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TECHNICAL NOTE

Pamela C. McDonough,[1] B.S.; Barry Levine,[1,2] Ph.D.; Shawn Vorce,[1] B.S.; Rebecca A. Jufer,[2] Ph.D.; and David Fowler,[2] M.D.

The Detection of Hydromorphone in Urine Specimens with High Morphine Concentrations*

ABSTRACT: A previous study suggested that small amounts of morphine are metabolically converted to hydromorphone. In the present study, morphine positive urine specimens obtained from a postmortem laboratory and a random urinalysis program were tested for morphine, codeine, hydromorphone, hydrocodone, oxymorphone, and oxycodone to assess the possibility that small amounts of hydromorphone are produced from the metabolism of morphine. The opioids were analyzed by gas chromatography–mass spectrometry as their respective trimethylsilyl derivatives following solid phase extraction. The limit of detection for hydromorphone was 5 ng/mL. A total of 73 morphine positive urine specimens were analyzed, with morphine concentrations ranging from 131 to 297,000 ng/mL. Hydromorphone was present at a concentration ≥5 ng/mL in 36 of these specimens at concentrations ranging from 0.02% to 12% of the morphine concentration. Hydrocodone was not detected in these specimens at the assay detection limit of 25 ng/mL. These results support earlier work suggesting that the detection of hydromorphone in urine specimens does not necessarily mean that exogenous hydromorphone or hydrocodone was used.

KEYWORDS: forensic science, toxicology, urine drug testing, hydromorphone, morphine, metabolism, opiates

Heroin remains one of the most widely abused opioids in the United States. Heroin itself is a prodrug, as it has little affinity for the opioid receptor in the brain. Its greater analgesic potency relative to morphine is due to its increased ability to cross the blood brain barrier.

Heroin is metabolized within minutes to 6-acetylmorphine and then to morphine. The major metabolic route of morphine is well established, as it undergoes phase II metabolism to morphine-3-glucuronide and morphine-6-glucuronide. Nevertheless, as analytical methodologies increase in sensitivity, minor metabolic products of drugs become more likely to be detected. In 2006, Cone et al. (1) reported evidence that a small amount of morphine is converted to hydromorphone. Hydromorphone is a hydrogenated ketone derivative of morphine and is available as a drug itself. In a precursor study, Oyler et al. (2) reported the identification of hydrocodone in urine following controlled codeine administration. Hydrocodone is the 6-ketone derivative of codeine and this metabolism is analogous to the conversion of morphine to hydromorphone.

In this study, urine specimens obtained from a postmortem laboratory and a random urinalysis program were analyzed for morphine, codeine, hydromorphone, hydrocodone, oxymorphone, and oxycodone by gas chromatography/mass spectrometry. The purpose of the study was to assess the possibility that small amounts of hydromorphone are produced from the metabolism of morphine. As a result, the detection of hydromorphone does not necessarily mean that exogenous hydromorphone or hydrocodone was used.

[1]Division of Forensic Toxicology, Office of the Armed Forces Medical Examiner, 1413 Research Blvd, Bldg 102, Rockville, MD 20850.
[2]Office of the Chief Medical Examiner, 111 Penn Street, Baltimore, MD 21201.

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Experimental

Urine specimens were collected from cases investigated by the Office of the Chief Medical Examiner, State of Maryland, and from the Fort Meade Drug Testing Laboratory. All specimens had already screened positive for opiates and confirmed for morphine by GC/MS, prior to receiving.

Chemicals and Reagents

All solvents were high-performance liquid chromatography (HPLC) grade and were purchased from Fisher Scientific (Pittsburgh, PA). Glacial acetic acid, concentrated ammonium hydroxide, concentrated hydrochloric acid, concentrated potassium hydroxide, and hydroxylamine hydrochloride (99%) were also purchased from Fisher Scientific. Certified reference standards of codeine, morphine, hydrocodone, hydromorphone, oxymorphone, oxycodone, and their respective deuterated analogs were obtained from Cerilliant (Round Rock, TX). Bis(trimethylsilyl)triflouroacetadmide (BSTFA) with 1% trimethylchlorosilane (TMS) was purchased from Sigma Chemical (St. Louis, MO). Clean Screen™ (ZCDAU020) 10 mL solid phase extraction columns were purchased from United Chemical Technologies (Bristol, PA).

