Distribution of Embutramide and Mebezonium Iodide in a Suicide after Tanax Injection

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Tanax is a veterinary formulation for euthanasia comprising embutramide, mebezonium iodide and tetracaine. A 37-year-old female was found dead on her bed, with three empty used syringes and a bottle of Tanax beside her body. Three needle puncture marks were observed on the body. The aim of this study was to evaluate the distribution of embutramide and mebezonium iodide in different biological matrices (femoral and cardiac blood, liver, muscle and vitreous humor) using a chromatographic method for the simultaneous determination of the two drugs. A direct and sensitive liquid chromatography–tandem mass spectrometry method was developed in multiple reaction monitoring mode with positive ionization. Lidocaine was used as an internal standard. Limits of detection and quantitation of 0.01 and 0.05 mg/L, respectively, were reached for both compounds. Embutramide levels ranged from 2.74 mg/L in vitreous humor to 5.06 mg/L in femoral blood, while mebezonium iodide was found at widely differing concentrations (ranging from 2.80 mg/kg in muscle to 24.80 mg/kg in liver). The chromatographic method developed for this study provides a very simple and sensitive means for the simultaneous determination of embutramide and mebezonium iodide, the emetic concentrations of which were consistent with suicides reported in the literature.

Introduction

Tanax is a veterinary pharmaceutical preparation for euthanasia that primarily comprises three substances: embutramide, a strong sedative drug; mebezonium iodide, a quaternary ammonium salt that paralyzes skeletal muscle; and tetracaine, a local anesthetic drug used to reduce painful tissue reaction at the injection site (1). In the literature, there are few articles describing suicide attempts and deaths by self-injection of Tanax or T-61 (another veterinary formulation of the same mixture) (2–4). In all these cases, embutramide and mebezonium iodide were therefore performed with a specific LC–MS–MS method. Screening and quantitative measurement of these compounds in horse serum (5), but none in human biological matrices.

Case Report

A female veterinarian, 37 years of age and affected by a major depressive disorder, was found dead on her bed. Three apparently used empty syringes, a bottle of the veterinary drug Tanax, used for euthanasia, and a bottle of Minias (lormetazepam) were found near the body. Examination of the body revealed three needle-puncture marks: one on the back of the left hand, the second on the front of the left upper arm and the third on the left buttock. Reflection of the skin showed hemorrhage underneath all the marks. The wound tracks penetrated deeply in the soft tissues and the muscles, sparing the superficial vessels. Bloodstained frothy fluid had leaked from the mouth and both nostrils. No injuries were found on the body to give any cause for concern. The autopsy revealed no natural disease or trauma to account for death. Pulmonary edema and congestion were very prominent.

Materials and Methods

Chemicals

All solvents were of HPLC grade or better and were obtained from Panreac (Milan, Italy). Water was purified by filtering deionized water on a Milli-Q Simplicity 185 filtration system (Millipore; Bedford, MA). Pure formic acid for mass spectrometry (MS) was purchased from Sigma-Aldrich (St Louis, MI). Methanolic solutions of standards (1 mg/mL) were prepared using pure solid material available in stock, and stored at −20 °C.

Mobile phase components were 0.1% formic acid (A) and acetonitrile (B). The aqueous solution was filtered on 0.45 μm PTFE filters (SUN-Sri, Duluth, GA). Filters were ultrasonicated for 20 s in acetonitrile before use.

Systematic toxicological analysis

Femoral blood was initially subjected to a systematic toxicological analysis by gas chromatography–mass spectrometry (GC–MS). The method used for sample preparation and instrumental analysis is described in detail in a previous published study by Poletti et al. (6). The method is able to identify over 300 drugs of forensic interest at therapeutic and toxic levels. The analysis confirmed the presence of lormetazepam that has undergone quantitative measurement using liquid chromatography–tandem mass spectrometry (LC–MS–MS) (7). None of the primary Tanax compounds were detected by the GC–MS screening. Detection and quantitation of embutramide and mebezonium iodide were therefore performed with a specific LC–MS–MS method.

Sample preparation

The following biological matrices were submitted for the analysis: cardiac and femoral blood, liver, muscle and vitreous humor. Liver and muscle were initially homogenized with water (1:1). Lidocaine, used as an internal standard (IS), was added at 20 μg/mL to 2 mL of the liver and muscle mixture and to 1 mL of blood and vitreous humor. Samples were then deproteinized, first with 1 mL methanol and second with 1 mL acetonitrile, centrifuged at 10,800 g for 10 min
finally diluted with deionized water (1:10). A 10-μL aliquot was injected in the LC–MS-MS system.

**Instrumental parameters**

The method was developed with an Agilent 1100 Series system (vacuum degasser, binary pump and column compartment) and an Agilent 1200 Series isocratic pump and autosampler maintained at 4°C (all from Agilent Technologies, Palo Alto, CA) interfaced to a 4000 QTrap (Applied Biosystem SCIEX, Foster City, CA) by an electrospray (ESI) Turbo V Ion Source.

