Pethidine Acid: Corroboration of a Doctor’s Denial of Pethidine Re-Use

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Pethidine (meperidine), a synthetic opiate, formally used as an analgesic in surgery and obstetrics, has been an abused drug of choice for some doctors. A case is presented in which a doctor, who previously admitted to using pethidine, was suspected of re-using, following a second positive urine test. A laboratory had reported the presence of pethidine in the doctor’s urine; however, the doctor denied re-use. The norpethidine (normeperidine) metabolite, normally found in urine, had not been detected, raising concern over the laboratory’s conclusion and necessitating an independent investigation. Because the major metabolite of pethidine is pethidinic acid (meperidinic acid), accounting for approximately 40% of the excreted dose, its presence or absence were deemed to be important criteria in interpreting the laboratory result. Pethidinic acid was synthesized by alkaline hydrolysis of pethidine and used as a control. Urine samples from a patient receiving pethidine for pain, from the previous pethidine use of the doctor, and the urine under question plus the control were analyzed for the presence of pethidine acid using electrospray mass spectrometry. Pethidinic acid was found in all samples except the one under dispute. The absence of pethidinic acid appeared to corroborate the doctor’s denial of re-use.

Introduction

Pethidine (meperidine) is a synthetic opiate, first developed as an antispasmodic in 1932. Although traditionally used as an analgesic in surgery and obstetrics, its therapeutic use has declined in recent years, due to many adverse side effects combined with a lack of clinical efficacy over other analgesics (1). The abuse potential of pethidine was reported by Mather and Meflin (2), who likened its effects to those of morphine. Although street addicts rarely use pethidine, it has been a favored drug of choice for some doctors, who have had ready access to this restricted and addictive substance.

A case is presented in which a doctor, who had previously admitted to using pethidine, was suspected of re-using, following a second positive urine test. A laboratory had reported the presence of pethidine in the doctor’s urine; however, the doctor denied any re-use. Norpethidine (normeperidine) metabolite, normally found in urine at an equal or greater concentration than the parent drug, had not been detected, thus raising concern over the laboratory’s conclusion and necessitating an independent investigation. Because the major metabolite of pethidine is pethidinic acid (meperidinic acid) accounting for approximately 40% of the excreted dose (3), its presence or absence were deemed to be important criteria in interpreting the validity of the laboratory result. The metabolism of pethidine is shown in Figure 1.

Methods and Materials

Because pethidinic acid was not commercially available, it was synthesized by the alkaline hydrolysis of pethidine (4, 5). Because it was extremely difficult to obtain pethidine hydrochloride directly from pharmaceutical companies, six ampoules of pethidine hydrochloride (50 mg/mL) were obtained from a hospital pharmacy and freeze-dried. The resultant pethidine was then recrystallized and checked for purity by melting point, Fourier transform infrared spectroscopy (FTIR), mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR). Pethidinic acid was synthesized by refluxing 300 mg of pethidine hydrochloride (1.21 mM) with 5 mL of methanol and 10 mL of 2 M sodium hydroxide for 2 h. After cooling the reaction to room temperature, the solution was washed twice with benzene to remove unreacted pethidine. The solution was then neutralized using 2 M of HCl. The white precipitate (pethidinic acid monohydrate) was filtered and recrystallized from ethanol (mp 86–87°C, yield 58%). 1H-NMR (500 MHz, D2O) δ ppm 2.10 (m, 2H), 2.84 (m, 2H), 2.90 (s, 3H, >N-CH3), 3.18 (m, 2H), 3.63 (m, 2H) and 7.39–7.56 (m, 5H); FTIR (cm−1) 3,485, 3,045, 1,718, 1,632, 1,466, 1,373, 1,227, 1,150 and 985.

As a control, a urine sample was obtained anonymously from a patient receiving pethidine at a hospital pain clinic. The remaining stored urine from the investigated doctor’s previous admitted use and the urine under question were obtained from the original testing laboratory after de-identification. These urine specimens (2 mL), together with blank urine specimens (2 mL), spiked with varying amounts of pethidine, norpethidine or the synthesized pethidinic acid standards, were lyophilized and redissolved in 0.5 mL of methanol; the methanolic extract was mixed with 1.5 mL of water containing 0.5% formic acid for electrospray ionization (ESI)-MS analysis.

ESI-MS

The previously described prepared urine specimens were introduced into a PerkinElmer SCIEX API365 liquid chromatography–tandem mass spectrometry (LC–MS-MS) instrument equipped with a Turbo IonSpray source via infusion through a syringe pump operated at a flow rate of 2.5 mL/h. The MS was run in positive scan mode (m/z 90 to 800) using the Sample Control 1.4 program. Nitrogen, at a flow rate of 1.25 L/min, was used as the nebulizing gas of the Turbo IonSpray and curtain gas. The electrospray ion source voltage was +5,000 V, the source temperature was 300°C, the orifice voltage was +31 V and the ring voltage was +110 V. A total of 10 scans were performed for each analysis, with the dwell time set at 10 ms and the step size at 0.2 amu.