Sample Preparation and Extraction

Initial Opiate Quantitation—An opiate assay using a single-point calibrator and three controls was utilized to quantitate morphine, codeine, hydrocodone, oxycodone, and oxymorphone. Annual method validation shows the assay has a linear range of 50–3000 ng/mL with correlation coefficients >0.99. The limit of detection (LOD) and limit of quantitation (LOQ) determined at 25 and 50 ng/mL, respectively. The inter- and intra-day coefficient of variance (CV) for all analytes were <3.4%, and all analytes had

<11.5% difference between theoretical and measured values. Urine samples (3 mL) were prepared by the addition of 100 μL of 0.01 mg/mL deuterated internal standard solution and 250 μL of concentrated hydrochloric acid for hydrolysis. The samples were autoclaved at 130°C at 15 psi for 30 min. The samples were cooled and neutralized with 2 mL of 0.3 M pH 6 phosphate buffer and 200 μL of concentrated KOH. The samples were converted to their oxime derivatives by the addition of 500 μL of 10% (w/v) hydroxylamine solution, incubated for 15 min at 70°C. The SPE columns were conditioned with 3 mL methanol, 3 mL deionized water, and 2 mL 0.1 M pH 6 phosphate buffer successively. The samples were applied and allowed to pass through the columns gravimetrically. The columns were washed with 2 mL deionized water, 2 mL 0.1 M acetic acid, and 2 mL of methanol. The columns were dried under about 25 psi of nitrogen for 10 min. The analytes were eluted with 3 mL of 2% NH_4OH in ethyl acetate and evaporated to dryness under nitrogen at 55°C. The opiates were reconstituted in 100 μL of acetonitrile, derivatized with 25 μL of BSTFA with 1% TMCS, and incubated for 20 min at 70°C. The samples were transferred into glass autosampler vials with 250 μL conical inserts. Any samples containing morphine concentrations greater than the assay's upper limit of linearity (LOL) (3000 ng/mL) were repeated with the necessary dilution.

Hydromorphone Quantitation—A separate 3 mL aliquot of sample was required for the quantitation of hydromorphone to detect lower concentrations of the analyte. A five-point calibration curve and two controls were prepared using a certified reference standard. Method validation results show the assay is linear from 10–500 ng/mL with a correlation coefficient above 0.999. The calibrator concentrations were 10, 25, 50, 75, and 100 ng/mL, 5 and 10 ng/mL established as the LOD and LOQ, respectively. The inter- and intra-day CV for all analytes were <6.1%, and all analytes had <11.9% difference between theoretical and measured values. An internal standard solution was prepared at 0.01 mg/mL of hydromorphone-d_3 in methanol and 50 μL was added to the samples. The same procedure used for opiate analysis was used for the extraction of hydromorphone with a small variance in derivatization. The samples were reconstituted in 50 μL of acetonitrile and derivatized with 25 μL of BSTFA with 1% TMCS for a more concentrated extract.

Instrumental Analysis

Initial Opiate Quantitation—Samples were analyzed using an Agilent 6890 gas chromatograph with a 5973N mass selective detector. The analytes were separated with a J&W DB-1MS 30 m × 0.25 mm × 0.25 μm capillary column. For the analysis of morphine, codeine, hydrocodone, oxycodone, and oxymorphone, 2 μL of sample was injected at 250°C operating in pulsed split mode. A split injector with a 10:1 ratio was used with a 35.0 psi pulse pressure for 0.70 min, and a split flow of 9.5 mL/min. The GC oven was initially set at 100°C, then ramped to 250°C at 18°C per minute and then to 300°C at 10°C per minute, holding for 2 min. The injections were run in select ion monitoring mode (SIM), using the ions listed in Table 1.

Hydromorphone Quantitation

The hydromorphone quantitation was conducted on the same instrument using the same column. Small adjustments to the data acquisition parameters were made to detect lower quantities of the analyte. The GC oven parameters remained the same; however, a pulsed splitless injection was used, with an injection temperature of 250°C and pulse pressure at 35.0 psi for 0.80 min. The samples were analyzed in SIM mode, using the ions listed in Table 1.

TABLE 1—*Ions used for selected ion monitoring (SIM).*

Analyte	SIM ions
Codeine	371*, 343, 372
Codeine-d_3	374*, 346
Morphine	429*, 430, 401
Morphine-d_3	432*, 417
Hydromorphone	444*, 355, 429
Hydromorphone-d_3	447*, 358
Oxycodone	474*, 475, 401
Oxycodone-d_3	477*, 462
Oxymorphone	532*, 517, 533
Oxymorphone-d_3	535*, 520
Hydrocodone	386*, 297, 387
Hydrocodone-d_3	389, 374

*Ion used for quantitation.

Results

A total of 73 morphine-positive urine specimens were tested. The morphine concentrations ranged from 131 to 297,000 ng/mL. Three specimens were excluded because they were positive for hydrocodone (about 10% of a dose of hydrocodone is metabolized to hydromorphone). Nine other specimens were excluded due to poor deuterated hydromorphone recovery and were unable to be reanalyzed because the sample had been expended.

Certified negative urine was spiked with morphine to a concentration of 300,000 ng/mL and tested for hydromorphone; no hydromorphone was detected.

