Anesthesiologist Suicide with Atracurium

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Abstract

Atracurium is a nondepolarizing skeletal muscle relaxant used to facilitate endotracheal intubation and to induce skeletal muscle relaxation during surgery or mechanical ventilation. The drug undergoes a spontaneous non-enzymatic biotransformation, yielding laudanosine and an acrylate moiety. This report documents the case of a 45-year-old anesthesiologist who was found dead at the hospital where he worked. The victim was known to be depressed and undergoing treatment with venlafaxine. An empty syringe was found near the body. Toxicological analysis revealed the presence of laudanosine in the syringe, 0.6 mg/L of laudanosine in heart blood, 0.3 mg/L in urine, and 0.02 mg/L in vitreous humor. Meanwhile, concentrations of venlafaxine and O-desmethyl-venlafaxine, its active metabolite, were 0.7 and 1.1 mg/L in heart blood, 1.7 and 5.2 mg/L in urine, 0.5 and 0.7 mg/L in vitreous humor, and 400 and 20 mg in gastric content, respectively. All drugs and metabolites involved in the case were detected using gas chromatography with nitrogen-phosphorus detection (GC-NPD) and confirmed using GC-mass spectrometry in full scan mode after solid-phase extraction using Bond-Elut Certify columns. Additional high-performance liquid chromatography coupled to diode-array detection screening also obtained the same results. Quantitation of laudanosine and venlafaxine together with its metabolite was carried out using GC-NPD. No other drugs, including ethanol, were detected. Recoveries for laudanosine and venlafaxine were 89% and 86%, respectively, at 0.5 mg/L; intraday and interday precisions were 2% and 6%, and 3% and 7%, respectively; and limits of detection and quantitation were 6 and 20 ng/mL and 18 and 59 ng/mL, respectively. The linearity of the blood calibration curves was excellent for both drugs with r² values of > 0.999 (range 0.1-2.0 mg/L). Based on the autopsy findings, case history, and toxicology results, the forensic pathologists ruled that the cause of death was an overdose of atracurium, and the manner of death was suicide.

Introduction

Atracurium (Laurak®, Tracrium®) is a nondepolarizing skeletal muscle relaxant. Chemically it is a quaternary nitrogen muscle relaxant of the structural class of benzylisoquinolines. Atracurium was introduced in 1980. It is used to facilitate endotracheal intubation and induce skeletal muscle relaxation during surgery or mechanical ventilation. In Spain, atracurium is available as a 5 mg/mL solution of the besylate salt of this diquaternary ammonium compound for intravenous administration. A 0.3-0.6 mg/kg injection is the recommended initial dose for most patients (1).

Atracurium undergoes spontaneous degradation via Hofmann elimination, a non-enzymatic breakdown process occurring in plasma at physiological pH and temperature, to produce laudanosine and a quaternary monoacylate (Figure 1). An alternative elimination pathway is enzymatic ester hydrolysis. None of the products of the enzymatic hydrolysis possess pharmacologic activity, although laudanosine produces excitation of the central nervous system in animals. The drug is excreted in urine and bile, mostly as metabolites, and the elimination half-life is about 20 min for atracurium and 2 to 21 h for laudanosine. More than 90% of a dose of atracurium, mainly as metabolites, is excreted in the urine within 7 h following intravenous injection (2).

Muscle relaxation begins in about 2 min after intravenous administration and lasts for 15-35 min depending on the dose. Recovery is generally 95% complete 1 h postinjection (2,3).

Most adverse effects of atracurium may be related to the drug's histamine-releasing property. Among them, hypertension and tachycardia and hypotension and bradycardia have been described (4,5). Atracurium has unique properties that separate it from other available nondepolarizing agents such as pancuronium and tubocurarine. It has more potent neuromuscular blocking properties with minimal cardiovascular effects, and it is a less potent releaser of histamines. Atracurium does not depend on hepatic or renal function for metabolism and elimination.

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Figure 1. Chemical structures of atracurium and laudanosine.
Venlafaxine (Dobupal®, Vandra®) is a phenethylamine derivative that inhibits the reuptake of certain neurotransmitters, including serotonin and norepinephrine. It is available for use as an antidepressant in the form of 37.5-150-mg tablets as the hydrochloride salt. Daily oral doses are normally in the range of 75–200 mg (1).

Previous reports suggested that venlafaxine is relatively safe in overdose, although combination with other pharmaceuticals or abuse drugs could increase its toxicity (6).

