Laxative Poisoning: Toxicological Analysis of Bisacodyl and Its Metabolite in Urine, Serum, and Stool

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Abstract

An 11-year-old boy was hospitalized because of severe diarrhea (over 5 L/day). The child survived; however, the diarrhea continued while he was given intravenous fluids and his electrolyte balance was closely supervised. Based on observation of the patient and his family in the hospital, surreptitious administration of a poison by his mother was suspected, and toxicological analysis was carried out on stools from the boy and on medicine administered to him by his mother. Bisacodyl, a cathartic with a direct effect on the colon, was detected in the medicine, and a metabolite of bisacodyl was present in the stool. We devised a sensitive and reliable method to quantitate the bisacodyl metabolite in urine and serum. The sample was incubated with β-glucuronidase at 37°C for 2 h; bisacodyl metabolite was extracted with tert-butyl methyl ether, then derivatized by methylation, and subjected to gas chromatography–mass spectrometry. Bisphenol A was used as an internal standard. The calibration curve was linear in the concentration range from 2 to 1000 ng/0.2 mL, and the lower limits of detection were 1 ng/0.2 mL for the urine and 2 ng/0.2 mL for the serum. As concentrations of bisacodyl metabolite in the urine and serum of the patient were clearly defined, perhaps such investigations are warranted before extensive clinical therapy is prescribed.

Introduction

Laxative abuse is a common cause of chronic diarrhea in adults, and there have been several pediatric cases that were due to the administration of laxatives such as phenolphthalein (1–3), magnesium (4), and sodium sulfate (5) by a parent. In such cases, extensive and potentially harmful investigations were made before the actual cause was discovered. Therefore, such cases are suspect, and toxicological analysis of fecal fluid and urine should be made.

We report here findings in an 11-year-old boy with intermittent but severe diarrhea. The cause proved to be surreptitious administration of bisacodyl, an over-the-counter laxative, to the boy by his mother. In addition, we developed a sensitive and reliable method to determine bisacodyl diphenol, a metabolite of bisacodyl, in human urine and serum using gas chromatography–mass spectrometry (GC–MS). Concentrations of bisacodyl diphenol in the urine and serum of the patient were clearly defined.

Case History

An 11-year-old Japanese boy, usually in good health, was hospitalized with diarrhea exceeding 5 L/day. After being in another hospital for 10 days, he was sent to our University Hospital on the 11th day; severe dehydration and hypernatremia were present. Intravenous fluids were administered, with close attention paid to electrolyte balance. The volume of...
diarrhea gradually decreased, but it continued. A significant decrease in the volume of diarrhea was evident only when his mother was not at the hospital for several days. Therefore, surreptitious administration of a cathartic by his mother was suspected. Because we learned that the mother had re-wrapped a powdered medicine that was prescribed for the boy in our hospital in a medicinal wafer, an admixture of some drug at this time was suspected, and toxicological analyses were carried out on the powder he ingested and on his stool, urine, and serum.

When we confronted his mother with our analytical results in December, she admitted that she had given bisacodyl to her son continuously from April to December as an excuse to care for her son in the hospital and escape from farming.

**Experimental**

**Reagents**

Bisacodyl (Figure 1) was provided by Shizuoka Caffeine (Shizuoka, Japan). Bisacodyl diphenol (Figure 1), a metabolite of bisacodyl, was prepared by alkali hydrolysis of bisacodyl as follows: 100 mg of bisacodyl was mixed with 1M sodium hydroxide in 50% methanol solution and heated at 70°C for 2 h. After cooling, the mixture was neutralized by 2M hydrochloric acid. A crystalline powder of bisacodyl diphenol was precipitated in the mixture, and this compound was characterized by GC-MS. Bisphenol A (4,4'-isopropylidene diphenol, Figure 1) was purchased from Tokyo Kasei (Tokyo, Japan) and served as an internal standard (IS). Iodomethane (CH3I), dimethyl sulfoxide, and tetrabutylammonium hydroxide (TBAH, 40% in water) were purchased from Ishizu Seiyaku (Osaka, Japan). TBAH was dissolved in dimethyl sulfoxide (TBAH-dimethyl sulfoxide reagent [2:98, w/v]). TBAH-dimethyl sulfoxide reagent was divided into small volumes for use during a single day and stored in a refrigerator under protection from light. β-Glucuronidase obtained from bacteria (E. coli) was purchased from Sigma Chemical Company (St. Louis, MO). Isooctane and tert-butyl methyl ether were of analytical reagent grade and were purified by distillation. The other chemicals were of reagent grade.

**Biological samples**

Human urine and serum were obtained from a healthy volunteer, and human serum, urine, and stool for toxicological analyses were obtained from the patient. All samples were kept at -20°C until analysis.

