

CASE REPORT

Giovanna Tassoni, Ph.D.; Claudio Cacaci, M.D.; Massimiliano Zampi, Ph.D.; and Rino Froidi, Ch.D.

Bile Analysis in Heroin Overdose

ABSTRACT: Following its metabolism in the liver, morphine and its metabolites can be directly eliminated in bile. Then, they undergo the enterohepatic cycle (EHC) and mostly reappear in the circulation. We report a case showing the presence of morphine in bile (21.3 µg/mL) and hair (4.8 ng/mg) but not in blood, urine or the liver of an addict who survived in hospital for about 144 h (6 days). These data would indicate that the EHC does not play any role about 144 h after the last injection, and directly confirms that gall bladder is a storage depot for morphine. They constitute the first report of a demonstration of the effect of the EHC on morphine bioavailability in an addict, and could be considered as indication, without supporting circumstantial evidence, that the morphine level in bile is related to chronic opiate use.

KEYWORDS: forensic science, toxicology, heroin death, bile, enterohepatic circle

Biliary excretion is an important route for the elimination of some drugs and drug metabolites in man (1,2). A drug or drug conjugate excreted in bile may be reabsorbed from the gastrointestinal tract. The conjugate may be hydrolyzed by gut bacteria liberating the original drug which can be returned to the general circulation determining an enterohepatic cycle (EHC). The EHC may prolong the pharmacological effect of the drug or drug metabolites (3–5). Furthermore, many of these drugs accumulate in bile, and the gall bladder serves as a collection and storage depot for xenobiotics (6–8). In fact, it has been shown that these drugs have significantly higher concentrations in bile than in blood (7,8). Among the xenobiotics excreted in the bile, there are some drugs of abuse such as opioids (7,9). Bile concentrations of morphine, the main metabolite of heroin, have been shown to be often several-fold higher than in blood (7,8,10). Morphine is mainly metabolized in the liver by conjugation with glucuronic acid to form morphine-6-glucuronide (M6G) and morphine-3-glucuronide (M3G). In humans, about 10–20% of morphine metabolites undergo EHC (11), and both metabolites accumulate in bile to a greater extent (12,13). The EHC, which prolongs the presence of morphine and its metabolites in systemic circulation, complicates their pharmacokinetics and consequently their detection time. In humans, very few studies have analyzed the role of EHC on morphine pharmacokinetics (12). According to some authors, EHC may contribute to the maintenance of blood and tissue levels of morphine and its metabolites in chronic use (14). Moreover, morphine bile results are commonly used to differentiate acute and chronic use of heroin (15–17). Epidemiological studies have evidenced higher bile morphine concentrations in established addicts, while newcomers to the practice or those who had returned after a period of withdrawal showed low values (15), thus indicating that the presence of high values of morphine in bile could be associated with chronic opiate use (18). However, according to some authors, bile could be a poor indicator of chronic opiate use. They state that hair analysis could provide a more accurate measure for determination of chronic opiate consumption (19). We report a case in which a high value of bile

morphine was detected in an established addict who survived about 144 h after hospitalization due to heroin overdose. This case could help us to clarify the role of bile in the presence of chronic use of heroin and give some information about the role of EHC in morphine pharmacokinetics of a chronic user.

Case History

A 39-year-old man known as a regular user of heroin for at least 2 years, arrived in hospital in a coma caused by a cardiocirculatory failure due to heroin overdose, as supported by a witness statement and confirmed by the hospital toxicological analysis. After 5 days, his health gradually deteriorated and on the sixth day of hospitalization the man died. Death was attributed to cardiac arrest.

Materials and Methods

Biological fluid (blood, urine, and bile) and samples of hair and liver were collected during autopsy. A Triage screening was performed on the urine. The urine was negative for opiates. Five gram of liver tissue was crushed and homogenized in distilled water (1:1) and sonicated for 30 min. The hair³nd stored in a plastic bag at room temperature until analyzed. The four most proximal centimeters of the hair were used to analyze the opiate use over the 3 months preceding death. The hair was washed once with distilled water and twice with methanol and cut into small pieces less than 1 mm long. Fifty milligram of the cut hair was weighed in conical vials where 1 mL of 0.1 M hydrochloric acid was added together with internal standard (nalorphine 100 µL). After incubation at 45°C overnight, the sample was neutralized with 2 N sodium hydroxide and buffered with 2 mL of phosphate buffer at pH 7. Then the sample was centrifuged at 3500 × g for 5 min.

One milliliter of hydrochloric acid (37% in water) and 1 mL of nalorphine solution (1 mg/L), as internal standard, were added to the samples (5 mL of blood, 3 mL of bile, and 5 g of homogenized liver). The mixture was incubated at 125°C for 20 min in a 10-mL sealed glass tube and then kept overnight at room temperature. After centrifugation, the solution was alkalized to pH 8.9–9 with sodium hydroxide (25% in water). SPE C-18, 130 mg cartridge for hair and 200 mg for blood and bile and liver from International Sorbent Technology (Tucson, AZ) were used. The columns were

Institute of Legal Medicine, University of Macerata, via Don Minzoni 9, I-62100 Macerata, Italy.