A literature search for papers on poisoning by atracurium cited in Medline revealed very few cases (4,5,7,8). Several fatal intoxications following atracurium administration have been reported, but analytical data are generally poor (8,9). A recent case involving the suicide of an anesthesiologist is presented in this work.

Case History

The deceased was a 45-year-old anesthesiologist. He had been on call the night before and was found dead in the doctor’s room of his hospital after being on duty the previous day. Information about the victim’s medical history was obtained from both the victim’s family and colleagues. The victim was known to be depressed and undergoing treatment with venlafaxine; he also had access to muscle relaxants. An empty syringe was found near the body. The medical examiner performed an autopsy. No evidence of violence was noted. Heart blood, vitreous humor, urine, gastric contents (250 g), and the empty syringe were collected and submitted to our laboratory for toxicological analysis.

The manner of death was listed as suicide, in view of the circumstances.

Experimental

Materials

Laudanosine and mepivacaine, the internal standard (IS), were purchased from Sigma Aldrich (Buch, Switzerland), and venlafaxine was kindly provided by Wyeth Laboratories (Hants, U.K.). O-Desmethyl-venlafaxine (ODV) was unavailable. Individual stock solutions were prepared by dissolving 10 mg of each pure compound in 10 mL of methanol. These stock solutions were stored in glass tubes and maintained at −27°C. Appropriate dilution with methanol yielded the working solutions containing all compounds involved in the study. The IS solution was prepared by diluting the stock solution of mepivacaine with deionized water to 16 mg/L.

All chemicals (Merck, Darmstadt, Germany) and solvents (Scharlau, Barcelona, Spain) were of analytical grade. Phosphate buffer (0.1M, pH 6.0), 0.01M acetic acid, acetone/dichloromethane (1:1), and dichloromethane/isopropanol/ammonia (78:14:8) were used for the solid-phase extraction (SPE) procedure.

Bond Elut Certify columns (130 mg of sorbent mass, 3 mL of column reservoir volume) and a VAC-ELUT SPS 24 vacuum manifold system for the manual mixed-mode bonded silica SPE were purchased from Varian Sample Preparation Products (Harbor City, CA).

Extraction

All blood calibrators, controls, blank, and case specimens (including appropriate dilutions with deionized water when necessary) were prepared following an SPE procedure based on our previously published work (10). The extraction was performed on a VAC-ELUT SPS 24 vacuum manifold system. To each 2.5 mL of sample, 7.5 mL of 0.1M phosphate buffer (pH 6.0) and 125 μL of a mepivacaine aqueous solution of 16 mg/L as IS were added, sonicated for 5 min, and centrifuged at 4000 rpm for 10 min. Then 8 mL of the supernatant, equivalent to 2 mL of whole blood, was used for further extraction. The columns were preconditioned with 1 mL methanol, followed by 1 mL 0.1M phosphate buffer (pH 6.0) under light vacuum, approximately 2 in. Hg, to avoid the columns becoming dry before the application of the sample. Then the samples of pretreated whole blood were applied onto the columns and drawn through completely at a flow rate of approximately 1.5 mL/min. The columns were washed with 2 mL of deionized water. The columns were acidified by passing through 0.5 mL of 0.01M acetic acid. The columns were then dried under full vacuum (15 in. Hg) for 4 min. Methanol (60 μL) was added, and the columns were dried under full vacuum for 1 min. Drugs were eluted from the column by adding first 3.5 mL of acetone/dichloromethane (1:1) and then 3 mL of dichloromethane/isopropanol/ammonia (78:14:8). The eluents were pulled through completely at a flow rate of 0.8 mL/min and 0.5 mL/min, respectively, and the combined eluates were evaporated at 50°C under a nitrogen stream. The extraction residues were reconstituted with 200 μL of methanol.

Instrumentation

Gas chromatography with nitrogen-phosphorus detection (GC–NPD) analysis used for screening and quantitation was performed with an Agilent 6890 N apparatus controlled by a ChemStation software with a 25-m (0.20 mm i.d., 0.11-μm film thickness) Ultra-1 methyl siloxane capillary column, all from Agilent Technologies (Avondale, PA). The GC conditions were as follows: carrier gas, helium (constant column pressure of 35 psi); split mode (split ratio 1:20); injector temperature, 280°C; oven temperature, programmed from 180°C (initial time 1 min) to 300°C (final time 3 min) at 10°C/min; and detector temperature, 300°C. The chromatographic time was 16 min.

