily of lipophilicity and pKa [*14,15*]. CSF is normally slightly more acidic than blood and might be expected to favor some partition of basic drugs like morphine [*16*].

Within the cerebrospinal compartment there is slow passive cephalad flow of the CSF from the lumbar region, but active flow and rapid recirculation of the intracranial CSF. Morphine concentrations in the cisternal CSF would therefore be temporally in equilibrium with the brain morphine concentration when the direction of flow is from the blood to the CSF. Only minor amounts of drug enter the CSF from the blood elsewhere in the spinal column and consequently morphine concentrations in the lumbar CSF will be less representative of the concentrations at the respiratory centers in the brain, following a single intravenous injection. Following chronic administration of morphine, when pseudo steady state conditions are achieved, morphine concentrations throughout the CSF have been shown to be uniform [*16*]. As a central nervous system (CNS) depressant, morphine acts on the brain, and the respiratory depressant effects are the most common fatal side effect of opiate use. The respiratory center in the brain is situated in the hind brain on the floor of the fourth ventricle [*15*], and the accumulation of significant amounts of morphine in surface tissue in this region are thought to be required for the development of respiratory depression. It has been shown in animal studies that the route of administration is critical in determining the extent to which respiratory depression develops, with intravenous administration producing significant respiratory depression, while intrathecal or intracerebroventricular administration produced little or none [*17*]. Human studies have shown cisternal CSF morphine concentrations in patients receiving morphine epidurally, intrathecally, or intracerebrovestibularly such as postpartum, during spinal surgery, or in treatment of chronic cancer pain, to result in peak CSF morphine concentrations as high as 0.747 and 1.025 μg/mL [*15,18*], often without pronounced respiratory depression.

In spite of the fact that all the cases described in our study had succumbed following intravenous injection of morphine or heroin, there was poor correlation between blood and CSF morphine concentrations, and obvious scatter (Fig. 1). Postmortem blood morphine concentrations can be very high if large amounts are injected immediately prior to death, allowing little time for distribution. As a result, the upper end of the range of blood morphine concentrations in compendia of cases associated with IV drug use can be very high [*4–6*], resulting in confusion as to whether lower blood morphine concentration could be consistent with overdose.

Just as blood morphine concentrations are heavily dependent on the amount of morphine administered immediately prior to death, and the interval between administration and death, so obviously are CSF/blood morphine ratios. Based on the wide range observed for this value in these cases and elsewhere [*11*], we recommend that CSF morphine concentrations not be used to predict quantitative blood morphine concentrations.

We do note however that morphine was detected in the cisternal CSF in all cases where it was detected in the blood, and inspection of the data in Table 1 suggests that

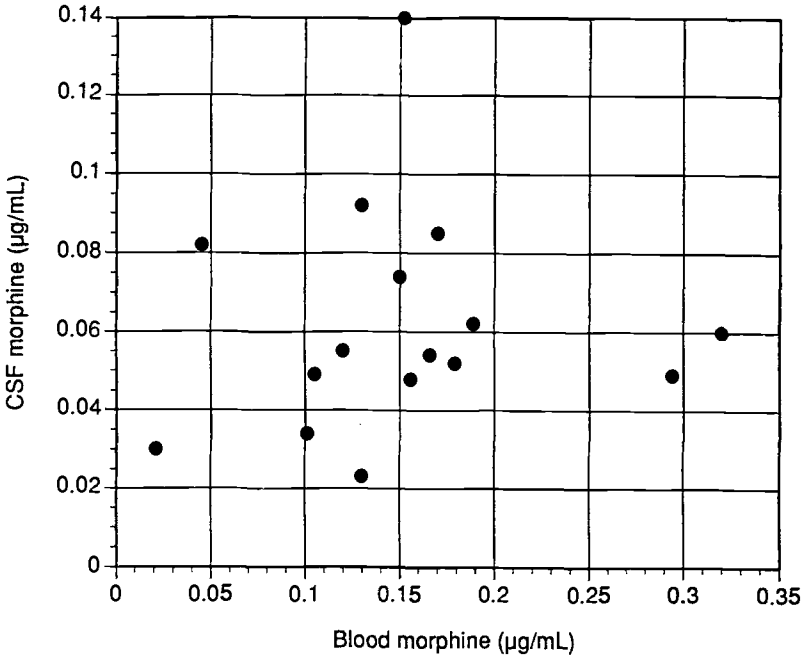

FIG. 1—*Scatterplot showing lack of good correlation between blood and CSF morphine levels in morphine deaths.*

the CSF morphine concentrations themselves may be helpful in determining the role morphine played in death, when the route of administration is known to be other than intrathecal, epidural or intracerebroventricular. In our cases presented here, all those subjects dying from intravenous morphine or heroin overdose had CSF morphine concentrations greater than 0.020 µg/mL, which appear to be associated with brain morphine concentrations sufficient to result in death through respiratory depression.

Other workers have reported [*19*] that following therapeutic intravenous dosing, mean CSF morphine concentrations in patients receiving a single therapeutic dose of morphine to be much lower, of the order of 0.001 to 0.002 µg/L for intramuscular and oral administration respectively. Other patients showed peak cisternal CSF morphine concentrations of 0.016 µg/mL within 2 minutes of a single intravenous therapeutic dose of 79 mg morphine base [*20*]. Patients receiving intracerebroventricular morphine for cancer pain showed essentially uniform lumbar and cisternal CSF morphine concentrations of up to 0.120 µg/mL [*20*]. CSF/blood morphine ratios in these patients were similar to those in our cases. Morphine receptors have been identified in the spine, and analgesia following intrathecal or epidural administration is probably a combination of local and central effects, with relatively little transport of the hydrophilic morphine from the CSF to the lipophilic brain tissue.

Therefore, in contrast to intravenous administration of morphine, when morphine is administrated epidurally or intrathecally, CSF morphine concentrations will be misleadingly high, and the blood morphine concentration will be a better indicator of the equilibrium brain morphine concentration, and therefore of toxicity.

Conclusions

Based on the above considerations, CSF morphine concentrations should be interpreted with care. Following intravenous or oral administration, the presence of morphine in the

CSF arises predominantly from circulation from the brain, and is therefore a good indicator of morphine concentration in the hind brain, particularly at the respiratory center (when CSF is drawn cisternally), and consequently a good indicator of morphine toxicity. When morphine is administered epidurally, intrathecally, or intracerebroventricularly, the majority of the drug is redistributed to the circulatory system with relatively little partitioning directly into the brain, and the CSF morphine concentration is likely to be meaningless in terms of assessing effective brain concentrations.

All the analytical methods evaluated were found to be suitable when calibrated in the expected range for CSF morphine. Although CSF morphine levels were typically half the blood morphine concentration, the CSF/blood morphine ratio extended from 0.10 to 1.82. Blood morphine concentrations could not be accurately predicted from CSF concentrations, however, cisternal CSF morphine concentrations of above 0.02 μg/mL in these IV drug users were consistently associated with death from morphine overdose.

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