**Extraction procedure**

The medicine given to the boy by his mother was of mixed particles with two different shapes. The particles with a different shape from the medicine prescribed in our hospital were picked out and dissolved in tert-butyl methyl ether, and an

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**Table 1. Precision and Accuracy for Analysis of Bisacodyl Diphenol in the Patient’s Urine and Serum**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Amount determined* (ng/0.2 mL)</th>
<th>CV¹ (%)</th>
<th>Amount determined* (ng/0.2 mL)</th>
<th>CV¹ (%)</th>
<th>Amount determined* (ng/0.2 mL)</th>
<th>CV¹ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>9.78±0.57</td>
<td>5.8</td>
<td>107.5±8.07</td>
<td>7.5</td>
<td>999.7±44.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Serum</td>
<td>9.61±1.11</td>
<td>11.6</td>
<td>105.1±7.26</td>
<td>6.9</td>
<td>1069.7±58.8</td>
<td>5.5</td>
</tr>
</tbody>
</table>

* Mean plus or minus standard deviation.
¹ Coefficient of variation.
aliquot of the solution was subjected to GC–MS. The stool (2 g) collected on admission to our hospital was centrifuged. The supernatant was mixed with 5 mL 0.1M phosphate buffer (pH 6.0) and extracted with 10 mL tert-butyl methyl ether. The organic layer was dried with sodium sulfate and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 µL tert-butyl methyl ether, and a 2-µL aliquot of the solution was injected to a GC–MS apparatus.

Urine or serum sample (0.2 mL) was mixed with 1 mL 1M phosphate buffer (pH 6.8) in a 10-mL centrifuge tube and incubated at 37°C for 2 h with 2 mg β-glucuronidase. After adding 4 mL tert-butyl methyl ether and 5 µL internal standard (IS) solution (bisphenol A, 50 ng), the preparation was shaken for 10 min and centrifuged at 850 × g for 20 min. The organic layer was transferred to another 10-mL centrifuge tube and evaporated to dryness under a stream of nitrogen. Derivatization was then performed by the following steps: 150 µL TBAH-dimethyl sulfoxide reagent was added to the residue, and the preparation was stirred for 10 s. After 2 min at room temperature, 50 µL iodomethane was added, and the sample was stirred again for 10 s. After standing for 5 min at room temperature, the reaction was stopped by adding 350 µL 0.1M hydrochloric acid. Two milliliters of isooctane was then added to the mixture, and the preparation was shaken for 10 min and centrifuged at 850 × g for 20 min. The organic layer was evaporated to dryness under a stream of nitrogen, and the residue was dissolved in 30 µL tert-butyl methyl ether. A 2-µL aliquot of the solution was injected onto the GC–MS. Urine samples from the patient that contained a high concentration of bisacodyl diphenol were diluted with distilled water and submitted to analysis.

**Table II. Concentrations of Bisacodyl Diphenol in the Patient's Serum and Urine.**

<table>
<thead>
<tr>
<th>Date*</th>
<th>Sample</th>
<th>Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. 6</td>
<td>serum</td>
<td>1.05</td>
</tr>
<tr>
<td>Sept. 4</td>
<td>urine</td>
<td>63.1</td>
</tr>
<tr>
<td>Sept. 22</td>
<td>urine</td>
<td>91.2</td>
</tr>
<tr>
<td>Oct. 20</td>
<td>urine</td>
<td>61.9</td>
</tr>
<tr>
<td>Dec. 1</td>
<td>urine</td>
<td>7.2</td>
</tr>
<tr>
<td>Dec. 6</td>
<td>urine</td>
<td>8.7</td>
</tr>
<tr>
<td>Dec. 20</td>
<td>urine</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* Date of admission to the hospital.

**GC–MS conditions**

Analyses were made using a Hewlett-Packard 5989A GC–MS system operated in the EI mode. A fused-silica capillary column, HP-1 (13 m × 0.2-mm i.d., 0.33-µm film thickness), coated with 100% dimethylpolysiloxane stationary phase was used. Splitless injection mode was selected with a valve-off time of 2 min. The GC–MS conditions are as follows: The
initial temperature of 100°C was held for 2 min, and the temperature was programmed to 300°C at a rate of 20°C/min. This temperature was maintained for 2 min. Injection port and transfer line temperatures were maintained at 260 and 280°C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/min. Scan mode was used to analyze extracts from the medicine given to the boy by his mother and from the patient’s stool, and selected ion monitoring (SIM) mode was used to determine bisacodyl diphenol using monitoring ions of \( m/z \) 305 for bisacodyl diphenol and \( m/z \) 256 for the IS.