Gas chromatography–mass spectrometry (GC–MS) used for confirmation analysis was performed with an HP 1800 A GCD system controlled by ChemStation software (Hewlett-Packard, Avondale, PA) with a 30-m (0.25-mm i.d., 0.25-μm film thickness) HP-5 capillary column (Agilent Technologies). The chromatographic conditions were as mentioned previously, with the exception of the oven temperature that was programmed from 175°C (initial time 1 min) to 300°C (final time 8 min). The chromatographic time was 21.5 min. The MS
conditions were as follows: full scan (m/z 50–425) mode, electron impact ionization with a 70 eV energy, and transfer line and ion-source temperatures were both maintained at 280°C.

**Results**

A comprehensive toxicological screening was performed on the deceased's biological samples. This included ethanol and other volatiles (methanol, acetone, n-propanol, and isopropanol) in heart blood and urine by headspace GC–flame ionization detection (FID). The results obtained ruled out the presence of these volatiles. Immunoassay screening of the urine sample was performed on a Hitachi 902 Automatic Analyzer (Tokyo, Japan) using Cedia reagents (Microgenics, Fremont, CA) and was negative for propoxyphene, cocaine and benzoylecgonine, methadone, opiates, cannabinoids, benzodiazepines, amphetamine and related compounds, barbiturates, and tricyclic antidepressants.

The empty syringe was rinsed with methanol and submitted to a GC–MS full scan mode screening in which laudanosine was detected. Acidic-neutral and basic drug screening of the deceased's heart blood, vitreous humor, urine, and gastric content was carried out using GC-NPD after SPE (Figure 2) and revealed the presence of laudanosine, venlafaxine, and ODV, which was confirmed using GC–MS full scan mode (Figure 3). An additional high-performance liquid chromatography–diode-array detection screening also obtained the same results. Quantitation of laudanosine, venlafaxine, and its metabolite, ODV, was carried out using GC–NPD after re-extraction of the case samples using whole blood calibration curves of each drug (range 0.1 to 2 mg/L). Quantitation of ODV was performed using venlafaxine as reference standard considering its unavailability in the market, and its structural similarity. Drug concentrations were calculated by comparing the peak-area ratio of each drug with that of the IS against the blood calibration curve. Toxicological findings are shown in Table I. Good recoveries, reproducibilities, sensitivities, and lineairties were obtained, respectively, for the drugs involved in this case; validation data are shown in Table II.

**Discussion**

A suicide case due to atracurium is reported here. The deceased was a 45-year-old anesthesiologist under venlafaxine therapy for his depression. Literature lacks sufficient data concerning fatal poisoning by atracurium, and even atracurium overdoses seem to be uncommon (4,5,8,9). Because no particular or specific anatomical changes are present with central nervous system depressants, analytical data are essential in these cases (8).

**Table 1. Toxicological Findings in the Suicide Case**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Laudanosine (mg/L)</th>
<th>Venlafaxine (mg/L)</th>
<th>ODV (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart blood</td>
<td>0.6</td>
<td>0.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Urine</td>
<td>0.3</td>
<td>1.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>0.02</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Gastric content</td>
<td>ND*</td>
<td>400 mg</td>
<td>20 mg</td>
</tr>
<tr>
<td>Total amount (mg)$^*$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syringe</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*$^*$ ND: not detected.
$^*$ Total amount (mg)/total amount of gastric content.
Atracurium contains two quaternary amine functions, therefore it will neither extract by conventional liquid–liquid extraction procedures with nonpolar organic liquids, nor chromatograph on most GC columns. Atracurium was found to be chemically stable when stored in disposable syringes in its undiluted form at room temperature for a period of up to six weeks; however, when diluted to be administered, it degrades to its degradation products (11). In addition, atracurium is highly unstable in vitro, even after acidification of plasma. On these bases, laudanosine, which is much more stable, has been used as a marker of atracurium usage by forensic investigators (8,12). In our case laudanosine together with other drugs were detected in postmortem samples as part of systematic toxicological analysis using a mixed SPE procedure and a GC–NPD/MS toxicological screening procedure used routinely in our laboratory, which is more convenient and practical for the forensic toxicologists.

Only laudanosine was found in an apparently empty syringe near the body. Although it has been demonstrated in vitro and in vivo that one molecule of atracurium degrades spontaneously to two molecules of laudanosine (13,14), there was no measurable residue volume of fluid to extrapolate to the original dose applied. Furthermore, extrapolations in biological or nonbiological samples cannot be done because part of atracurium can be sequestered in deep compartments, and other molecules of degradation would not have been measured under the present chromatographic conditions.

