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Elevated Morphine Concentrations Determined During Infant Death Investigations: Artifacts of Withdrawal of Care

ABSTRACT: Three cases are reported of elevated postmortem blood morphine concentrations (189–3036 ng/mL) that were observed during the course of death investigations involving three children ranging in age from 1 week to 2 years, all of whom underwent withdrawal of life support. In all three cases, the presence of opiates in postmortem blood was indicated by immunoassay (ELISA) and quantitative confirmatory analysis of free morphine concentrations in postmortem blood was performed by solid-phase extraction followed by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode. While the practice of withdrawing life support from terminally ill patients, with the accompanying administration of narcotics/analgesics has been reported in the medical literature, it has not been adequately described in the forensic literature. The implications of this practice on the forensic toxicological interpretation of morphine findings are discussed. To our knowledge, this is the first report of postmortem morphine concentrations arising directly from administration in conjunction with withdrawal of care in pediatric patients.

KEYWORDS: forensic science, forensic toxicology, morphine, withdrawal of care, postmortem, blood, pediatric

Morphine is a narcotic analgesic that is often used both illicitly and in the treatment of moderate-to-severe pain. The pharmacological properties of morphine and other opioids include analgesia, sedation, nausea, vomiting, pupillary constriction, and respiratory depression, and have been described extensively (1–3). Morphine has also found widespread use accompanying the withdrawal of life support, particularly mechanical ventilation, in both adult and pediatric patients (4–9). Other drugs used in this application include fentanyl (4), midazolam, and lorazepam (7). The clinical objective in these cases is to improve comfort and diminish pain in the patient, provide sedation, reduce anxiety, and to alleviate ''air hunger'' (10). Although this practice has been documented in the medical literature, there is a paucity of forensic literature that makes note of this application. Consequently, in the absence of a detailed case history, a finding of an opioid such as morphine or fentanyl in postmortem fluids or tissues at concentrations that are greater than those expected with therapeutic administration may cause significant alarm in the forensic scientists and other parties involved in the death investigation. To compound this, in the event of a very rapid death subsequent to drug administration and withdrawal of care, the possibility of incomplete drug distribution exists and consequently, very high blood drug concentrations may be observed, depending upon the site of blood collection.

We report herein the results of toxicological analyses obtained as part of the investigation of three deaths of children 2 years of age and younger. The children in question were approximately 1 week, 2 weeks, and 2 years of age, and each underwent withdrawal of life support by mechanical ventilation following unsuccessful treatment attempts. In all of these cases, morphine was detected in postmortem samples (blood and liver) that were submitted to the Centre of Forensic Sciences (CFS) in Toronto, Canada, for toxicological analysis subsequent to autopsy. In cases in Ontario where pediatric patients are on life support and death is imminent, postmortem toxicological analysis is not normally performed. Consequently, these cases provided a unique opportunity for the analysis of postmortem samples from pediatric patients who underwent withdrawal of life support.

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Methods

In Ontario, the investigation of deaths in children 2 years of age and younger involves an extensive toxicological analysis which generally includes a basic drug screen by gas chromatography (GC-NPD) and gas chromatography-mass spectrometry (GC/MS) that detects approximately 150 basic drugs and drug metabolites; analysis for alcohols and other associated volatiles (ethanol, methanol, acetaldehyde, acetone, isopropanol, and *n*-propanol) by headspace gas chromatography; an immunoassay screen for drugs of abuse (cocaine metabolite, barbiturates, cannabinoid metabolites, opioids) as well as for acetaminophen and salicylates; and a benzodiazepine screen that detects the presence of approximately 20 benzodiazepines and related metabolites by LC/MS/MS. The opioids that may be detected in this battery of analyses include morphine, oxycodone, hydromorphone, codeine, hydrocodone, methadone, meperidine, normeperidine, and levorphanol. The basic drug screen by GC-NPD with GC/MS confirmation was adapted from the method of Koves and Wells (11) and was performed as described elsewhere (12). This screen detects the opioids codeine, hydrocodone, methadone, and oxycodone.

