Case Report

Tissue Distribution of Loperamide and N-Desmethylloperamide Following a Fatal Overdose*

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Abstract

We report a case involving a fatal intoxication with loperamide (Imodium®). Loperamide is a synthetic opioid of the phenylpiperidine class used as an over-the-counter antidiarrheal. It exerts its effects through interaction with μ-opiate receptors in the intestine to reduce peristalsis. Loperamide lacks the typical euphoric opiate effects when administered at recommended doses. Both loperamide and its major metabolite, N-desmethylelperamide, were isolated by liquid–liquid extraction into n-butyl chloride from alkalized samples. Extracts were analyzed by liquid chromatography–electrospray–mass spectrometry in selected-ion-monitoring mode. Rapid separation of the drug, metabolite, and internal standard (diphenoxylate) was achieved using a high-resolution C18 column with 1.8-μm particle diameter. The mobile phase consisted of 0.1% formic acid in deionized water (60%) and acetonitrile (40%) at a flow rate of 0.5 mL/min. Heart blood concentrations for loperamide and its metabolite were 1.2 mg/L and 3.3 mg/L, respectively. In contrast, reported peak plasma concentrations of loperamide after administration of recommended daily doses of 16 mg did not exceed 0.012 mg/L in controlled trials. Because the heart blood ethanol concentration was 0.08 g/dL, the medical examiner ruled that the cause of death was loperamide and ethanol intoxication, and the manner of death as undetermined.

Introduction

Loperamide (Imodium), 4-(4-chlorophenyl)-4-hydroxy-N,N-dimethyl-α-α-diphenyl-1-piperidine butyramide hydrochloride, is a piperidine opioid structurally related to diphenoxylate (Figure 1). Loperamide is administered orally for the management of both acute and chronic diarrhea through its reduction of gastrointestinal motility and, possibly, intestinal secretion (1,2). Loperamide was removed from Schedule V of the Controlled Substances Act in 1982 due in part to its efficacy and
displayed potential (3). Doses are available as 2-mg caplets, with or without 125 mg simethicone; cherry-mint-flavored liquids at 1 mg loperamide/5 mL; and mint-flavored chewable tablets also with simethicone. Also, until 1990, loperamide was sold in a drop formulation at a concentration of 2 mg/mL, but was withdrawn after reports of misuse in treating infant diarrhea (4).

The pharmacokinetics of loperamide have been reviewed in detail (2,5–9) with reported peak plasma times of 4–5 h and elimination half-lives between 7 and 19 h. As little as 0.3% of an administered dose of loperamide was present in plasma owing to first-pass metabolism (2). In addition to poor bioavailability, loperamide does not penetrate the central nervous system well, and in recommended doses lacks the analgesia typical of opioids. Single 4-mg oral doses resulted in peak plasma concentrations less than 0.001 mg/L for 3 individuals (10), and 6 individuals receiving 8-mg doses in either caplet or liquid form produced peak serum concentrations of 0.00224 and 0.00219 mg/L, respectively (6). N-Desmethylloperamide is a major inactive metabolite, and the primary route of lop-

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Figure 1. Chemical structure of loperamide (A) and diphenoxylate (B).
Loperamide has been implicated in numerous overdoses, particularly when administered to infants and children (11–14). In a 5-year review of 8 poison control centers, 216 cases of loperamide ingestions were reported (15). The majority of the cases involved children under the age of 3 with the most common symptoms including drowsiness, vomiting, abdominal pain, and nausea. Treatments have included gastric decontamination and administration of naloxone. Loperamide therapy has also been contributory in cases of toxic megacolon (16), pancreatitis (17), and lethal gastroenteritis (18).

Methods for the analysis of loperamide in biological samples have included radioimmunoassay (RIA) (5,10), liquid chromatography (LC) (8,19), LC-mass spectrometry (MS) (9), and LC-MS-MS (20,21). In this report, an LC-MS method was developed for the analysis of loperamide and N-desmethylloperamide in biological specimens and applied to a postmortem case.

Case History

A 26-year-old Caucasian male with a medical history of schizophrenia, bipolar disorder, depression, and alcohol abuse was found unresponsive, face-down on the side of the road at approximately 0600 hours. Reportedly, the deceased was unable to sleep and went to a nearby convenience store to buy groceries at approximately 0300 hours that morning. When his roommate awoke, he found that the deceased had not returned and located him a short distance from their residence as described. His prescription medications at the time of his death included lithium carbonate (300 mg), trazodone (100 mg), and aripiprazole (10 mg). Prescription dates and amounts of remaining tablets were consistent with proper use. An empty blister pack of Equate loperamide (commercial name of U.S. generic drug line) was found with the body. The roommate stated the deceased “frequently” took the over-the-counter anti-diarrheal medication for self-treatment of “stomach” problems.