Twenty-five of the remaining 61 specimens had no detectable hydromorphone at a detection limit of 5 ng/mL. For these cases, the morphine concentrations ranged from 640 to 103,000 ng/mL.

Thirty-six of the remaining 61 specimens had hydromorphone concentrations >5 ng/mL. The concentrations ranged from <10 to 1440 ng/mL. In these specimens, the morphine concentrations ranged from 810 to 297,000 ng/mL.

Table 2 lists the morphine, codeine, and hydromorphone concentrations in the 36 specimens containing hydromorphone. The percentage of hydromorphone to morphine ranged from 0.02% to 12%; however, only six had a ratio ≥2.0%.

Nine of the 36 specimens had codeine concentrations much greater than the morphine concentrations, likely indicating codeine use. In these cases, it is possible that the measured hydromorphone arose from hydrocodone metabolism, as hydrocodone is a minor metabolite of codeine (2).

Discussion

The urine specimens included in this study were collected outside of a controlled setting. As a result, the drug used and the purity of the drugs taken are unknown. Therefore, there are several alternative explanations for the presence of hydromorphone in these cases that must be considered. For instance, one obvious explanation for the presence of hydromorphone in these specimens is that the individual used hydromorphone or hydrocodone, which is metabolized to hydromorphone.

The opiate assay used in the analysis of these specimens tested for hydrocodone at a LOD of 25 ng/mL. There were three specimens in this project that tested positive for hydrocodone and were excluded from further study. It is also possible that hydrocodone

TABLE 2—*Morphine, codeine, and hydromorphone concentrations.*

Sample ID	Morphine (ng/mL)	Hydromorphone (ng/mL)	Codeine (ng/mL)	HMOR/MOR (%)
1	74,200	130	1210	0.18
2	297,000	230	130	0.08
3	30,200	510	720	1.7
5	6700	6.3	0	0.09
7	2570	28	0	1.1
9	30,700	100	290	0.33
10	9610	10	250	0.10
11	31,500	200	850	0.63
12	14,100	160	150	1.1
13	43,000	34	1460	0.08
15	2930	60	39	2.0
16	8750	6.2	260	0.07
17	810	7.3	0	0.90
19	74,100	18	1100	0.02
20	2200	270	0	12
21	14,500	61	190	0.42
23	5200	250	0	4.8
29	81,600	29	1200	0.04
30	1950	13	39	0.67
33	6090	20	33,500	0.33
35	9770	290	0	3.0
36	5950	96	22,200	1.6
40	2180	16	18,000	0.73
41	990	25	0	2.5
43	6320	6.4	0	0.10
45	5480	70	35,600	1.3
46	35,600	1440	0	4.0
47	4540	38	36,300	0.84
48	6560	27	50,100	0.41
50	1060	12	22,200	1.1
51	5860	56	45,900	0.96
53	60,500	380	0	0.63
61	5430	130	0	2.4
67	7440	15	20,200	0.20
71	45,000	18	0	0.04
73	6400	41	0	0.64

HMOR, hydromorphone; MOR, morphine.

was present in some of these specimens at concentrations <25 ng/mL which in turn could cause the detection of hydromorphone at concentrations around the limits used for hydromorphone. Smith et al. (3) found peak total urine concentrations of hydromorphone of 4300 ng/mL following intramuscular administration of 4 mg of hydromorphone. Urine concentrations of total hydromorphone above 50 ng/mL were observed up to 3 days after dosing.

One other explanation for the presence of hydromorphone in these specimens is that hydromorphone is a contaminant of the illicit drugs that were taken. For instance, codeine was found in 64% of these urine specimens. However, acetylcodeine is a known contaminant in heroin and the body converts acetylcodeine to codeine in an analogous process to the deacetylation of heroin to 6-acetylmorphine and then to morphine.

Overall, alkaloid analysis of heroin has identified eight substances (4). Morphine and codeine are both found in opium; acetylation of these compounds produces heroin (diacetylmorphine) and acetylcodeine. Two monoacetylated products of morphine, 3-acetylmorphine and 6-acetylmorphine have also been identified. Noscapine and papaverine are two other alkaloids present in opium. Various ratios of these compounds in addition to the presence of adulterants such as quinine, diphenhydramine, and acetaminophen have been used to ascertain the source of heroin. The authors were unable to find any reports of the presence of hydromorphone in any heroin samples. Therefore, it is unlikely that the hydromorphone detected in these urine specimens came from heroin administration.

The results of this study support earlier work suggesting that the detection of hydromorphone in urine specimens does not necessarily reflect the use of hydrocodone or hydromorphone. However, one would expect to see elevated concentrations of morphine associated with the detection of this "nonexogenous" hydromorphone.

Disclaimer

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Additional information and reprint requests:
Barry Levine, Ph.D.
Office of the Chief Medical Examiner
111 Penn Street
Baltimore, MD 21201
E-mail: blevi001@umaryland.edu