Tizanidine Distribution in a Postmortem Case*

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Abstract

The case of a 57-year-old white female who was found deceased at home by her husband is presented. A suicide note was found at the scene. No remarkable findings were observed at autopsy. Comprehensive toxicological analysis of the heart blood identified ethanol (0.16 g/dL), diazepam (1.1 mg/L), and tizanidine (2.3 mg/L). Blood concentrations of tizanidine following therapeutic use do not exceed 0.025 mg/L. The medical examiner ruled that the cause of death was combined ethanol and multiple drug intoxication, and the manner of death was suicide.

Introduction

Tizanidine (Zanaflex®), is a centrally acting imidazoline muscle relaxant that is structurally similar to clonidine but not to other muscle relaxants such as baclofen or the benzodiazepines. Tizanidine is an α2-adrenergic agonist used to treat symptomatic muscle spasms and chronic spasticity associated with central nervous system disorders such as multiple sclerosis (1,2). It affects mainly the polysynaptic pathways, reducing the facilitation of spinal motor neurons. Some off-label uses include tension headache and trigeminal neuralgia (3).

Tizanidine (Figure 1) contains a cyclic guanidine moiety and can exist as two tautomers (4). It is available in 2- and 4-mg tablets as the hydrochloride salt with doses titrated from 4 mg/day to a maximum recommended daily dose of 36 mg (5). Capsule formulations in doses of 2, 4, and 6 mg are pending approval for sale (6).

Tizanidine is rapidly absorbed from the gastrointestinal tract with peak plasma concentrations reached within 1 to 2 h after oral administration (2). It is widely distributed throughout the body and undergoes extensive first pass metabolism in the liver (7). The elimination half-life has been reported between 2.1 and 4.2 h and up to 13.6 h for subjects with renal impairment (2). Multiple oxidative metabolites have been identified, but none with apparent pharmacological activity (8,9). In multi-dose tizanidine studies, the most frequent adverse effects reported were dry mouth, somnolence, lethargy, and dizziness (2). A retrospective study of 45 ingestions of tizanidine reported to a poison control center detailed lethargy, bradycardia, hypotension, and agitation as the most frequently cited clinical effects (3). Data on blood and tissue concentrations of toxic tizanidine doses are absent from the literature; however, peak plasma concentrations in living individuals following single and multiple doses did not exceed 0.025 mg/L (2). We report a case of tizanidine intoxication in a single individual in which measurements of postmortem tissues and fluids were performed.

Case History

A 57-year-old white female was found unresponsive at home by her husband. The subject was last seen 6 h earlier after attending a football game and becoming intoxicated at a bar. The husband went to sleep on the sofa and awoke to find the deceased bent over opposite the sofa. The subject had a history of ethanol abuse, prescription drug abuse, and smoking. The...
The decedent also had a prior history of suicide attempts, and a suicide note was found on a nearby table.

External examination identified fixed lividity on the posterior surfaces of the body, except in areas exposed to pressure. There was no evidence of trauma or medical intervention. Internal examination was limited to the brain and the liver; no remarkable findings were observed. Heart blood, bile, urine, vitreous humor, liver, and stomach contents were submitted for toxicological analysis.

**Experimental**

**Materials**

Tizanidine hydrochloride was obtained from Novartis (Basle, Switzerland). Internal standard 7-aminoclonazepam-d4 was purchased from Cerilliant (Austin, TX). STRATA X-C™ 200 mg/6 mL solid-phase extraction (SPE) columns were purchased from Phenomenex (Torrance, CA). N-Methyl-N-[trimethylsilyl]-tri-fluoroacetamide (MSTFA) was purchased from Pierce Chemical (Rockford, IL). All other chemicals were high-performance liquid chromatography grade and purchased from Fisher Scientific (Pittsburgh, PA).

**Extraction**

Standard curves were prepared in both urine and blood using certified negative sources. Calibrators were spiked with tizanidine at 0.01, 0.05, 0.1, 0.5, 1.0, and 5.0 mg/L. One gram of liver was homogenized in 3 mL of 0.01M HCl after the tissue was cut into small (2-4 mm) pieces. One milliliter of homogenate was used for analysis. Additionally, 1:1000, 1:100, 1:10, and 1:2 dilutions in deionized water were made for the gastric contents. One-milliliter volumes of samples or calibrators were added to individually labeled tubes, and 3 mL of 0.01M HCl was added to each. Fifty microliters of 7-aminoclonazepam-d4 in methanol (10 µg/mL; 0.5 mg/L final concentration) was added to all tubes. The tubes were capped, vortex mixed, and then centrifuged for 5 min at 3000 rpm.

The SPE cartridges were conditioned with 3 mL of methanol, 3 mL of deionized water, and 2 mL of 0.01M HCl. The samples were applied to the column and aspirated at a flow of 1–2 mL/min. The columns were then washed with 3 mL of deionized water, 3 mL of 0.01M HCl, and 3 mL of methanol. Columns were dried under vacuum for 10 min. Tizanidine and internal standard were eluted with 3 mL of ethyl acetate containing 4% concentrated ammonium hydroxide. The eluates were collected in clean tubes and evaporated to dryness under nitrogen at 40°C. The residue was reconstituted with 50 µL of MSTFA and incubated at 70°C for at least 90 min, which was sufficient to fully convert to the di-TMS derivative. The extracts were cooled to room temperature and transferred to glass autosampler vials for analysis.

