Emetine Identified in Urine by HPLC, with Fluorescence and Ultraviolet/Diode Array Detection, in a Patient with Cardiomyopathy

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A 15-year-old girl with a four-month history of cardiac failure from undetermined cause was admitted to the hospital with weakness, fatigue, and weight loss. During her hospitalization she was found to have abused diet aids, laxatives, and cathartics. Although an electrocardiogram revealed nonspecific T-wave abnormalities and laboratory studies showed supranormal enzyme test results for creatine kinase and lactate dehydrogenase, no definite explanation of the cardiomyopathy was forthcoming. Ipecac abuse leading to cardiomyopathy was suspected early in the hospitalization. HPLC analysis of a urine sample showed emetine, a principle component of ipecac, the presence of which was later confirmed by more-specific HPLC analysis with photodiode array detection.

Additional Keyphrases: abused drugs • toxicology • ipecac

Ipecac syrup is an emetic prepared from the dried roots of Cephaelis ipecacuanha or C. acuminata. In the United States it is primarily used to induce emesis, but the syrup is also used outside this country in the treatment of amebiasis (1, 2). The pharmacological actions of ipecac are ascribable to its principal alkaloids, emetine and cephaeline. Emetine makes up more than half of the total alkaloid content of ipecac (2). The ipecac alkaloids irritate the gastric mucosa and stimulate the medullary chemoreceptor trigger zone (1, 3). Vomiting occurs only when the medullary chemoreceptor trigger zone is active (3).

Deaths from ipecac poisoning have been reported in patients receiving syrup of ipecac and those given fluid-extract of ipecac, which is 14 times more potent than ipecac syrup (4, 5). Such mortality was more common before 1970, the year the United States Pharmacopeia withdrew the extract from the market in an attempt to eliminate the accidental dispensing of fluid-extract of ipecac in place of syrup of ipecac (1).

In the 1980's, reports of toxicity due to syrup of ipecac intake among individuals with eating disorders such as anorexia nervosa and bulimia have increased (6–9). An important dose-limiting toxic effect, severe muscle weakness, reportedly begins at 15 mg of emetine per kilogram body weight (10). The toxicity, largely attributable to the emetine, has been associated with various symptoms, including diarrhea, nausea, fatigue, dyspnea, ataxia, hypotension, muscle aches, and myositis (8). The use of ipecac syrup to induce vomiting by patients with eating disorders has been associated with cardiac and skeletal myopathy (8, 9) and death (5, 6). Signs and symptoms associated with emetine cardiotoxicity include supraventricular tachycardia, atrial premature contractions, flattened or inverted T waves, prolonged QT and PR intervals, alterations of the QRS complex, decreased contractility, ventricular tachycardia, fibrillation, cardiac arrest, and unexplained heart failure (1). The exact biochemical mechanism remains to be defined, but appears to be related to depression of metabolic activity in myofibrils and resulting loss of contractility (1).

Case Report

A 15-year-old girl was admitted to Hartford Hospital with shortness of breath, generalized muscle weakness, dysphagia of solid foods, and slurring of speech. She had been healthy until four months before admission, when she had been hospitalized (also at Hartford Hospital) for pedal edema and orthopnea. The left ventricular ejection fraction was then 33% (normal range: 55–75%). An endomyocardial biopsy showed changes compatible with a congestive cardiomyopathy. She was discharged with prescriptions for furosemide, digoxin, and potassium supplements.

The patient's vital signs included a blood pressure of 110/80 mm Hg and heart rate of 120 per minute. Deep-tendon reflexes, as well as shoulder and arm strength, were subnormal bilaterally. The patient was unable to squat and her gait was unsteady. Review of systems was remarkable for nonbloody diarrhea, weight loss of 10.5 kg during the previous year, anemia, and history of using diet pills for one month, nine months previously. She denied use of ipecac syrup, although family members found empty Ex-Lax® (phenolphthalein) boxes in her possession.

Laboratory results obtained within the first week of hospitalization included a normal value for serum sodium, but the serum potassium concentration was 5.2 mmol/L, just within the reference interval (3.0–5.2 mmol/L). Total serum protein concentration was within normal limits, but the β-globulin fraction was subnormal, 4.0 g/L (normal = 7.0–11.0). Values for alanine aminotransferase and aspartate aminotransferase were within the normal reference interval. The following enzymes in serum had high values: creatine kinase 562 U/L (ref. interval for females: 15–75), the MB isoenzyme 22 U/L (ref. interval: <8), and lactate dehydrogenase 474 U/L (ref. interval: 100–300). Results of thyroid studies and an anti-double-stranded DNA titer were within normal limits, but the anti-nuclear antibody titer was positive at 1:80, and showed a "diffuse" pattern. The electrocardiogram revealed a sinus tachycardia, and nonspecific T-wave abnormalities. An echocardiogram demonstrated septal hypokinesia. An electromyelographic study of the deltoid and gastrocnemius muscles did not indicate a typical myopathy.
Potential emetine toxicity was suggested to be an explanation for these changes when they were reviewed along with the patient's history of weight loss and admitted laxative use. A urine sample was sent to the clinical chemistry laboratory for general toxicology along with a specific request to look for emetine. Although the patient denied having used ipecac, the laboratory reported the presence of emetine in this urine sample. On the 13th day of hospitalization the patient's father recalled finding several empty ipecac bottles outside his home a month before the last admission. A second urine specimen was sent to the laboratory for toxicological workup on the 18th day of hospitalization. Both emetine and cephaline were detected in this urine. After a second psychiatric consultation, the patient confessed to using ipecac as an emetic. A skeletal muscle biopsy (deltoid) showed nonspecific myopathic changes with cytoplasmic inclusions consistent with those seen in emetine toxicity. Because the patient seemed willing and able to cooperate with the psychiatrist as an outpatient, she was discharged on the 22nd day of hospitalization.

