TECHNICAL NOTE

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Concentrations of Unconjugated Morphine, Codeine and 6-Acetylmorphine in Urine Specimens from Suspected Drugged Drivers

REFERENCE: Ceder G, Jones AW. Concentrations of unconjugated morphine, codeine and 6-acetylmorphine in urine specimens from suspected drugged drivers. J Forensic Sci 2002;47(2): 366–368.

ABSTRACT: Concentrations of unconjugated morphine, codeine and 6-acetylmorphine (6-AM), the specific metabolite of heroin, were determined in urine specimens from 339 individuals apprehended for driving under the influence of drugs (DUID) in Sweden. After an initial screening analysis by immunoassay for 5-classes of abused drugs (opiates, cannabinoids, amphetamine analogs, cocaine metabolite and benzodiazepines), all positive specimens were verified by more specific methods. Opiates and other illicit drugs were analyzed by isotope-dilution gas chromatography-mass spectrometry (GC-MS). The limits of quantitation for morphine, codeine and 6-AM in urine were 20 ng/mL. Calibration plots included an upper concentration limit of 1000 ng/mL for each opiate. We identified the heroin metabolite 6-AM in 212 urine specimens (62%) at concentrations ranging from 20 ng/mL to > 1000 ng/mL. The concentration of 6-AM exceeded 1000 ng/mL in 79 cases (37%) and 31 cases (15%) were between 20 and 99 ng/mL. When 6-AM was present in urine the concentration of morphine was above 1000 ng/mL in 196 cases (92%). The concentrations of codeine in these same urine specimens were more evenly distributed with 35% being above 1000 ng/mL and 21% below 100 ng/mL. These results give a clear picture of the concentrations of unconjugated morphine, codeine and 6-acetylmorphine that can be expected in opiate-positive urine specimens from individuals apprehended for DUID after taking heroin.

KEYWORDS: forensic science, drugs of abuse, codeine, DUID, GC-MS, heroin, morphine, opiates, 6-acetylmorphine, urine

Well-known difficulties exist in interpreting the concentrations of drugs of abuse measured in urine in relation to the prevailing blood or plasma concentrations (1–3). Nevertheless, reliable information about the concentrations of drugs excreted in urine is needed when required to interpret toxicological reports and make a statement about the likely time-frame of exposure, which is often necessary in urine-drug testing programs (4,5).

Although information about the concentrations of various drugs and toxins in body fluids can be gleaned from reference books or various tabulations of therapeutic and toxic levels, these compilations have limitations. Such sources of information are secondary and cannot substitute for reading the original articles cited or better still practical experience from routine casework in one's own laboratory. The problem posed by relying on reference books for "expert information" was highlighted in a recent letter to the editor of this journal (6).

The article concerned the concentration of 6-acetylmorphine (6-AM) in urine from a person involved in a multiple vehicle crash, who was subsequently treated with morphine for pain-control of his injuries. A suspicion arose that the man had abused heroin, which was confirmed by finding 6-AM in urine at a concentration of 267 ng/mL. The question posed was whether the concentration of 6-AM in urine was high or low for individuals suspected of DUID after taking heroin.

The use of standardized routines for sampling, transport and storage of body fluids submitted for toxicological analysis together with accurate, precise and specific methods of analysis are essential requirements for boosting confidence in the analytical reports (7). Information about the concentrations of drugs of abuse in body fluids from toxicology reference books is sometimes based on old experimental work using methods of analysis no longer used today. Indeed, the concentrations of drugs of abuse in blood and urine determined by less specific methods such as RIA, GC-EC, and GC-NP need updating. The methodology for measuring drugs of abuse in body fluids has improved considerably over the years and today isotope-dilution gas chromatography-mass spectrometry (GC-MS) or LC-MS are the methods of choice for quantitative analysis of opiates (morphine, codeine, and 6-acetylmorphine) as well as other abused drugs (8,9).

The half-life of 6-AM in blood is only about 10–15 min (10), which makes it very difficult to confirm the presence of this heroin metabolite above the limits of quantitation with current GC-MS methods (~5 ng/g blood) (11). By contrast, 6-AM can be identified in urine for a much longer time (2–8 h) after last use of heroin (4). Although identification of 6-AM in urine gives unequivocal proof of heroin use, at least within 24 h of specimen collection, making a definite statement about time of drug administration or the size of the dose taken is quite a different matter (1–4). What can be said is that the concentration reported was high or low in comparison with a similar population of individuals, whether DUID suspects, opiate addicts entering detoxification, or heroin overdose deaths (2).

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Received 10 July 2001; and in revised form 17 Aug. 2001; accepted 17 Aug. 2001.

