Diltiazem and Pentoxifylline Determination in Postmortem Specimens

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Abstract

A 78-year-old woman was found dead in her basement. Qualitative screening of available postmortem specimens detected the presence of diltiazem and pentoxifylline. Quantitations were carried out by gas chromatography using nitrogen-phosphorus detection and confirmed by gas chromatography–mass spectrometry with the following results: blood, 0.59 mg/dL diltiazem and 0.63 mg/dL pentoxifylline; urine, 1.17 mg/dL diltiazem and 0.08 mg/dL pentoxifylline; bile, 0.40 mg/dL diltiazem and 0.22 mg/dL pentoxifylline; gastric contents, 0.28 mg/dL diltiazem and 0.02 mg/dL pentoxifylline. Both drugs were found qualitatively in formalin-fixed tissues.

Introduction

Diltiazem (Cardizem®) is a calcium channel blocker used in the treatment of hypertension, angina, and supraventricular arrhythmias. Typical doses range from 120 to 300 mg/day, and therapeutic plasma concentrations range from 0.005 to 0.020 mg/dL (1). The primary metabolite of diltiazem, desacetyldiltiazem, also has potent pharmacological activity as a coronary vasodilator (2). Although numerous diltiazem overdoses have been reported, only ten have included fatalities (3–9).

Pentoxifylline (Trental®) is used in the treatment of peripheral and cerebrovascular diseases. It is a vasodilator that is also used to reduce blood viscosity. Typical doses, administered three times a day, range from 300 to 600 mg resulting in plasma concentrations which usually do not exceed 0.04 mg/dL (10–13). The major metabolites are (1-[5-hydroxyhexyl]-3, 7-dimethylxanthine) and (1-[3-carboxypropyl]-3,7-dimethylxanthine), and plasma concentrations of the metabolites are 5 and 8 times greater than pentoxifylline, respectively (14). Only one pentoxifylline overdose has been reported, and it was not fatal (15).

Case History

A 78-year-old, 5 ft 3 in, 125 lb female was found lying face down on the basement floor, 10 ft from the bottom of the stairwell in her home, by her son. Blood was pooled under her face. She had a history of hypertensive heart disease. Partially filled Cardizem (180 mg), Lanoxin® (125 mg), and Trental (400 mg) prescription containers for the decedent were obtained at the house and accompanied the body. The quantities of missing pills suggested that she had been following the prescribed dosage schedule. An autopsy showed rib fractures and contusions of the head, trunk, and extremities that were probably due to an apparent fall. She also had chronic liver disease characterized by portal tract fibrosis and fatty degeneration.

Experimental

Chromatography

Diltiazem and pentoxifylline were quantitated by gas chromatographic (GC) analysis using a Hewlett-Packard model 6890 GC with a Restek RTX-50 (30.0 m x 0.32 mm, 0.25-μm film thickness) capillary column and a nitrogen-phosphorus detector. Helium was the carrier gas programmed to hold at an initial flow rate of 1.3 mL/min for 12 min, increase to 3 mL/min over 0.5 min, and hold for 3.5 min. The inlet and detector temperatures were 250 and 300°C, respectively. The oven temperature was programmed to hold at 220°C for 3 min, increase to 280°C at 10°C/min, hold for 2 min, increase to 300°C at 40°C/min, and then hold for 5.5 min. The injector was in the split mode with a split ratio of 1:10.

Drug confirmations and metabolite identifications were performed by GC–MS with a dual column Hewlett-Packard model 5890 GC with dual capillary columns interfaced into a single model 5970 mass selective detector (16). The columns were J&W Scientific DB-5 (30 m x 0.25 mm, 0.25-μm film thickness). Helium at a flow rate of 1.0 mL/min was the carrier gas, and the inlet temperature was 200°C. The oven temperature was programmed to hold at 150°C for 3 min, increase to 200°C at 50°C/min, increase to 290°C at 10°C/min, and then hold for 2 min.

Specimens

Postmortem blood, urine, bile, vitreous, liver, and gastric
contents were obtained during the autopsy, which was performed approximately 12–18 h after the time of death. Blood samples, which were taken from the heart, were stored in 50-mL glass screw-top tubes containing approximately 0.6 g of sodium fluoride/potassium oxalate (5:1, w/w) and sealed with Teflon®-lined caps. Blood plasma was obtained by centrifugation of a whole blood sample at 2500 rpm for 10 min at which time the plasma layer was transferred to a separate 50-mL glass screw-capped tube and sealed with a Teflon-lined cap. Urine was placed in a 50-mL glass screw-capped tube and sealed with a Teflon-lined cap. Vitreous specimens were placed in 7-mL red-capped Vacutainer® tubes. Bile and gastric contents were collected in Nalgene™ polypropylene wide-mouth bottles. Subsequent to the analyses of these specimens, formalin-fixed tissue samples were obtained from autopsy storage vessels because fresh tissue samples were no longer available.

