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Johan Duflou,[1,2,3] *M.Med.Path. (Forens.), F.R.C.P.A.; Shane Darke,*[4] *Ph.D.; and Jennifer Easson,*[5] *B.Sc. (Hons.)*

Morphine Concentrations in Stomach Contents of Intravenous Opioid Overdose Deaths

ABSTRACT: Death caused by heroin overdose is almost always the result of intravenous injection of the drug in Australia. We briefly describe a case where a heroin overdose was initially thought to be the result of oral ingestion of the drug, primarily as a result of higher concentrations of morphine in stomach contents than in blood. During the subsequent criminal trial and investigation, however, the issue of the entero-hepatic circulation of morphine was raised as a possible reason for the presence of morphine in the stomach contents. In this study, we report on the distribution of opioids in blood, stomach contents, urine, liver, and bile in 29 deaths caused by intravenous heroin overdose. The mean total and free blood morphine concentrations were 0.60 and 0.32 mg/L, respectively, and the mean stomach contents total morphine concentration was 1.16 mg/kg. All cases had detectable morphine in the stomach contents, and 24 of 29 cases (83%) had higher concentrations of total morphine in stomach contents than in blood. The mean total morphine concentration in bile was *c.* 100 times that in blood, and the liver total morphine concentration averaged twice that of blood levels. We conclude that the entero-hepatic circulation of morphine and subsequent reflux of duodenal contents back into the stomach can result in the deposition of morphine in gastric contents. Consequently, the relative levels of opioids in blood and stomach contents cannot be used to determine the site of administration of the drug.

KEYWORDS: forensic science, toxicology, autopsy, morphine, entero-hepatic circulation, site of administration

Accidental heroin overdose deaths in Australia in non-Asian men are almost exclusively caused by intravenous injection of the drug, which is often taken in association with other psychotropic drugs, especially alcohol (1). Occasionally, allegations are made that the deceased was given a "hot shot" of heroin—in other words, the deceased was intentionally administered an excessive amount of the drug for the purposes of killing that person. Such "hot shots" are said to be administered either intravenously, usually by the deceased person but without his knowledge of it being a "hot shot," or orally, as in the form of a spiked drink. To cause death, oral ingestion of heroin requires significantly larger doses of the drug, and is a very unusual method of homicide in Australia. However, it may be the only way that the offender is able to administer the drug to the victim.

In 2001, a known illicit drug user died in Queensland following what was initially thought to be an accidental heroin overdose. Needle puncture marks were noted in the antecubital fossae of the arms, but there had been attempted intravenous access by paramedics during the failed attempted resuscitation, and it was not clear whether these needle puncture marks were the result of administration of heroin or as a consequence of attempted resuscitation. Toxicological analysis revealed a blood-free morphine concentration of 0.3 mg/L, a total blood morphine concentration of 0.5 mg/L, and a blood codeine concentration of 0.1 mg/L. In addition to opioids, low levels of alcohol, methamphetamine and MDMA, and therapeutic levels of venlafaxine were detected in blood at autopsy. As a result of further information received, a detailed examination of the various retained autopsy specimens revealed a higher level of morphine in stomach contents (total morphine 2.6 mg/kg) than in blood (total morphine 0.5 mg/L) and it was concluded that the drug was administered orally. On that basis, a person was charged with murder and the case proceeded to trial. Initially, a number of pathologists and clinical toxicologists gave evidence that the drug was in all likelihood administered orally. The trial was aborted when a toxicologist gave evidence in relation to metabolism of heroin, specifically relating to the entero-hepatic circulation of the drug, where the drug is excreted into the bowel via the bile and then reabsorbed on a number of occasions before being finally excreted via feces (2). It was the opinion of the toxicologist that a higher opioid level in stomach contents than in blood could readily be explained on the basis of the entero-hepatic circulation, and therefore that the deceased could have administered the drug to himself accidentally. Prosecuting authorities concluded there was no prospect of a conviction and the trial was abandoned.

Because opioid levels are very infrequently assayed in stomach contents, there are almost no scientific data from "real world" cases to confirm or refute this conclusion (3,4). Previously conducted animal research is similarly largely unhelpful in answering this question, given the differences in entero-hepatic metabolism of opioids between species (5).