The aim of this paper is to provide information about the concentrations of 6-acetylmorphine determined in urine specimens from a well-defined population of DUID suspects apprehended in Sweden. The specimens were not hydrolyzed so the concentrations of unconjugated morphine, codeine, and 6-acetylmorphine were determined by a well-established isotope-dilution GC-MS method involving selected ion monitoring.

Methods

In addition to blood samples, urine specimens were available from a large number of DUID suspects apprehended in Sweden during 1999 and 2000. The blood and urine specimens were submitted for toxicological analysis to the National Laboratory of Forensic Chemistry in Linköping, Sweden. A blood and urine sampling kit for use during DUID investigations was prepared at our laboratory and sent to police forces throughout Sweden. Venous whole blood arrived in two 10 mL Vacutainer tubes (Terumo Europe N.V., Belgium) containing 100 mg NaF and 25 mg potassium oxalate as preservatives. Urine arrived in a 10 mL plastic screw-capped tube that contained 100 mg NaF as preservative. The blood and urine specimens were sent to our laboratory by overnight mail, therefore, 1–3 days elapsed after sampling. The biological specimens were registered on the day of arrival and stored at +4°C until analyzed sometimes up to 3 weeks later depending on any special priority requested and laboratory workload. After an initial screening analysis for 5-classes of abused drugs (opiates, cannabinoids, amphetamine analogs, cocaine metabolites, and benzodiazepines) by EMIT and CEDIA with Hitachi 717, all positive results were verified by more specific methods. Urine specimens were screened as positive for opiates if the concentration exceeded a cut-off limit of 300 ng/mL. Quantitative analysis of opiates entailed making a solid phase extraction with BondElut Certify columns (Varian Inc., Harbor City USA) followed by preparation of trimethylsilyl derivatives with bis-trimethylsilyltrifluoroacetamide (BSTFA) before chromatographic analysis by capillary column GC-MS with deuterium labeled internal standards. The urine samples were not hydrolyzed so the concentrations of unconjugated opiates are reported.

To the urine specimen (1 mL) was added internal standards (deuterium (d_3) labeled analogs of morphine, codeine and 6-AM) purchased from Radian International, Austin Texas and then pH was adjusted to 6.1 by addition of 2 mL 0.2 M phosphate buffer. The opiates were eluted from the BondElut Certify columns with a freshly prepared mixture of dichloromethane:isopropanol:ammonia (80:20:2). After evaporation to dryness under nitrogen gas, a 100 μL mixture of BSTFA and acetonitrile (1:2) was added before heating at 60°C for 30 min. The reaction product was evaporated to dryness under nitrogen and reconstituted with 100 μL ethyl acetate in preparation for GC-MS analysis. Quantitative analysis of the opiates was done with a single injection of 1 μL to a Hewlett-Packard (HP) gas chromatograph 5890II fitted with a HP 5MS capillary column (30 m, 0.25 mm, and 0.25 μm). The temperature program was 150°C (1 min), 35°C per min to 200°C (0.2 min) and then 5°C per min to 285°C (0.1 min). The mass detector was HP 5972A, with electron-impact ionization, and for quantitative and qualitative analysis the following mass fragments were used; morphine m/z ratio 429/432 (qualifying ions 414 and 401), codeine m/z ratio 371/374 (qualifying ions 343 and 234) and 6-AM m/z ratio 399/402 (qualifying ions 340 and 287). A 6-point calibration curve was constructed by analysis of standards containing from 20 to 1000 ng/mL of each of the opiates purchased from Radian International (Austin, Texas). The limit of quantitation (LOQ) for each opiate was 20 ng/mL urine.

Results

From among 339 opiate-positive urine specimens from DUID suspects, 212 (63%) were shown to contain 6-AM above 20 ng/mL (LOQ of the method). Figure 1 shows a frequency distribution plot of 6-AM concentrations in urine. Because of the unusual nature of this distribution, citing a mean value and standard deviation or even median and centiles is not recommended. Instead, what can be said is that 79 cases (37%) had a 6-AM concentration above 1000 ng/mL and in 31 cases (15%) the concentration was between 20 and 99 ng/mL. When 6-AM was present in urine, the concentration of unconjugated morphine exceeded 1000 ng/mL in 196 specimens (92%) with a range from 150 to >1000 ng/mL. There were 74 specimens (35%) with codeine concentrations above 1000 ng/mL urine and 21 cases (10%) were between 30 and 99 ng/mL. In two cases urinary codeine was below LOQ whereas 6-AM was above LOQ. In 18 cases, 6-AM was verified present in urine at a mean concentration of 163 ng/mL (median 90 ng/mL, range 20 to 700 ng/mL) although the concentrations of unconjugated morphine and codeine in blood were below LOQ (5 ng/g).