The limit of detection (LOD) for the detection of pethidinic acid in urine was estimated at 50 ng/mL using a signal-to-noise.
ratio of at least 3:1 for the protonated pethidinic acid [M + H]$^+$ at $m/z$ 220.

To investigate the matrix effect, blank urine (2 mL) was lyophilized and extracted in the same manner as described previously. Pethidinic acid standard was then added to the urine extract (urine) or a mixture of 0.5 mL of methanol and 1.5 mL of water containing 0.5% formic acid (solvent) at a concentration of 50 ng/mL (LOD) and 500 ng/mL. ESI-MS analyses of these samples were performed and absolute peak heights of the signal at $m/z$ 220 were compared. The experiments were repeated with blank urine from three different donors. The average peak heights of pethidinic acid in urine were calculated at 114% (at 50 ng/mL) and 103% (at 500 ng/mL) relative to those of the solvent alone. There was a slight ion enhancement effect from the urine matrix at a low concentration of pethidinic acid; this ion enhancement effect became insignificant at a higher analyte concentration.

**Results**

Pethidinic acid was prepared through base hydrolysis of pethidine; its identity (as the monohydrate) was confirmed by melting point determination and spectroscopic analyses including NMR, FTIR, ESI-MS, and gas chromatography (GC)–MS on its methyl ester derivative (data not shown). The synthesized pethidinic acid with [M + 1]$^+$ at $m/z$ 220 is shown in Figure 2A.
Pethidinic acid, together with norpethidine, was found in all samples except the one under dispute. Due to the small volumes of urine remaining from the doctor, no attempt was made to quantify the amount of metabolites found. Protonated molecular ions ([M + H]+) due to pethidine at m/z 248, norpethidine at m/z 234 and pethidinic acid at m/z 220, are shown in Figure 2B for the urine taken from a patient receiving pethidine; Figure 2C shows the sample taken from the doctor’s previously admitted pethidine use. The spectrum of the doctor’s disputed urine is shown in Figure 2D, with missing ions of m/z 248, 234 and 220.

Discussion
Stambaugh et al. (6) reported the relative amounts of pethidine and norpethidine excreted into urine over 48 h following oral administration. They found approximately twice the level of norpethidine to pethidine. Thus, it was reasonable to conclude that a finding of pethidine without norpethidine in urine raised concern over the validity of the laboratory’s finding of pethidine alone.

The original laboratory did not quantitate their results, but merely reported the presence of pethidine. This study’s review of their GC–MS total ion chromatography trace was that the peak purporting to be pethidine was very small and appeared to have a signal-to-noise ratio of 4:1. The retention time and mass spectrum of the peak appeared to match those of the reference standard. Their GC–MS method was based on the injection of urine extract without any derivatization and MS detection in full scan mode (m/z 50–700). The laboratory did not reveal the LOD of their method. Based on the experience of the authors following consideration of their method, the LOD was estimated to be in the range of 50 to 100 ng/mL. The absence of pethidine in the current analysis tends to suggest that the small amount of pethidine reported by the original laboratory may have been a false positive.

Wainer and Stambaugh (7) found that over a 24 h period, pethidinic acid was excreted at a significantly higher concentration than either pethidine or norpethidine when analyzed at 1, 2, 4, 6, 8, 12 and 24 h. Therefore, it was apparent that an absence of pethidinic acid in this doctor’s urine was strong evidence that he had been telling the truth.

In the tested samples, the urine from a patient who had received pethidine had clearly shown the presence of all three metabolites, as did the doctor’s initial urine. The absence of pethidinic acid in the disputed urine allowed the doctor to be given the benefit of the doubt.

It is also worth noting the simplicity of the analytical method used in this study. Due to the unavailability of the LC component of the LC–MS instrument and the urgency of the case at the time of analysis, sample analysis was conducted by MS without LC separation. The satisfactory LOD and the minimum matrix effect observed deem the method suitable for the purpose of this study. Given that the study was not aimed at quantifying the level of pethidinic acid in urine, efforts to synthesize its deuterated counterpart as internal standard were not attempted.

Although pethidine is now rarely used and is probably not a significant drug of abuse due to the difficulty in obtaining it, this study has demonstrated that by testing for an unlikely metabolite, albeit the most commonly excreted one, it is possible to look independently at a laboratory result, especially where there is a dispute. The study further highlights the need for toxicologists to objectively review laboratory results, and if they do not appear to be straightforward, then further investigation must be commenced.

Conclusions
Although pethidine has largely been surpassed as an opiate in pain management and is not usually included in routine drugs-of-abuse testing, there are instances in which it can be abused, particularly by health care professionals who have ready access to the drug. In the presented case, a doctor had previously admitted to abusing pethidine, following a positive urine test. In a second disputed test, the lack of norpethidine in his urine raised doubts as to the laboratory’s conclusions. By looking for the presence or absence of the major metabolite of pethidine in the remaining urine sample, it was possible to substantiate the veracity of either the original analysis or the doctor’s claim of innocence.

The absence of pethidinic acid appeared to corroborate the doctor’s denial of re-use. The case highlights the need to sometimes look for alternative markers of drug metabolism for situations in which the results appear irregular. Simply relying on MS database matching can often produce erroneous results with serious consequences.

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References