Materials and Methods

Reagents and standards. Methanol and acetonitrile (both from J.T. Baker Chemical Co., Phillipsburg, NJ 08865) were of "HPLC" grade. Emetine hydrochloride was obtained from Sigma Chemical Co., St. Louis, MO 63178; cephaline was a gift from D. E. Rollins (University of Utah, Salt Lake City, UT). Water for the mobile phase was purified by elution from a C18 radial compression column (Waters Associates, Milford, MA 01757) before acidification with phosphoric acid. All other chemicals and standards were ACS reagent grade or better.

Sample preparation. An extract of the patient's urine was prepared for HPLC analysis according to a protocol developed in our laboratory for the confirmation of drugs and toxins (11, 12). Aliquot portions of urine or emetine standard were extracted in Toxi-Lab® (Marion Laboratories, Laguna Hills, CA 92636) tubes containing the manufacturer's specified mixture of aqueous buffer, methylene chloride, and ethylene chloride. The organic phase of the extract was then concentrated by evaporation onto Toxi-Lab-A® tabs supplied with the kit. Two of the tabs were then placed onto a Toxigram and chromatographed as specified by Marion Laboratories. After development of the chromatogram in the Toxi-A solvent, one of the two channels containing the patient's sample was removed with a Toxi-Lab Chromatogram Strip Punch® (Marion Laboratories) and set aside. The remaining chromatogram was then taken through a series of staining steps according to the Toxi-Lab-A protocol. The chromatogram was then used as a template to locate the suspected emetine band from the unstained strip. The portion of the chromatogram containing this band was excised and extracted for 5 min at room temperature into 1 mL of methanol, and the extract was evaporated under a stream of air. The residue was then reconstituted with 500 μL of acetonitrile (11, 12). A similar extraction/chromatographic separation was conducted for the emetine and cephaline standards.

HPLC chromatography. The preliminary HPLC system was a modification of the method of Crouch et al. (13). We utilized a μBondapak™ phenyl column, 3.9 mm × 30 cm, packed with 10-μm particles, a Model 6000 pump, and a UK6 sample injector (all from Waters Associates) and an RF-535 HPLC fluorescence detector (Shimadzu Corp., Kyoto, Japan). Analysis of 15 μL of sample at a flow rate of 2 mL/min was by isocratic elution with a 34/66 (by vol) mixture of acetonitrile and potassium phosphate buffer (6 mmol/L, pH 3.3). The detector was operated at the optimal excitation and emission wavelengths of 285 and 315 nm, respectively, as described by Crouch et al. (13). Recordings were made with an Omniscrbr® recorder (Houston Instrument, Austin, TX 78753).

Extracts of urine were also analyzed by use of a second HPLC system involving a photodiode array ultraviolet detector for more precise identification of the urinary toxins. The system consisted of a Model 5000 chromatographic pump (Varian Associates, Walnut Creek, CA 94089); a Waters UK6 sample injector; and a Varian Polychrom® 9060 photodiode array detector interfaced to an AT computer (IBM Corp., Boca Raton, FL 33432). The chromatographic column was a 4 mm × 30 cm modified C18 Micropak SP supplied by Varian. The mobile phase was composed of acetonitrile and 3 mmol/L phosphoric acid, pH 2.5 (35/65 by vol). Data were collected from the Polychrom detector via the AT computer.

Chromatograms were assessed for peak height and retention time. We did not use an internal standard in this analysis, because rapid qualitative identification of emetine was needed by the cardiologist. Instead, ultraviolet absorbance and fluorometric retention time data were compared with those for aqueous standards. Spectral data obtained from the Polychrom 9060 were also compared with the library of standards in the AT as previously described (11, 12).

In some later experiments, this Varian HPLC system was extended by placing the RF-535 fluorometer in series with the diode array. In these last experiments, we used a mobile phase of acetonitrile and 3 mmol/L phosphoric acid, pH 2.5 (20/80 by vol), for a cleaner separation of emetine and cephaline. This design, used for the analysis of the second urine sample, also allowed a more precise comparison of retention time and of the relative sensitivities of the two detectors.

Results

Materials migrating as emetine and the emetine standard were individually visible as a single spot on the Toxi-Lab-A chromatogram at Stage 1 (sulfuric acid), Stage 3 (fluorescence), and Stage 4 (Dragendorff's solution). The ratio of migration of emetine to that of the solvent front (Rf) was 0.25. Quinidine also migrates at a similar Rf at Stages 3 and 4, so we also included quinidine in the assays with the two HPLC systems.