The LC injector needle was externally washed with water before any injection. Two Chrompack Inertsil ODS-3 columns (100 x 3 mm, 3 μm particle size), both equipped with a Chrompack (10 x 2 mm) RP guard column (Varian, Walnut Creek, CA), were kept at 28°C during the analysis. The flow rate was kept constant at 0.2 mL/min and the gradient was set as follows: from 90% A (formic acid 0.1%) to 90% B (acetonitrile) in 10 min, rinsing step at 100% B for 3 min. The column oven was kept at 25°C. MS was performed using a 4000 Q-TRAP (Applied Biosystems/MD5 SCIEX). LC flow was directed into the ion source using the following settings: ion-spray voltage, 4,500 V; source temperature, 350°C; nebulization and heating gas (air), 30 and 35, respectively; and curtain gas (nitrogen), 15. Multiple reaction monitoring (MRM) was performed using nitrogen as collision gas (with pressure set at a medium level) and a dwell time of 100 ms. Table 1 shows MRM parameters for embutramide, mebenzonium iodide, and lidocaine (IS). The following transitions were used as quantifier ions: m/z 147.8 → 60.1 for mebenzonium iodide; m/z 294.2 → 121.2 for embutramide; and m/z 234.7 → 86.1 for lidocaine.

**Validation**

Validation parameters were evaluated for blood only. Ten blank blood samples collected from other autopsy cases were used for validation. A five-point linear calibration curve ranging from 0.05 to 5.00 mg/L was used to quantify concentrations of the three compounds in the biological matrices and the linearity was evaluated by the measurement of the regression coefficient. Accuracy and intra-day and inter-day imprecision were measured at two different quality control levels (0.1 and 1 mg/L). Ion suppression was measured by comparing blood samples spiked at the concentrations of 0.1 and 1 mg/L and water solutions of embutramide and mebenzonium iodide spiked with the same standard concentrations.

**Results**

Validation parameters of the method fulfilled the suggested international threshold values (8) for both mebenzonium iodide and embutramide, as shown in Table II. A limit of detection (LOD) of 0.01 mg/L was calculated for both substances by evaluating a signal-to-noise ratio of 3, while a limit of quantitation (LOQ) of 0.05 was measured by calculating the linearity of the curve at those levels. Accuracy and precision measured on five different replicates showed percentages lower than 10% for both inter-day and intra-day assays.

Mebenzonium iodide and embutramide concentrations in samples analyzed are reported in Table III (Figure 1 shows a chromogram of the case sample). Embutramide levels ranged from 2.74 mg/L in vitreous humor to 5.06 mg/L in femoral blood. Mebenzonium iodide was found at widely differing concentrations (ranging from 2.80 mg/kg in muscle to 24.80 mg/kg in liver).

**Discussion and Conclusions**

A simple, rapid, robust and validated method for the direct simultaneous determination of mebenzonium iodide and embutramide in biological matrices has been developed. The method has been successfully applied to a real-life positive case. This study, for the first time to the best of our knowledge, measured mebenzonium and embutramide concentrations in different human matrices and tissues.

In the case presented here, lormetazepam was also detected and measured in femoral blood at a concentration of 0.22 mg/L. Although toxic levels of lormetazepam in whole blood have not been reported, the same concentration in

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**Table I**

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRM* Transition</th>
<th>DP*</th>
<th>EP*</th>
<th>CE*</th>
<th>CXP*</th>
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</thead>
<tbody>
<tr>
<td>Mebenzonium iodide</td>
<td>m/z 147.8 → 60.1</td>
<td>15</td>
<td>27</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m/z 147.8 → 95.1</td>
<td>75</td>
<td>27</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Embutramide</td>
<td>m/z 294.2 → 121.2</td>
<td>10</td>
<td>41</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m/z 294.2 → 148.3</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Lidocaine (IS)</td>
<td>m/z 234.7 → 86.1</td>
<td>50</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m/z 234.7 → 57.9</td>
<td>50</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>


**Table II**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Linearity (R²)</th>
<th>LOD (mg/L)</th>
<th>LOQ (mg/L)</th>
<th>QC (mg/L)</th>
<th>Accuracy (CV%)</th>
<th>Precision (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mebenzonium iodide</td>
<td>0.9999</td>
<td>0.01</td>
<td>0.05</td>
<td>0.1</td>
<td>15.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Iodide</td>
<td>1.0</td>
<td>0.6</td>
<td>8.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embutramide</td>
<td>0.9999</td>
<td>0.01</td>
<td>0.05</td>
<td>0.1</td>
<td>15.0</td>
<td>9.9</td>
</tr>
</tbody>
</table>
| QC: Quality Control

**Table III**

<table>
<thead>
<tr>
<th>Biological Matrix</th>
<th>Mebenzonium Iodide (mg/L – mg/Kg)</th>
<th>Embutramide (mg/L – mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral blood</td>
<td>13.80</td>
<td>5.10</td>
</tr>
<tr>
<td>Cardiac blood</td>
<td>14.00</td>
<td>4.90</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>3.10</td>
<td>2.70</td>
</tr>
<tr>
<td>Liver</td>
<td>24.80</td>
<td>3.80</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.80</td>
<td>3.40</td>
</tr>
</tbody>
</table>

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serum would lead to a level tenfold higher than the therapeutic level (0.02 mg/L) (9). Hence, it is probable that this concentration would have a significant neurodepressant effect; this fact could explain the second (and lethal) self-injection of Tanax in the gluteus region after the failed injection attempt in the biceps area, as reported in the autopsy.

Figure 1. Chromatograms obtained from cardiac blood sample.
Although a high level of lorazepam was measured in blood, concentrations of embutramide and mebezonium iodide were consistent with other reported suicides, and therefore death was judged to be a consequence of Tanax injection.

References