In this paper, we examine a range of tissue specimens usually sampled during autopsy in suspected heroin overdose deaths, for the presence and amount of various drugs in stomach contents, blood, bile, urine, and liver, to determine whether stomach contents concentrations of morphine can be reliably used to demonstrate the route of administration of the drug.

[1]Department of Forensic Medicine, Sydney South West Area Health Service, NSW, Australia.
[2]School of Medical Sciences, University of New South Wales, NSW, Australia.
[3]Department of Pathology, University of Sydney, NSW, Australia.
[4]National Drug and Alcohol Research Centre, University of New South Wales, NSW, Australia.
[5]Division of Analytical Laboratories, Sydney West Area Health Service, Lidcombe, NSW, Australia.

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Methods

Twenty-nine consecutive cases of confirmed heroin overdose where there was death scene evidence of intravenous use (typically syringes combined with a history of prior intravenous drug use) and autopsy features of injecting drug use (typically recent and old needle puncture marks) were selected. It was an inclusion requirement that there be either death scene evidence of intravenous use and/or autopsy features of injecting drug use.

As part of the medicolegal autopsy in cases of suspected heroin overdose, the standard protocol at the Department of Forensic Medicine Glebe is to retain samples of peripheral blood, urine, liver, stomach contents, and bile for toxicological analysis. Generally, the blood, urine, and bile are examined in detail, and stomach contents and liver are kept in reserve in the event of specific issues being raised in relation to the death. In this study, in addition to the routine toxicological analysis, an aliquot of stomach contents and, where available, a sample of liver tissue were analyzed to determine the distribution of heroin and its metabolites in cases of intravenous heroin overdose.

In all cases, a broad-ranging screen for drugs of abuse and other commonly ingested drugs was performed on blood as well as on urine, where the latter was available. Quantification of free and total morphine was performed on blood, and total morphine quantification was performed on stomach contents, urine, liver, and bile. Urine was assayed for the presence of 6 mono-acetyl morphine (6-MAM), the primary metabolite of heroin. Total morphine was measured after conversion to the free form by hydrolysis at pH 5.1 by β-glucuronidase. Liver specimens were minced before weighing aliquots for extraction. The free morphine was extracted from all specimens after the addition of the deuterated morphine analogue (D3-Morphine) to an aliquot of the relevant sample (blood, urine, bile, stomach, or liver). Buffer pH 6.0 was added to the specimens which were mixed and then applied to mixed mode solid phase extraction cartridges. After cleanup, the analytes of interest were eluted with dichloromethane:isopropanol (80:20) containing 2% concentrated ammonia solution. The extracts were analyzed by liquid chromatography-mass spectrometry (LC-MS) (positive electrospray). All blood specimens were analyzed for alcohol by gas chromatography-flame ionization detector. Immunoassay screening was performed on all blood specimens utilizing ELISA for methylated amphetamines, cocaine, benzodiazepines, and opiates. A broad screen was carried out on blood using liquid chromatography-photodiode array where possible or gas chromatography with nitrogen phosphorus detection (GC-NPD). Urine screening was performed by GC-NPD with GC-MS confirmation. 6-MAM was extracted similarly to morphine and GC-MS and LC-MS were used for analysis. The morphine limit of quantitation was 0.05 mg/L (or mg/kg) for blood, bile, urine, stomach, and liver samples. Limit of detection for blood and urine morphine was 0.01–0.02 mg/L.

Ethical approval for the research was obtained from the Sydney South West Area Health Service Human Research Ethics Committee, and permission was granted by the New South Wales State Coroner to perform the study.

Statistical Analyses

For all distributions, means and standard deviations (SD) were reported. For bivariate comparisons of means, *t*-tests were conducted. All analyses were conducted using SPSS for Windows, release 14.0 (6).