A material migrating as emetine in the first urine sample was easily detected fluorometrically during the preliminary HPLC analysis (Table 1). We identified emetine in the fluorescence system by reference to the retention times of standards. Quinidine was not observed when the fluorome-

| Table 1. Retention Times for Standards and the Unknown in Two Different HPLC Systems |
|---------------------------------|------------------|------------------|
|                                 | Waters: fluorescence | Varian: diode array |
| Unknown (patient's first urine) | 3.90             | 4.90             |
| Emetine standard                | 4.00             | 4.90             |
| Quinidine standard*             | 0                | 1.31             |

* Injected directly.
* Not detected at wavelength optimal for emetine (see text).
ter was set to detect emetine (Table 1), although quinidine was shown to have a retention time similar to that of emetine when assayed separately with excitation and emission wavelengths of 350 and 450 nm, respectively (data not shown).

A second peak preceded emetine on the chromatogram (retention time: 3.25 min), but it could not be identified at that time. Re-analysis of this sample in the second HPLC system (where the ultraviolet/diode array was the sole detector) confirmed the presence of emetine and clearly eliminated quinidine from further consideration (see Table 1 and Figure 1).

Both emetine and cephaeline were identified in the second urine sample analyzed on the Varian dual-detection system (Figure 2) after their isolation from an Rf 0.25 spot on the Toxi-gram. Although we emphasize qualitative results in our diode array studies, we nevertheless estimate that the urinary concentrations of emetine and cephaeline in the second urine specimen were <30 µg/L.

Discussion

This case is an example of serious intoxication with a relatively uncommon drug of abuse. During the patient’s first hospitalization, emetine intoxication was not suspected as a cause of her cardiac dysfunction. Other possible causes, such as viral myocarditis, were ruled out. To also rule out the more common drugs of abuse as the cause of the cardiotoxicity in this patient, a general drug screen was performed early in the second hospitalization. Both urine specimens gave negative test results for sympathomimetic amines such as amphetamines and phenylpropanolamine (14–17). No other drugs were detected that could not be attributed to her therapy during hospitalization. Only during this second admission was ipecac use suspected. Typically for these cases, its use was denied by the patient.

Using thin-layer chromatography, we detected emetine in the urine specimen collected from the patient at the time of admission. [We performed this test despite the recommendation by the manufacturer that the Toxi-Lab-A system be reserved for vomitus and gastric fluids, where the drug concentration is relatively high (18).] In our experience, the Toxi-Lab-A system can detect emetine in concentrations below the manufacturer’s suggested limit of detection (2 mg/L); therefore, it may be suitable for screening urine samples for ipecac abuse. However, other fluorescing compounds (e.g., cephaeline and quinidine) may have a Toxi-gram mobility identical to that of emetine, so identification of this compound cannot be based entirely on its Toxi-gram pattern.

Crouch et al. (13) showed that HPLC with fluorescence detection is a precise, sensitive method for detecting and quantifying emetine and cephaeline in plasma and urine. While fluorometry is the more sensitive mode of detection, the use of ultraviolet/photodiode array detection offers advantages through increased specificity. To offset the lower sensitivity of ultraviolet detection, we included in our studies a preliminary isolation by the Toxi-Lab-A system, which resulted in a more interference-free extract for the HPLC analysis and concentrated the emetine to 50 times that found in the urine itself. With this approach we confirmed the identity of both emetine and cephaeline in the patient’s urine and also could conclusively distinguish emetine from quinidine, a possible interferent.

Emetine is slowly released from body fluids into the urine, which may allow ipecac abuse to be detected long after the period of drug ingestion. Studies by Gimble et al. (19) suggest that emetine is retained unusually long in the mammalian body, with as much as 35% of the drug being present after 35 days. In addition, short-term therapy with emetine as an amebicide has shown the drug to be detectable in urine 40 to 60 days after its administration (20). Although ipecac abuse by the patient during her hospitalization cannot be totally excluded in this study, it is quite possible that the emetine and cephaeline found in the

Fig. 1. Overlay of normalized spectra derived by diode array processing of the apex portions of peaks associated with HPLC of urine extracts and standards

The patient’s urine extract was from the earlier collection, and both it and emetine standard were carried through a Toxi-Lab-A isolation step (see Methods).
second urine sample arose from drugs taken before her admission 18 days earlier.

Various authors have emphasized the need for considering ipecac toxicity when individuals with eating disorders present with myopathy and (or) nonspecific complaints (5, 6, 8, 9). While some claim that the relationship of emetine abuse to cardiotoxicity is exaggerated (21), experimental evidence indicates that emetine is an inhibitor of protein synthesis (22) and a mitochondrial toxin (23). Until more complete information is available regarding its effects, the toxicology laboratory can provide a valuable service by positive identification of the drug in urine or other body fluids (13, 24, 25). We demonstrate that the combination of thin-layer chromatography and HPLC with spectral analyses for identification of emetine is a useful tool in identifying and confirming ipecac intoxication.

References