A good correlation has been observed between the plasma concentration of atracurium and its neuromuscular blocking action after both bolus injections and continuous infusions. Injection of 0.5 mg/kg of atracurium gave an average plasma concentration of 9.7 mg/L after 2 min, dropping to 0.2 mg/L in 60 min (15). Steady-state plasma concentrations in major surgery ranged from 0.73 to 1.47 mg/L (16). The measured laudanosine concentration in our case was similar to a fatal case reported by Kintz et al. (8). These authors found 0.917 mg/L of laudanosine in cardiac blood, 0.597 mg/L in urine, 0.018 mg/L in vitreous humor, and 0.084 mg/L in gastric contents. After an error, 37 mg was administered to an infant over 75 min. Plasma concentrations of laudanosine after cessation of infusion were 0.59 mg/L at 11.3 h, 0.18 mg/L at 14.5 h, and undetectable at 17.3 h (5). Laudanosine was subject to postmortem redistribution as shown by a heart-blood-to-peripheral-blood ratio of 2.4 (8). In fact, the high volume of distribution suggests a distribution outside the systemic circulation in other tissues and a possible redistribution after death. Therefore, our data could have overestimated the amount of circulating drug.

Any concentration in the absence of mechanical, controlled ventilation could be potentially fatal because of respiratory paralysis and hipoxia (3). Therapeutic administration of curare and related agents may cause respiratory paralysis, prolonged apnea, cardiovascular collapse, and respiratory arrest. The differences in concentrations between blood and urine could be considered a sign of short survival time after drug intake. Other cases of atracurium overdoses reported in the literature were iatrogenic because of a mistake with the solution (4,5). Even at a fivefold overdose, there was no problem because the patient was intubated during neuromuscular block, and neuromuscular blocking effects were reversed with intravenous neostigmine (7).

In clinical trials, serum venlafaxine concentrations in subjects measured within 2 h postdose ranged from 0.08 to 0.29 mg/L. Multiple-dose trials have yielded venlafaxine steady-state plasma concentrations of 0.07–0.27 mg/L and 0.24–0.52 mg/L of ODV, its active metabolite. Postmortem tissue concentrations studied in 12 postmortem cases for venlafaxine and ODV were 0.1–36 and < 0.05–3.5 mg/L (peripheral blood), < 0.05–55 and < 0.05–21 mg/L (urine), and < 0.05–10 and < 0.05–1.5 mg/L (vitreous), respectively, and 0.1–200 mg of venlafaxine in the gastric contents (6). Our concentrations were 0.7 and 1.1 mg/L, respectively, which are high for a therapeutic range, but not so high as in other overdoses. As the sample was heart blood, postmortem redistribution could have contributed (17). Besides, there are differences in the ratio of parent drug to metabolite in overdoses and therapeutic use, 10:1 and 0.5:2, respectively. The detection of only slightly elevated venlafaxine concentrations with significant concentrations of ODV is more likely indicative of the decedent having taken a high therapeutic dose of venlafaxine over an extended period of time than an acute overdose. Besides, even at extremely high concentrations, venlafaxine alone was not sufficient to cause death (6), and no interaction in metabolism pathways is possible with atracurium.

When people think of committing suicide, they use substances present in their environment. The literature with quaternary nitrogen muscle relaxant injections is scarce because they are substances not available to the general public. The detection and identification of laudanosine as a marker of

<table>
<thead>
<tr>
<th>Compound</th>
<th>Level of Fortification (mg/L)</th>
<th>Percent Recovery (n = 6)</th>
<th>Intraday Precision RSD* (%) (n = 6)</th>
<th>Interday Precision RSD (%) (n = 9)</th>
<th>Linearity r² (0.1–2 mg/L)</th>
<th>Limit of Detection (ng/mL)</th>
<th>Limit of Quantitation (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laudanosine</td>
<td>0.5</td>
<td>89</td>
<td>2</td>
<td>6</td>
<td>0.999</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>0.5</td>
<td>86</td>
<td>3</td>
<td>7</td>
<td>0.999</td>
<td>18</td>
<td>59</td>
</tr>
</tbody>
</table>

* RSD: relative standard deviation.
Atracurium poisoning is of importance in forensic toxicology because pathological findings are unspecific; however, there is a history of the presence of needles, syringes, and a recent needle mark in the victim.

References


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