At autopsy, the deceased was a well-nourished, well-developed male, 5 feet, 10 in. in height, and weighing 183 pounds. Superficial abrasions were on the right side of the forehead, the right infraorbital ridge, and the right side of the upper lip. There were no associated injuries to the facial skeleton, the skull, or brain. There was no other external evidence of injury and cut downs failed to identify trauma to the lower extremities. The internal examination was significant for a heart weight of 490 g associated with a left ventricular free wall and septal thickness of 1.5 cm and scattered 0.2-cm calcified nodules in the spleen. Regional lymph nodes were unremarkable. There were no gross abnormalities of the upper or lower gastrointestinal tract. Microscopic examination identified left ventricular myocyte hypertrophy, non-specific reactive lobular hepatitis, moderate to focally severe chronic esophagitis, and fibrocalcified, non-caseating granulomatous inflammation of the spleen. Special stains for acid-fast bacilli and fungi were negative. Appropriate specimens were collected for toxicological analysis.

Methods

Materials

Loperamide hydrochloride, diphenoxylate hydrochloride, and sodium borate decahydrate were obtained from Sigma-Aldrich (St. Louis, MO). N-Desmethylloperamide was donated by Dr. David Hachev of the Mass Spectrometry Research Center, Vanderbilt University School of Medicine (Nashville, TN). Formic acid was obtained from ICN Biomedicals (Aurora, OH). All solvents were HPLC grade and purchased from Fisher Scientific (Pittsburgh, PA).

Sample preparation

Blood (central and peripheral), urine, gastric contents, bile, kidney, and liver were collected at autopsy and stored, unrefrigerated, at −15°C. Standard curves were prepared in blood and urine at 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, and 1.0 mg/L for loperamide and N-desmethylloperamide.

The diphenoxylate internal standard was prepared at a concentration of 0.01 mg/mL in methanol. Two-hundred microliters of each blood, urine, and bile were initially assayed, but final quantitation was based on dilutions tailored for each. Tissue samples and gastric contents (1.0 g) were homogenized in 10 mL of saturated sodium borate buffer using a Brinkmann PT3000 tissue homogenizer (Westbury, NY). Two-hundred-microliter aliquots of homogenate or dilutions were extracted.

Two hundred microliters of blood or urine calibrators and 200 μL of the appropriately diluted specimens were added to clean, labeled 11 × 100-mm tubes, and 2 mL saturated sodium borate was added. Thirty-five microliters of the diphenoxylate internal standard (1.75 mg/L) was added to each tube along with 3 mL of n-butyl chloride. The tubes were capped and mixed for 10 min on an orbital mixer. After centrifuging the tubes for 5 min at 3000 rpm, the solvent was transferred to 10-mL conical tubes and evaporated to dryness under nitrogen at 40°C. The residue was reconstituted with 300 μL of HPLC mobile phase (deionized water/acetonitrile/formic acid, 60:40:0.1) and transferred to autosampler vials. One microliter was injected for analysis.

**Table I. Tissue Distribution of Loperamide and N-Desmethylloperamide**

<table>
<thead>
<tr>
<th></th>
<th>Central Blood (mg/L)</th>
<th>Peripheral Blood (mg/L)</th>
<th>Gastric (mg)</th>
<th>Bile (mg/L)</th>
<th>Kidney (mg/kg)</th>
<th>Liver (mg/kg)</th>
<th>Urine (mg/L)</th>
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<tr>
<td>Loperamide</td>
<td>1.2</td>
<td>2.6</td>
<td>3.9</td>
<td>8.9</td>
<td>8.3</td>
<td>12.5</td>
<td>9.2</td>
</tr>
<tr>
<td>N-Desmethylloperamide</td>
<td>3.3</td>
<td>6.4</td>
<td>1.2</td>
<td>44.6</td>
<td>31.7</td>
<td>30.6</td>
<td>44</td>
</tr>
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Instrumentation

Biological extracts were analyzed using an Agilent 1100 LC–mass selective detector equipped with an orthogonal electrospray ionization interface. Separation was performed using a Zorbax StableBond-C18, high-throughput cartridge column (30 ×...
2.1 mm, dp = 1.8 μm, Agilent, Palo Alto, CA) held at 35°C. The mobile phase consisted of 0.1% formic acid in deionized water (60%) and acetonitrile (40%) at a flow rate of 0.5 mL/min.