Results

The mean age of cases was 35.17 (SD 10.24, range 18–54 years). Full autopsies were conducted in all cases, and the primary cause of death in all cases was opioid toxicity. The mean total and free blood morphine concentrations were 0.60 mg/L (SD 0.40 mg/L, range 0.07–1.60 mg/L) and 0.32 mg/L (SD 0.23 mg/L, range <0.05–0.86 mg/L), respectively. The mean stomach contents total morphine concentration was 1.16 mg/kg (SD 0.71 mg/kg, range 0.27–3.30 mg/kg). All cases had detectable morphine in the stomach contents. Twenty four of 29 cases (83%) had higher concentrations of total morphine in stomach contents than in blood. In only four cases (14%), there were higher concentrations of total morphine in blood than in stomach contents (Table 1).

The mean total morphine concentration in bile was *c.* 100 times that in blood (0.60 vs. 53.70 mg/L, $t_{28} = 7.5$, $p < 0.001$), and the liver total morphine concentration averaged twice that of blood levels (mean 1.27 mg/L vs. 0.60 mg/L, $t_{28} = 2.5$, $p < 0.05$). 6-MAM, the primary metabolite of heroin was detected in 16 of 28 (57%) of urine samples.

In all but one case (97%), drugs other than heroin/morphine were also detected. Alcohol was detected in blood in 14 cases (48%), with a mean concentration of 0.08 g/100 mL (SD 0.08, range 0.01–0.23 g/100 mL) among alcohol positive cases. Other drugs detected were: benzodiazepines (55%), antidepressants (21%), cocaine (17%), methamphetamine (10%), and methadone (7%).

Discussion

Identification of route of administration of a drug or poison at autopsy is important because there can be important medicolegal ramifications, as was demonstrated in the case discussed above. The current study shows that stomach morphine levels cannot be relied upon to determine whether heroin had been orally ingested or injected intravenously. It should be noted that the demographic and toxicological characteristics of these cases were typical of fatal heroin overdose cases (1,7). In many cases, in this study, there appears to be strong evidence of duodeno-gastric reflux, with resultant high levels of morphine in stomach contents. Given the large number of drugs and poisons which undergo entero-hepatic circulation, it would therefore appear prudent not to make comment on route of administration of such drugs unless definite evidence of oral ingestion of the drug can be obtained, for example through visualization of appropriate pill fragments.

Although toxicological testing of a variety of autopsy specimens is performed as a routine in opioid-related deaths, there are surprisingly few studies which detail the distribution of these drugs in the various substrates. Moriya et al. (4), in a single case report of death following intravenous injection of heroin, detail the distribution of heroin metabolites in a range of body fluids and organs, including blood, urine, liver, and stomach contents. In their reported case, the femoral blood free and total morphine concentrations were 1.35 and 1.57 mg/L, respectively, and the stomach contents total morphine concentration was 5.2 mg/kg, compared with mean levels in the present study of 29 cases of 0.32 mg/L free morphine and 0.60 mg/L total morphine in blood and 1.16 mg/kg total morphine in stomach contents. Kerrigan et al. (8) report on a single case, where a patient who died of pancreatic cancer had been administered intravenous morphine through a continuous infusion pump. They reported free morphine concentrations in heart blood and stomach contents of 96 and 82 mg/L, respectively, and the total morphine concentrations in heart blood and stomach contents were 421 and 325 mg/L, respectively. Both these studies clearly demonstrate that the contents of the stomach can contain morphine following intravenous administration of opioid, and in the Moriya study there is a 3.3 times higher concentration of morphine in stomach contents than in blood (4). In the present study, the mean stomach contents total morphine concentration was almost double the mean blood total morphine concentration.