Extraction
A single-step extraction technique was used to extract pentoxifylline using N-propionyl-p-aminophenol as the internal standard. Two milliliters aqueous standard, blood, urine, bile, gastric, or tissue sample, respectively; 1 mL of 10 mg/dL internal standard, 2–3 g of NaCl; and 10 mL of ethyl acetate were added to 16 x 125-mm glass culture tubes with Teflon-lined screw caps (tissue samples were prepared by homogenizing 6 g of tissue in 30 mL of deionized water). The tubes were placed in a rotator for 20 min, removed, and centrifuged for 5 min at 2500 rpm. The ethyl acetate layer was separated and evaporated under a stream of nitrogen at 60°C. Each residue was reconstituted in 100 µL of methanol, and 1 µL was injected into the GC. A standard curve of drug concentration versus the peak-area ratio of drug and internal standard was used for quantitation. The extraction efficiency was greater than 92% for each matrix analyzed versus aqueous controls.

Quantitation of diltiazem, using promazine as the internal standard, involved the following extraction procedure. Three milliliters aqueous standard, blood, urine, bile, gastric, or tissue sample, respectively; 0.1 mL of 0.5 mg/dL internal standard; 2 mL of a saturated aqueous NaCl solution; 2 mL of a saturated aqueous NH₄Cl solution (pH adjusted to 9.2 with concentrated ammonium hydroxide); 0.1 mL of 6N NaOH; and 10 mL of ethyl acetate were added to 20 x 150-mm glass culture tubes with Teflon-lined screw caps. The tubes were placed in a rotator for 20 min, removed and centrifuged for 5 min at 2500 rpm. The ethyl acetate layer was transferred to 16 x 125-mm glass culture tubes with Teflon-lined screw caps containing 2 mL of 0.5N HCl. The tubes were placed in a rotator for 10 min, removed, and centrifuged for 5 min at 2500 rpm. The organic layer was removed by aspiration. Tris buffer (3 mL, 1.2M, pH 9.2) and 5 mL of hexane/isopropanol (9:1) were added to the acid. The tubes were placed in a rotator for 20 min, removed, and centrifuged for 5 min at 2500 rpm. The
organic layer was transferred to 13 × 100-mm glass culture tubes and evaporated to dryness under a stream of nitrogen at 60°C. Each residue was reconstituted in 50 μL of MeOH, and 2 μL was injected into the GC. A standard curve of drug concentration versus the peak-area ratio of drug and internal standard was used for quantitation. Specimens were diluted with deionized water when necessary to bring the concentration within the linear range of the standard curve. The extraction efficiency was greater than 94% for each matrix analyzed versus aqueous controls.

**Results and Discussion**

The quantitative analyses of pentoxifylline and diltiazem are given in Table I. Both drugs showed good linearity over the concentration ranges tested (0.016-2.000 mg/dL, \( r = 1.000 \) pentoxifylline and 0.006-0.200 mg/dL, \( r = 0.996 \) diltiazem). Examples of the gas chromatograms used for quantitation of pentoxifylline and diltiazem are shown in Figures 1 and 2. Identification of these compounds was confirmed on the GC-MS system. The major metabolites of pentoxifylline and diltiazem (1-[5-hydroxyhexyl]-3,7-dimethylxanthine) and desacetyldiltiazem were qualitatively identified using GC-MS by comparing the mass spectra obtained against the Pflegler library (17) with match qualities of 95% and 91%, respectively. Post-mortem distribution was not studied because of the limited sampling performed since there was no suspicion of drug involvement at the time of the autopsy. Again, because of the limited sampling at the autopsy, the only tissues available were formalin-fixed liver, kidney, and spleen. These specimens, along with the formalin solution in which they were stored, were analyzed 2-3 weeks after the autopsy; the results are shown in Table I.

Other drugs detected in the blood were caffeine (0.42 mg/dL) and ibuprofen (3.1 mg/dL). At these concentrations, neither of these drugs was significant to the cause of death.

The literature showed few data on the effects of the high levels of pentoxifylline found, which, in this case, were greater than ten times higher than therapeutic concentrations. The highest plasma concentration reported to the manufacturer was 0.096 mg/dL (18). The quantities of diltiazem found in the blood and urine were consistent with those reported in other fatalities in which diltiazem was present (3-9). The small amounts of diltiazem and pentoxifylline found in the gastric contents precludes consideration of an intentional overdose. It appears that impaired metabolism attributable to chronic liver disease is a reasonable explanation for the high drug concentrations. The Coroner ruled the cause of death as the "end result of hypertensive, hypertrophic and ischemic cardiomyopathy and other condition of blunt impact to the head, trunk and extremities, acute intoxication by diltiazem and pentoxifylline", and it was accidental in nature.

**Table I. Toxicological Findings**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Diltiazem* (mg/dL)</th>
<th>Pentoxifylline* (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.59</td>
<td>0.63</td>
</tr>
<tr>
<td>Urine</td>
<td>1.17</td>
<td>0.08</td>
</tr>
<tr>
<td>Bile</td>
<td>0.40</td>
<td>0.22</td>
</tr>
<tr>
<td>Gastric (200 mL)</td>
<td>0.28</td>
<td>0.02</td>
</tr>
<tr>
<td>Liver*</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>Spleen*</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>Kidney*</td>
<td>present</td>
<td>present</td>
</tr>
</tbody>
</table>

* Desacetyldiltiazem was qualitatively found in all specimens
† 1-[5-Hydroxyhexyl]-3,7-dimethylxanthine was qualitatively found in all specimens.
‡ Formalin-fixed tissues.

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**References**


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