Discussion

The results reported here will be useful when expert opinions are required about the concentration of 6-AM encountered in urine specimens from DUID suspects. We report the concentrations of unconjugated opiates in urine because the specimens were not hydrolyzed before analysis. However, this presumes that the glucuronide-conjugates remain stable after voiding and during transport and storage at room temperature for one to three days and then further storage in a refrigerator at 4°C until analyzed as much as three to four weeks later. We have no evidence to the contrary and the presence of enzyme inhibitor (NaF) and cold storage of the specimens at the laboratory should prevent microorganisms causing any spontaneous hydrolysis of glucuronide conjugates. Although we have not investigated this issue ourselves, a study by Lin et al. (12) demonstrated good repeatability of results when authentic urine samples were reanalyzed after various periods of storage

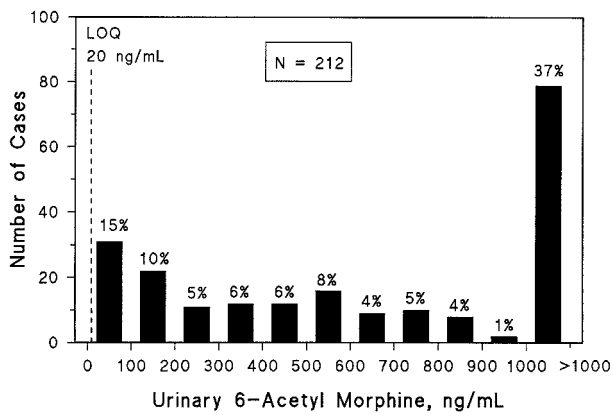

FIG. 1—*Distribution of the concentrations of 6-acetylmorphine (6-AM) in 212 urine specimens from drug impaired drivers determined by isotope-dilution GC-MS. LOQ is limit of quantitation and the highest concentration of 6-AM on the calibration plot was 1000 ng/mL.*

in a refrigerator even without preservatives. Both unconjugated morphine and codeine and their glucuronides were analyzed at intervals for several months after voiding. Moreover, considering the vigorous reaction conditions necessary to cleave glucuronides when analyzing total morphine, e.g., prolonged heating of specimens in strong hydrochloric acid, testifies to the stability of these metabolites (13,14).

In the report by Powers (6), the concentration of 6-AM in urine was 267 ng/mL for the motorist suspected of taking heroin. This concentration of 6-AM is relatively low compared with Swedish DUID suspects, because we found that 70% of heroin cases had concentrations of 6-AM exceeding 267 ng/mL. The use of expert testimony in civil and criminal litigation has been much discussed in the U.S. as exemplified by the Supreme court decision in Daubert vs Merrell Dow Pharmaceuticals (15) The Daubert opinion stressed, among other things, the importance of peer review and publication as criteria for admission of scientific evidence (16,17). As noted by Powers (6) it is crucial that the primary sources of information are checked and carefully evaluated and we echo this strategy. Such things as method of analysis used, number of subjects involved, kind of biological specimens analyzed (whole blood, serum or urine), whether data were derived from controlled pharmacokinetic studies, autopsy reports, patients entering detoxification, or impaired drivers are important to consider (1,2,18). Furthermore, whether the articles were published in peer-reviewed journals is also worthy of note (15–17).

Controlled dosing studies with heroin can help to establish the width of the detection window for identifying 6-AM in blood and urine (4,19–21). In this connection, the recent paper by Staub et al., (22) provides urinary excretion profiles for morphine, codeine and 6-AM after controlled administration of heroin. However the results are a bit confounded by the fact that heroin was administered every 6 h at a time when metabolites were still being excreted from the previous dose. Relating the urinary concentration of drugs to the time of last use is fraught with difficulties and depends on many factors including the dose taken, the frequency of urination, diuresis, and how often the drug was taken. From literature data, it seems that the presence of 6-AM in urine is likely to reflect fairly recent intake of heroin, at least within the previous 24 h (4,20–21). Paul et al. (23) suggested reporting the concentrations of unconjugated opiates in urine to simplify interpretations of results in urine-drug testing programs.

In Sweden, the presence of a scheduled narcotic drug in urine is not sufficient to charge the person with DUID. Therefore, the analysis of 6-AM in urine is important to verify that heroin was used because some DUID suspects maintain that positive findings of morphine and codeine in their blood is from the use of a prescription drug containing codeine (11). Accordingly, we are not so concerned about the exact concentration of 6-AM when this is very high (>1000 ng/mL). Police reports about the actual pattern of driving and the general appearance and behavior of the individual including signs and symptoms of illicit drug use are necessary to judge performance decrement. Results of field-sobriety tests and examination by drug-recognition experts provide a useful compliment to toxicological analysis of body fluids. Taken together with toxicologic findings, this kind of information allows making a statement about the person's ability to drive safely.

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