Received 28 Jan. 2007; and in revised form 1 June 2007; accepted 1 June 2007; published ■■■■.

conditioned with 2 mL of methanol followed by 2 mL of 0.1 M phosphate buffer at pH 7. The samples were slowly drawn through the columns under low vacuum (at least 2 min). The columns were washed sequentially with 2 mL of water, 3 mL of 0.1 N HCl, and 2 mL of methanol, and then dried for 5 min under full vacuum. The analytes were eluted twice with 1 mL of a methylene chloride/isopropyl alcohol solution (8:2) with 2% ammonium hydroxide, freshly made daily. The eluate was transferred to 3-mL silanized gradual tubes and evaporated to dryness at 50°C in a temperature bath under a slow stream of nitrogen. The residue was reconstructed with 50 µL of *N*-methyl-*N*-trimethyl-silyl-trifluoroacetamide, incubated for 15 min at 75°C in sealed gradual tubes and 1 µL was injected. All the analyses were performed using a gas chromatograph-mass spectrometry (GC/MS/MS) which consisted of a Fisons 8000 series GC connected to a quadrupole Polaris MS/MS. The following operating conditions were applied: a wall coated open tubular (WCOAT) column (fused silica, 30 m × 0.32 mm) was used and the carrier gas was helium at flow rates of 1.5 mL/min. The transfer line and the source temperature were respectively 250 and 230°C. The oven temperature profile was 100–180°C at 40°C/min, and then ramped at 10°C/minute to a final temperature of 310°C, which was held for 5 min. The injection volume was 1 µL (splitless mode). Electronic impact (70 eV) was used for ionization. Mass spectra were recorded in the range *m/z* 70–500. The ions chosen were *m/z* 429, 414, 401, 324 for morphine-bis-trimethyl-silyl and 455, 414, 440, 324 for nalorphine-bis-TMS.

Results and Discussion

Quantization was carried out by monitoring the abundance of *m/z* 429 ions (for morphine-bis-TMS) and *m/z* 455 ions (for nalorphine-bis-TMS, I.S). To verify the linearity of the detector and to calibrate the method, five calibrations were tested: 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L (morphine in blood and bile and liver previously tested to be negative). Linear regression analysis of the calibration data shows a correlation coefficient of 0.99. The limit of morphine quantization was set at 1 ng/mL with a signal to noise ratio of 15.

The quantitative results of the GC/MS analysis showed that after about 144 h from the injection of heroin there is a presence of morphine in the bile at the value of 21.3 µg/mL, but morphine was absent in the blood and the liver. Morphine was also detected in the hair at the value of 4.8 ng/mg.

Given the current levels of sensitivity, morphine or its metabolites should be detectable up to 48 h after administration in the blood and in the urine (11,20). Longer detection times have rarely been shown. Experiments on humans have shown that after a single administration, morphine is detected in plasma up to 72 h (12,13). This long circulation time of morphine and morphine glucuronide is probably maintained by EHC (11–13). The contribution of EHC to the total bioavailability of morphine is considerable (21), and it is influenced by different factors. One of the most important is the presence or absence of tolerance, which is known to influence the metabolic rate. Until now, it is not known with any certainty just how long it takes an addict, where tolerance is present, to clear morphine from the EHC (11). Lack of reliable information regarding the biliary excretion of drugs in man is partially due to the relative inaccessibility of the human's biliary tract (1). The importance of the EHC on drug pharmacokinetics is usually analyzed using animal models (22,23) or, in humans, it is derived indirectly by detecting the drug level in blood and urine (12,13). As far as we are aware, our results constitute a further contribution to what we know about the effect of the EHC on morphine bioavailability in

an addict. The presence of morphine in bile after a long time from the assumption with the absence of morphine in blood, urine and liver, would indicate that EHC does not play any role about 144 h after the last injection, and confirms directly that gall bladder is a storage depot for morphine. Moreover, the person who died was known to be an addict. The substantial amount of bile morphine found allows us to conclude that a high value of bile morphine can be associated with chronic opiate use (11,15). This statement is supported by the quantity of morphine in the hair observed in frequent or chronic heroin abusers (24,25). Our results could be considered as rare evidence, without circumstantial evidence, supporting the statement that the level of morphine in bile is related to previous opiate use (26).

In conclusion, bile analysis can provide important information about morphine use, especially in cases when morphine is not detected in blood and/or hair, and, for this reason it should be used as a routine specimen in toxicological analysis.