Screening for drugs of abuse is performed using Minilyser® automated liquid handling robotics for immunoassay in an ELISA format. ELISA-based assays are of the competitive binding format, using kits (Diagnostix, Inc., Mississauga, ON, Canada; Neogen, Inc., Lexington, KY; Immunalysis Corp., Pamona, CA) designed

for the detection of acetaminophen, barbiturates, cocaine metabolite, cannabinoids, benzodiazepines, opioids, and salicylate. All immunoassay results positive for opioids underwent further quantitative analysis for morphine and hydromorphone by GC/MS.

Quantitative Morphine and Hydromorphone Analysis in Postmortem Samples

Case blood samples were analyzed along with prepared standards at concentrations of 0, 15.8, 31.3, 62.5, 125, 250, and 500 ng/mL (morphine/hydromorphone). Blood samples (1 mL) were combined with deuterated internal standards (morphine and hydromorphone) such that the final concentration for each internal standard was 125 ng/mL. Acetonitrile (3 mL) was then added to each of the blood samples. Each mixture was vortexed and centrifuged at 3800 rpm for 20 min. The supernatant was then decanted and evaporated under a nitrogen stream to approximately 0.5 mL. Phosphate buffer (4 mL, pH 6.0) was then added to each sample. Morphine and hydromorphone then underwent solid phase extraction using EZ-Extract Clean Screen® 200 mg/3 mL extraction columns. Columns were first sequentially conditioned with methanol, water, and phosphate buffer (pH 6). Samples were then loaded onto the columns, followed by sequential rinsing with water, phosphate buffer (pH 4), and methanol. Elution was achieved using a dichloromethane:isopropanol:ammonium hydroxide mixture (78:20:2). Collected samples were evaporated to dryness under nitrogen stream at 65°C. Solid residues were reconstituted in 1.0 mL toluene. Derivatization by acetylation was achieved by the addition of 5 μL acetic anhydride and 2 μL triethylamine to each sample. Each sample was then vortexed briefly, and the acetylation reaction was allowed to proceed by incubation of samples at room temperature for 60 min. The organic layer from each sample was then transferred to a tapered microvial, and evaporated to dryness under nitrogen at 65°C. Residues were then reconstituted in 50 μL toluene and analyzed by GC/MS in selected ion monitoring (SIM) mode. GC/MS analysis was performed using a Hewlett Packard 5890 Series II gas chromatograph equipped with a Hewlett Packard 5989 mass selective detector, single capillary column (DB-5, 30 m length, 0.25 mm I.D.). Ions monitored were m/z 285 and 327 amu, for both morphine and hydromorphone, and m/z 288 and 330 amu for their deuterated analogs. Differentiation between drugs was achieved by means of differences in retention time.

The method used for confirmation of morphine in these cases measured free morphine concentrations in blood by means of solid-phase extraction followed by GC/MS analysis of the acetyl derivative in the SIM mode. There is currently no validated method for the analysis of morphine-3-glucoronide or morphine-6-glucoronide available at the CFS.

Case Histories

Case 1

The deceased was an infant approximately 1 week of age, reported to have been delivered by Caesarean section because of fetal distress. The child was diagnosed with severe hypoxic damage to the brain. Therapeutic administration of phenytoin and phenobarbital within 3 days of birth ensued. It was reported that the child was neurologically unsalvageable, and withdrawal of care was initiated. An initial 5-mg dose of morphine was administered i.v. at the time of extubation. A second 5-mg dose of morphine was administered at approximately 15 min after extubation, and was followed by a 2-mg dose of midazolam. The child was pronounced dead 1 h and 50 min following extubation, after 2 min of absent respirations and heartbeat. Toxicological analyses determined morphine to be present in heart blood at a concentration of 3036 ng/mL, phenobarbital to be present at a concentration of 8.5 mg/L, phenytoin at a concentration of 6.2 mg/L, and midazolam to be detected. Quantitation of midazolam was not performed. There were no other significant toxicological findings.