**Preparation of calibration curves**

Urine and serum samples were prepared to contain bisacodyl diphenol at concentrations of 2–1000 ng/0.2 mL, each containing 50 ng IS. These samples were extracted in the same manner as described. Calibration curves were obtained by plotting the peak-area ratio of bisacodyl diphenol to IS versus the amount of bisacodyl diphenol.

**Results and Discussion**

**Analysis of the medicine and stool samples**

The total ion chromatogram of the extract from the particles with different shapes is shown in Figure 2A. Three peaks not found in the prescribed medicine were observed at retention times of 11–12 min. The mass spectrum of peak 3 shows the molecular ion at \( m/z \) 361 (Figure 2B), and this compound was determined to be bisacodyl using a Wiley data base built into the GC–MS system and direct comparison of retention time with standard bisacodyl. The other two peaks, 2 and 1, proved to be mono- and di-deacetylated bisacodyl, respectively, based on their mass spectra. Bisacodyl diphenol, a metabolite of bisacodyl corresponding to peak 1 in Figure 2A was also found in the patient’s stool. As bisacodyl is a commonly used over-the-counter laxative in Japan, surreptitious administration of this drug to the boy by his mother became evident.

**Determination of bisacodyl diphenol in human urine and serum**

Bisacodyl is rapidly deacetylated to its active metabolite, bisacodyl diphenol, in the intestinal cavity, and it is conjugated to the mono glucuronide in the wall of intestine or liver (6–8). The conjugated metabolite of bisacodyl corresponding to 20–30% of the original dose was excreted in the urine within 24 h (7). Therefore, determination of this metabolite in human urine and serum was attempted. A number of procedures have been published for the analysis of bisacodyl diphenol, using GC–MS (6,9) and high performance liquid chromatography (7). These methods, however, do not need an IS for quantitation, and the sensitivity is not high enough to detect small amounts of bisacodyl diphenol in human urine and serum. These problems were overcome by selecting bisphenol A as

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![Figure 4](image_url)"
the IS and derivatizing bisacodyl diphenol to its methylated compound. The sensitivity was significantly increased by methylation, and reliable quantitation was carried out using IS.

**GC-MS determination of bisacodyl diphenol**

Mass spectra of methylated bisacodyl diphenol and IS are shown in Figure 3. The molecular ion and fragment ion, respectively, were observed at m/z 305 and 227 for bisacodyl diphenol and m/z 256 and 241 for IS. The ions of m/z 305 and 256 were selected for determination of bisacodyl diphenol and IS, respectively. The selected ion monitoring chromatograms of the extract from human urine and serum containing bisacodyl diphenol (100 ng) and IS (50 ng) are shown in Figure 4. Each peak was clearly separated on the chromatograms, and there were no interfering peaks on chromatograms of control samples. The calibration curves were linear in the concentration range from 2 to 1000 ng/0.2 mL in both samples with correlation coefficients of over 0.99. The lower limits of detection, at a signal-to-noise ratio of 5, were 1 ng/0.2 mL for urine and 2 ng/0.2 mL for serum. The sensitivity was 50–100 times higher than that described in the literature (7). The absolute recoveries of bisacodyl diphenol in the urine and serum at three different concentrations, 10, 100, and 1000 ng/0.2 mL, were determined by comparing the peak areas of methylated bisacodyl diphenol in samples with those in standard solutions directly injected onto the GC-MS. The calculated recoveries were 70.7–90.4% in urine and 65.9–74.7% in serum. The precision of this method in urine and serum at concentrations of 10, 100, and 1000 ng/0.2 mL sample is summarized in Table I. The coefficients of variation ranged from 4.4 to 11.6%.

**Quantitation of bisacodyl diphenol in urine and serum**

Concentrations of bisacodyl diphenol in the patient’s urine and serum were determined by the established method and results are shown in Table II. Bisacodyl diphenol was detected in the serum that was collected on admission to our hospital and stored in a −20°C freezer until analysis; the concentration was 1.05 µg/g. The parent drug, bisacodyl, was not detected in the serum. Urine samples were taken at regular intervals and submitted to analysis after we found that bisacodyl was causing the diarrhea. Concentrations of bisacodyl diphenol in the patient’s urine, when the patient had been excreting about 1 L/day of loose stool, were 61.9 to 91.2 µg/mL. These values are significantly higher than the 1–5 µg/mL noted after administration of a single oral dose (10 mg) of bisacodyl (7). Therefore, the mother probably gave more than 10 tablets of the drug to her son at one time.

**Conclusion**

Based on toxicological analysis of the drug administered by the mother and of the stool of the 11-year-old boy, the cause of the severe diarrhea proved to be surreptitious administration of a common laxative. The concentrations of bisacodyl diphenol, a metabolite of bisacodyl, in the patient’s urine and serum were clearly determined when we used bisphenol A as the internal standard.

**Acknowledgments**

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**References**