TABLE 1—*Case details and toxicological findings in study population.*

Case	Age	Gender	Blood (mg/L)		Bile (mg/L)	Urine (mg/L)		Liver (mg/kg)	Stomach (mg/kg)	Blood	Other Drugs
			Morphine (total)	Morphine (free)	Total Morphine	Total Morphine	MAM	Total Morphine	Total Morphine	Alcohol g/100 mL	
1	23	M	0.88	0.46	110	17	Detected	2.6	0.66	ND	Methylamphetamine, Quetiapine, Diazepam
2	51	F	0.47	0.37	26	NS	NS	1.4	1.30	ND	ND
3	18	M	0.83	0.19	8.9	24	Detected	0.23	1.10	ND	Oxazepam, Promethazine
4	34	M	1.40	0.73	3.5	0.47	Detected	NS	2.40	0.084	ND
5	51	M	0.55	0.25	45	0.42	ND	1.7	0.44	0.008	7-amino Nitrazepam, Diazepam
6	54	M	0.40	0.10	NS	5.10	Detected	0.71	0.94	0.013	Sertraline
7	39	M	0.20	0.19	1.2	0.19	Detected	0.94	0.27	0.211	ND
8	36	M	1.60	0.84	63	8.8	Detected	3.9	1.20	ND	Cocaine & metabs
9	27	M	0.32	0.21	78	NS	NS	1.1	0.72	0.042	Diazepam, Paracetamol
10	24	F	0.38	0.37	20	3.7	Detected	NS	1.10	ND	Alprazolam, Oxazepam
11	28	M	0.73	0.64	3	0.08	ND	3.5	2.30	ND	Diazepam
12	46	M	0.87	0.65	200	57	Detected	4.8	3.30	0.009	Cocaine & metabs, Diazepam, Temazepam
13	26	M	0.30	0.05	85	22	Detected	0.33	0.32	0.006	ND
14	45	F	1.40	0.17	330	>20	Detected	1.5	1.80	ND	Cocaine & metabs, Olanzapine, Paroxetine
15	41	M	0.30	0.10	24	14	ND	NS	0.71	0.146	Citalopram, Diazepam, Oxazepam
16	38	M	0.39	0.17	NS	0.15	ND	NS	1.70	0.229	Cocaine & metabs
17	39	M	0.10	0.10	1.1	0.39	Detected	0.71	0.38	0.082	Citalopram, Methylamphetamine
18	29	F	1.20	0.86	6.6	2.5	ND	NS	1.20	ND	Methadone
19	26	M	0.56	0.39	3.8	5.6	Detected	1.2	1.70	0.124	Diazepam, Lignocaine, Metoclopramide, Paracetamol
20	25	M	0.07	<0.05	50	4.8	ND	NS	0.70	ND	Cocaine & metabs, Diazepam, Methadone
21	44	M	0.94	0.24	260	21	ND	NS	0.83	ND	Olanzapine, Diazepam
22	45	M	0.78	0.35	15	1.6	Detected	NS	1.80	ND	Promethazine
23	28	M	0.45	<0.05	99	1.5	ND	0.54	0.68	0.013	Citalopram, Diazepam, Paracetamol, Doxylamine
24	30	M	0.55	0.45	3	0.20	Detected	5.7	1.90	0.03	Diazepam
25	23	M	0.50	0.30	110	2.6	ND	1.4	1.00	ND	Cocaine & metabs, Venlafaxine, Naproxen, Diazepam, Olanzapine, Valproate
26	49	M	0.26	0.26	0.6	0.62	Detected	1.0	1.10	ND	Diazepam, 7-amino Nitrazepam, Temazepam
27	45	M	0.20	0.18	0.36	<0.05	ND	0.65	0.75	0.118	Nevirapine
28	30	F	0.32	0.25	0.63	0.17	ND	1.2	0.67	ND	Methylamphetamine, Olanzapine
29	26	M	0.39	0.24	9.7	1.6	Detected	1.8	0.58	ND	Alprazolam

ND, not detected; NS, no sample.

As illustrated in the case described above, there may be important medicolegal reasons for determining whether heroin was administered orally or parenterally. For many drugs and poisons, a simple way of making this distinction is to analyze the stomach contents and compare the levels of the drug in the stomach to those in blood; a higher stomach contents concentration of the drug would generally be strong supportive evidence for the assertion that the drug or poison was administered orally. Morphine, however, in common with a range of other drugs, undergoes entero-hepatic circulation as part of the metabolism and elimination of the drug (9). The entero-hepatic circulation is a complex mechanism, whereby chemicals that have undergone conjugation reactions in the liver, such as morphine, once in the gastrointestinal tract, may be subject to the action of hydrolytic enzymes that deconjugate the molecule. Deconjugation results in increased lipophilicity of the molecule and renders it once again subject to passive uptake. Reabsorbed morphine then enters the circulation via the hepatic portal vein, returning to the liver where the molecule can be biotransformed again and re-eliminated. Morphine may undergo several cycles of entero-hepatic circulation resulting in a significant increase in the retention time and its consequent duration of action (2,5). Not only is there pronounced variation in the effects of the entero-hepatic circulation between species, but there also appears to be variation between individuals and at different times in the same individual (5), adding to the difficulties associated with attempting to calculate the dose of opioid administered and its duration of action in an individual case.