Case 2

The deceased infant was approximately 2 weeks of age and was reported to have mild acute enteritis. The infant remained in a neonatal intensive care unit (ICU) for 14 days following birth and was subsequently discharged from hospital. The infant was then seen by a physician 2 days later, and was found to be in good health. The following evening, the child was placed to sleep in the supine position. The mother of the infant reported that the child was unresponsive with blood draining from the nose a couple of hours later. Despite successful resuscitation, the child remained unresponsive, had fixed and dilated pupils, and lacked reflexes. Following discussion between the attending physician and parents, it was decided to withdraw therapy. Morphine was administered as part of withdrawal of therapy, with 0.24 mg morphine (0.1 mg/kg) administered i.v. 3 min prior to withdrawal of care. 0.24 mg morphine was again administered i.v. 25 min following withdrawal of care, and a further dose of 0.24 mg morphine was administered i.v. 1 h and 5 min following withdrawal of care at the time of endotracheal tube removal. The child was pronounced dead 2 h and 55 min following withdrawal of care. The cause of death was ruled as sudden unexpected death by undetermined means, with no anatomical or toxicological cause of death established, and possible occurrence of a Sudden Infant Death Syndrome-like (SIDS) event. Toxicological analysis detected morphine in a liver homogenate sample, and was detected in postmortem blood at a concentration of 189 ng/mL. There were no other significant toxicological findings.

Case 3

The deceased infant was approximately 2 years of age and had an extensive surgical and hospitalization history. The child reportedly suffered cardiorespiratory arrest following surgical admission, and did not regain vital signs. Withdrawal of care was undertaken following irreversible brain damage and organ failure. A single 5-mg bolus of morphine was administered palliatively at the time of withdrawal of care, after brain death had been established. The reason cited for morphine administration at that time by the physicians was that the parents wished to hold the child, and, in the experience of the ICU physicians, gasping respiration may occur after extubation. Toxicological examination of submitted postmortem blood determined morphine to be present at a concentration of 1100 ng/mL, as well as traces of lorazepam (i.e., concentration below limit of quantitation, 10 ng/mL). There were no other significant toxicological findings. The toxicological findings in each of the cases are summarized in Table 1.

Discussion

Interpretation of postmortem morphine blood concentrations in infants and neonates presents a particular set of issues for consideration by the toxicologist. Typically, in adults and older children, interpretation of blood morphine concentrations in death investigations requires consideration of potential tolerance as a result of repeated or prolonged drug administration. Infants and neonates

TABLE 1—*Summary of positive toxicological findings in cases 1–3.*

Case	Sample Analyzed	Morphine Concentration (ng/mL)	Other Drugs (concentration)
1	Blood (heart)	3036	Phenobarbital (8.5 mg/L) Phenytoin (6.2 mg/L) Midazolam (detected*)
2	Blood (unspecified source)	189	No significant findings
2	Liver	Detected	No significant findings
3	Blood (unspecified source)	1100	Lorazepam (<10 ng/mL)

*Drug was detected by GC-NPD, and confirmed by GC/MS, but quantitative analysis was not performed.

generally represent an exception to this scenario, as chronic administration of morphine is rare in this population, and any tolerance to opioids that may have developed is likely the result of the clinical administration of these drugs, for which a medical history may be available. Thus, the case history may provide some insight into the extent to which tolerance has developed.

Although the confounding influence of tolerance may be lessened in pediatric fatalities, interpretation of morphine blood concentrations is complicated by the paucity of literature in pediatric subjects describing morphine toxicity and fatalities, with associated blood morphine concentrations. Furthermore, there is no clear distinction between therapeutic and fatal blood morphine concentrations within the existing body of literature. For example, a morphine blood concentration of 128 ng/mL was reported in the overdose of an 8-year-old child following accidental oral ingestion of morphine sulfate (13), while a serum morphine concentration of 94 ng/mL was determined in a single infant fatality of a 7-month-old child, attributed to the rectal administration of morphine *via* suppositories containing 4-mg morphine (15). In therapeutic applications, average steady state morphine concentrations of 167 ng/mL were determined in a study of 31 neonates following therapeutic administration of morphine by constant infusion over 2–4 days. However, it should be noted that all subjects in this study were supported with mechanical ventilation (3). Another study involving 62 postsurgical pediatric patients 0–4 weeks of age who received morphine infused at an average rate of 10 μg/kg/h following a bolus dose of 100 μg/kg for postoperative analgesia, reported measured trough plasma morphine concentrations ranging from approximately 2–90 ng/mL at 6 h following surgery. Although at that time, only 15 of those subjects were breathing spontaneously, there was no statistically significant correlation between morphine plasma concentration and analgesia or the presentation of respiratory depression. In contrast, nonfatal toxicity was reported in a study of 30 cardiac surgery patients ranging from 2- to 570-day old (mean age 155 days) who were administered i.v. morphine postoperatively. Signs of toxicity including respiratory depression were detected in a majority of infants at serum morphine concentrations above 20 ng/mL (14). Finally, generalized seizures were reported in two of 12 infants administered i.v. morphine for postoperative analgesia, in whom serum concentrations of 61 and 90 ng/mL were detected, respectively (15).