The positive ions of loperamide (m/z 477, 266, 210), N-desmethylloperamide (m/z 463, 252, 196), and diphenoxylate (m/z 453, 425) were formed by electrospray ionization. Pneumatic-assisted nebulization utilized nitrogen at 35 psi, and 350°C nitrogen drying gas was used at a flow of 12 L/min. Quantitation was based on multi-point, internal standard linear regression. The peak-area ratios for the [M+H] ions of N-desmethylloperamide (m/z 463) and loperamide (m/z 477) to that of the internal standard (m/z 453) were used to calculate response factors. Identification was based on retention time matching within ± 2% and ion ratios matching within ± 20% to the mean values of all calibrators.

Results

The heart blood and urine were tested for volatile substances (methanol, ethanol, acetone, and isopropanol) by headspace gas
N-desmethylloperamide that was applied to a fatal overdose injected to the same extraction, chromatographic, and mass spectral conditions. The central blood ethanol concentration was 0.08 g/dL. Subclavian blood, urine, and vitreous humor ethanol concentrations were 0.11, 0.05, and 0.12 g/dL, respectively. Diphenhydramine and pseudoephedrine were detected in the urine; no diphenhydramine was detected in the blood at a limit of quantitation of 0.05 mg/L. The heart blood pseudoephedrine concentration was 2.6 mg/L. In addition, two unidentified peaks were detected in both the blood and urine on the alkaline drug screen that eluted past all previously encountered drugs, including verapamil, trazodone, and mesoridazine. Subsequent mass spectral analysis tentatively identified the substances as loperamide and N-desmethylloperamide. The second peak was verified as loperamide when an authentic standard was subjected to the same extraction, chromatographic, and mass spectral conditions.

Quantitative results for the case specimens are shown in Table I. Loperamide and metabolite were found in all specimens. N-Desmethylloperamide concentrations were greater than loperamide concentrations by a factor of 2.4-5 in all specimens except the stomach contents. The greatest concentrations of drug and metabolite were found in bile with a near equivalent amount found in urine.

Discussion

Separation by LC was achieved in less than 1.5 min by using a combination of high flow, short column length, and high-efficiency column packing (Figure 2). The single-step extraction of only 0.2 mL of sample resulted in good specificity without discernable interference. Chromatographic resolution of drug, metabolite, and internal standard was acceptable (R_s > 3). Standard curves produced correlation coefficients better than 0.998 for both loperamide and N-desmethylloperamide.

Plasma and serum concentrations of loperamide have been published for pharmacokinetic and bioequivalence studies (5,6,8-10). Michiels et al. (10) used RIA to measure loperamide in the plasma of 3 subjects taking 4-mg capsule doses. Peak levels did not exceed 0.0075 mg/L for any participant. Weintraub et al. (5) gave single doses of between 18 mg and 54 mg of loperamide hydrochloride caplets to 6 subjects. They reported peak serum concentrations of approximately 0.012 mg/L (48-mg dose). The maximum recommended daily dose of 16 mg was administered to 24 subjects by Doser et al. (8). Using LC with electrochemical detection, the authors measured peak plasma concentrations in the range of 0.0015 mg/L to 0.011 mg/L.

Fatal overdoses of loperamide have been reported in the literature; however, quantitative values in tissues have generally not been available. Recently, Johansen and Jensen (21) presented an LC-MS-MS method for loperamide and N-desmethylloperamide that was applied to a fatal overdose case. The authors found 0.084 mg/kg (0.089 mg/L, estimated) of loperamide and 0.38 mg/kg (0.403 mg/L, estimated) of desmethylloperamide in postmortem whole blood. They also quantitated the liver at 0.87 mg/kg and 3.8 mg/kg for loperamide and metabolite, respectively. Loperamide concentrations in this case were fully two orders of magnitude higher than any peak level concentrations found in whole blood in previously reported controlled trials.

The medical examiner ruled that the cause of death was loperamide and ethanol intoxication. Hypertensive cardiovascular disease was listed on Part II of the death certificate. The manner of death was undetermined.

Acknowledgment

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References

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