Excreted conjugated morphine is found at high concentrations in bile, with a previously described average bile to blood concentration ratio of over 150 (10), and a ratio of 100:1 in the current study. The common bile duct empties bile and conjugated morphine into the duodenum, and from there the majority of biliary excretion products pass down into the distal small bowel. A limited quantity of bile also flows retrograde into the proximal duodenum to the region adjacent to the pylorus. The pyloric sphincter during life is a poor barrier to duodeno-gastric reflux, and reflux of duodenal contents into the stomach cavity is a normal phenomenon both following meals and in the fasting state (11). It can therefore be expected that either in the agonal phase or following death that small quantities of bile containing high concentrations of morphine could readily reflux from the duodenum into the stomach. Bile can also be detected in the stomach following meals in many cases, indicating not only the presence of duodeno-gastric reflux from the proximal duodenum but also the retrograde movement of bile into the stomach (11). If such bile contains excreted morphine at high concentrations, this would explain the presence of significant concentrations of morphine in the stomach contents of intravenous heroin users.

In the current study, morphine was detected in the stomach contents in all cases, and in 83% of cases the stomach morphine level was higher than that in blood. There were no obvious differences between these cases and the minority in which blood morphine concentrations were higher than or equal to stomach concentrations. The results indicate that reflux of morphine from the duodenum into the stomach appears to be the norm, at least after death. Also, it is likely that the gastro-duodenal sphincter is at best an incomplete barrier in death, and is probably quite ineffective in preventing reflux of morphine-rich semi-digested material from the duodenum back into the stomach, had it not occurred during life. Furthermore, even if the gall bladder had been removed surgically at some prior time, stomach morphine concentrations can still be higher than the blood total morphine levels, as illustrated in case 6 (the only such case). In a further two cases (cases 27 and 28), for reasons which were unclear, the bile morphine concentration was lower than the stomach morphine concentration, reflecting a dynamic process and consequent even greater difficulty in providing any firm opinions in a court setting.

As in all studies, caveats must be borne in mind. As far as could be ascertained from the death scene investigation and the autopsy findings, all cases in this study died following intravenous administration of opioids. We cannot exclude, however, as a remote possibility that all or some of the drug was taken orally in any of these cases. Also, 6-MAM measurement was only performed in urine, and total and free morphine quantification was only reported for blood, consistent with the protocols of the toxicology laboratory. Stomach contents is frequently not a homogenous medium, with semi-digested food and other material having the potential to provide spuriously high or low concentrations in aliquots relative to the overall stomach contents. However, we have shown that stomach contents morphine level in our cases is higher than blood morphine levels in 83% of cases, and that morphine was found in stomach contents in every case. This would indicate to us that the phenomenon of reflux is the norm in opioid-related deaths. We recommend that detailed further toxicological analysis of the various specimen types might provide further information on the mechanisms for the high stomach morphine concentrations and may answer questions in relation to the possible reflux and other effects of the various coadministered drugs.

Conclusion

The current study demonstrated that stomach morphine levels cannot be relied upon to determine whether heroin had been orally or intravenously administered. Given the large number of drugs and poisons which undergo entero-hepatic circulation, it would appear prudent to not make comment on route of administration of such drugs unless definite evidence of oral ingestion of the drug can be obtained, for example through visualization of appropriate pill fragments.

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Additional information—reprints not available from author:
Johan Duflou, M.Med.Path.
Associate Professor
Department of Forensic Medicine
PO Box 90
Glebe NSW 2037
Australia
E-mail: jo.duflou@sswahs.nsw.gov.au