Further complications in interpretation may arise in situations of possible morphine administration *via* breastfeeding. Again, there is a paucity of literature available on this topic. In a single case study, a serum concentration of 4 ng/mL was determined in an infant 1 h following commencement of breastfeeding. The breastfeeding mother was administered 30 mg oral morphine the day prior to the study, 5 mg of oral morphine 9 h prior to breastfeeding, and a further 5 mg of oral morphine 3 h prior to breastfeeding (16). While the morphine concentration in the plasma of the infant was not itself alarming, the doses reportedly taken by the mother were consistent with therapeutic use and suggest the possibility that larger plasma concentrations may result in breastfeeding infants of mothers who use morphine in much larger doses for illicit or therapeutic purposes. Based on these data it is clear that the interpretation of blood morphine concentrations in infants and neonates can be complex, and that substantial overlap exists between therapeutic, toxic, and potentially fatal blood concentrations.

At first glance, consideration of the magnitude of morphine concentrations reported in Table 1 and the published data with respect to morphine concentrations in pediatric decedents supports the notion of fatal morphine toxicity. However, a number of studies have been reported in the medical literature describing practices associated with withdrawal of life support (4,6–8,17–20). The use of morphine, alone or in combination with benzodiazepines, has been reported in several studies as a means of making the patient more comfortable in a majority of both adult and pediatric patients (69–89%) following the withdrawal of mechanical ventilation (10,18,19,21). Certainly, the use of drugs that are associated with respiratory depression in conjunction with the withdrawal of mechanical ventilation raises the question as to whether death occurred as a direct result of the administration of the drugs or as the natural sequelae to withdrawal of life support. This issue has been addressed in a number of studies through examination of the relationships between the dose of morphine used and the time interval between dosing, withdrawal of mechanical ventilation, and death. In studies by Chan et al. (6), Rocker et al. (7), Ankrom et al. (8), and Partridge and Wall (4), no significant association between morphine dose and time interval between drug administration and death was observed.

Further complexity in the interpretation of blood morphine concentrations from such cases may arise from the wide variations in the time interval between drug administration and death following withdrawal of ventilation. In consideration of data within and between studies, intervals as short as 1 min (6) and as long as approximately 46 h (20) were reported. Such significant variations in the time interval between drug administration and death would be expected to result in widely varying postmortem morphine blood concentrations from a given dose. In those cases where death occurred rapidly following withdrawal of ventilation, incomplete distribution of drug may result in very large observed blood morphine concentrations, and increase the risk of interpretation as a fatal overdose. In case 3 reported here, the proximity of morphine administration to time of death, coupled with extremely high postmortem morphine blood concentrations, suggests a possible incomplete distribution scenario. Conversely, a significantly delayed death would allow for much lower blood morphine concentrations as a result of continuing metabolism of the drug, which is itself highly variable in pediatric patients and dependent upon age, hepatic, and renal function. In case 2 reported here, the child survived almost 3 h following withdrawal of care, and just over an hour following a final i.v. morphine administration. The child in case 1 also survived for approximately 35 min following a second i.v. morphine administration, and following a total administration of 10 mg morphine. These two neonates, who presumably had no tolerance for morphine, survived for a considerable period of time despite the finding of postmortem morphine blood concentrations that could be presumed fatal under some circumstances. Unfortunately, there have been no data published specifically describing postmortem blood morphine concentrations from cases where morphine was administered following withdrawal of care to provide some range of reference values for

these cases. As such, the data presented here constitutes an important contribution to the groundwork for toxicological interpretation in such cases.

Conclusions

To our knowledge, this is the first report of postmortem toxicological analysis in cases involving withdrawal of life support in the forensic literature, and by extension, the first documentation of resultant postmortem morphine blood concentrations in a pediatric population who underwent withdrawal of mechanical ventilation. The data presented here highlight the possibility of elevated postmortem morphine concentrations in such cases, and underscore the need for a detailed case history in the interpretation of analytical results.

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