References

1. Rollins DE, Klaassen CD. Biliary excretion of drugs in man. *Clin Pharmacokinet* 1979;4:368–79.
2. Levine WG. Biliary excretion of drugs and other xenobiotics. *Ann Rev Pharmacol Toxicol* 1978;18:81–96.
3. Roberts MS, Magnusson BM, Burcznski FJ, Weiss M. Enterohepatic circulation: physiological, pharmacokinetic and clinical implications. *Clin Pharmacokinet* 2002;41:751–90.
4. Chen HS, Gross FJ. Pharmacokinetics of drugs subject to enterohepatic circulation. *J Pharm Sci* 1979;68:792–4.
5. Horkovics-Kovats S. Efficiency of enterohepatic circulation, its determination and influence on drug bioavailability. *Azneimittelforschung* 1999;49:805–15.
6. Agarwal A, Lemos N. Significance of bile analysis in drug-induced deaths. *J Anal Toxicol* 1996;20:61–3.
7. Vanbinst R, Koenig J, Di Fazio V, Hossoun A. Bile analysis of drugs in postmortem cases. *Forensic Sci Int* 2002;128:35–40.
8. Hepler BR, Sutheimer CA, Sunshine I, Sebrosky GF. Combined enzyme immunoassay-LCEC method for the identification, confirmation and quantitation of opiates in biological fluids. *J Anal Toxicol* 1984;8:78–90.
9. Tacqui A, Kintz P, Ludes B. Buprenorphine-related deaths among drug addicts in France: a report on 20 fatalities. *J Anal Toxicol* 1998;22:430–4.
10. Levine B. Postmortem forensic toxicology. In: Levine B, editor. *Principles of forensic toxicology*, 2nd ed. Washington, DC: AACC Press, 2003;2.
11. Karch SB. Opiates. In: Karch SB, editor. *Karch's pathology of drug abuse*, 3rd ed. Boca Raton, Florida: CRC Press LLC, 2002;3.
12. Hasselstrom J, Sawe J. Morphine pharmacokinetics and metabolism in humans. Enterohepatic cycling and relative contribution of metabolites to active opioid concentrations. *Clin Pharmacokinet* 1993;24:344–54.
13. Westerling D, Persson C, Hoglund P. Plasma concentrations of morphine, morphine-3-glucuronide, and morphine-6-glucuronide after intravenous and oral administration to healthy volunteers: relationship to nonanalgesic actions. *Ther Drug Monit* 1995;17:287–301.
14. Hanks GW, Hoskin PJ, Aherne GW, Turner P, Poulain P. Explanation for potency of repeated oral doses of morphine? *Lancet* 1987;2:723–5.
15. Baselt RC. Morphine. In: Baselt RC, editor. *Disposition of toxic drugs and chemicals in man*, 7th ed. Foster City, CA: Biomedical Publications, 2004;7.
16. Garrick TM, Sheedy D, Abernethy J, Hodda AE, Harper CG. Heroin-related deaths in Sydney, Australia. How common are they?. *Amer J Addiction* 2000;9:172–8.
17. Kalter HD, Ruttember AJ, Zack MM. Temporal clustering of heroin overdoses in Washington, DC. *J Forensic Sci* 1998;34:9–13.
18. Drummer OH. Postmortem toxicology of drugs of abuse. *Forensic Sci Int* 2004;142:101–13.
19. Darke S, Hall W, Kaye S, Ross J, Dufflou J. Hair morphine concentrations of fatal heroin overdose cases and living heroin users. *Addiction* 2002;97:977–85.
20. Spector S, Vessell ES. Disposition of morphine in man. *Science* 1971;174:421–2.

21. Rook EJ, Huitema AD, Van den Brink WV, Ree JM, Beijnen JH. Pharmacokinetics and pharmacokinetic variability of heroin and its metabolites: review of the literature. *Current Clin Pharmacol* 2006;1:109–18.
22. Ouellet DM, Pollack GM. Biliary excretion and enterohepatic circulation of morphine-3-glucuronide in rats. *Drug Metab Dispos* 1995;23:478–84.
23. Othani M, Kotaki H, Uchino K, Sawada Y, Iga T. Pharmacokinetic analysis of enterohepatic circulation of buprenorphine and its active metabolite, norbuprenorphine, in rats. *Drug Metab Disp* 1994;22:2–7.
24. Moeller MR, Fey P, Sachs H. Hair analysis as evidence in forensic cases. *Forensic Sci Int* 1993;63:43–53.
25. Tagliaro F, De Battisti Z, Smith FP, Marigo M. Death from heroin overdose: findings from hair analysis. *Lancet* 1998;27:1923–5.
26. Cravey RH, Reed D. The distribution of morphine in man following chronic intravenous administration. *J Anal Toxicol* 1977;1:166–7.

Additional information and reprint requests:

Rino Froidi, Ch.D.
Institute of Legal Medicine
University of Macerata
via Don Minzoni 9
I-62100 Macerata
Italy
E-mail: froldi@unimc.it