### Table 1. Distribution of Tizanidine in the Presented Case

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Tizanidine Concentration</th>
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</thead>
<tbody>
<tr>
<td>Heart blood</td>
<td>2.34 mg/L</td>
</tr>
<tr>
<td>Urine</td>
<td>0.055 mg/L</td>
</tr>
<tr>
<td>Liver</td>
<td>9.19 mg/kg</td>
</tr>
<tr>
<td>Bile</td>
<td>3.37 mg/L</td>
</tr>
<tr>
<td>Gastric</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

Figure 2. Extracted ion chromatograms of tizanidine (10.56 min) and 7-aminoclonazepam-d4 (11.51 min) extracted from case blood.
Instrumentation

One-microliter splitless injections were made onto an Agilent Technologies (Palo Alto, CA) 6890 gas chromatograph (GC) equipped with an Rtx-200 capillary column (30 m x 0.25-mm i.d., 0.25-μm film thickness, Restek, Bellefonte, PA). The GC oven was held for 1 min at 70°C, ramped to 290°C at 20°C/min, and held for an additional minute. The transfer temperature was heated to 280°C, and the injector temperature was kept at 250°C. Helium was used as the carrier gas with a constant flow of 1.0 mL/min. An Agilent 5973 mass selective detector was used for the selected-ion-monitoring of m/z 397, 362, and 214 for tizanidine and m/z 433 and 398 for the internal standard.

Identification was by retention time matching with a tolerance of ± 2% and ion ratio matching within ± 20% of those calculated for the calibrators. Quantitation was performed by internal standard linear regression using the peak areas of m/z 397 and 433 for tizanidine and internal standard, respectively. The quantitation of the urine specimen was done using the standard curve prepared in negative urine and all other specimens were calculated using the blood standard curve.

Results

The heart blood was tested for volatile substances, and the urine specimen was tested for therapeutic and abused drugs. This included volatile testing for methanol, ethanol, acetone, and isopropanol by headspace GC; acid/neutral drug testing by GC–nitrogen-phosphorus detection (NPD); alkaline drug testing by GC–NPD; morphine and benzodiazepines by immunoassay; and acetylmethionine, ethchlorvynol, and salicylate by color test. The heart blood ethanol concentration was 0.16 g/dL. The urine and vitreous humor ethanol concentrations were 0.21 and 0.19 g/dL, respectively; no other volatile substances were detected. The alkaline drug screen identified diphenhydramine, which was confirmed by GC–mass spectrometry (MS). No diphenhydramine was detected in the blood. An alkaline drug screen of the blood identified diazepam and tizanidine; each was confirmed by full scan electron ionization GC–MS. The blood diazepam concentration was 1.1 mg/L; no nordiazepam was detected at a limit of detection of 0.1 mg/L.

Tizanidine was detected in all samples analyzed. The toxicological results are listed in Table I. Tizanidine was quantitated in the heart blood (Figure 2), bile, gastric contents, and liver specimens using a six-point calibration curve of spiked blood. The limit of quantitation was 0.01 mg/L, and the upper limit of linearity was 5.0 mg/L. The calibration curves for liver, blood, bile, and gastric contents had R2 values of 0.999, 0.991, 0.991, and 0.983, respectively. The calibration curve for urine had an R2 value of 0.999.

Discussion

Pharmacokinetic studies have reported peak plasma concentrations following single or multiple oral doses of tizanidine between 0.003 and 0.025 mg/L, depending on the dose (2). The concentration of tizanidine in biological specimens following toxic episodes has not been included in published reports. The most commonly related symptoms of intoxication have been lethargy, bradycardia, hypotension, and agitation (3,10,11). These symptoms, particularly the hypotensive and sedative effects, are also noted with therapeutic doses of tizanidine when taken in combination with cytochrome P450 (1A2) inhibitors such as fluoxetine (12,13) or ciprofloxacin (14).

The structurally related agent clonidine and the phenylaminohiazine derivative xylazine (Figure 1) are both centrally acting α2-receptor agonists like tizanidine. Clonidine is prescribed as a hypotensive agent and as a treatment of attention-deficit hyperactivity disorder (ADHD); xylazine is used in veterinary practice for sedation and general anesthesia. In contrast to tizanidine, clonidine has a slower onset and longer duration of action in humans, whereas xylazine is exclusively used for veterinary practice because of the severe hypotension it produces in humans.

Numerous nonfatal overdoses have been reported for clonidine. Romano and Dinh (15) reported on a 5-year old with ADHD that received an oral dose of approximately 50 mg of clonidine due to a pharmacy compounding error. Seventeen hours postingestion, the serum clonidine concentration was measured as 0.064 mg/L. Clonidine was surreptitiously administered in four cases of criminal submission (16). Blood concentrations determined within 24 h after the administrations ranged from 0.013 to 0.068 mg/L with symptoms including hallucinations, dizziness, and hypotension. Domino et al. (17) reported the accidental ingestion of 100 mg of clonidine by a 28-year-old man. Gastric lavage was performed to remove 57 mg of the dose with a peak plasma concentration of 0.23 mg/L achieved 1 h after administration. Both hypertensive and hypotensive periods of toxicity were noted. Lukkari et al. (18) reported a fatal overdose of clonidine with postmortem blood, brain, and kidney concentrations of 0.023 mg/L, 0.024 mg/kg, and 0.086 mg/kg, respectively.

Overdoses with xylazine have been reported in instances of recreational abuse, suicide, and homicide. Hoffmann et al. (19) reported the accidental ingestion of 100 mg of clonidine by a 28-year-old man. Gastric lavage was performed to remove 57 mg of the dose with a peak plasma concentration of 0.23 mg/L achieved 1 h after administration. Both hypertensive and hypotensive periods of toxicity were noted. Lukkari et al. (18) reported a fatal overdose of clonidine with postmortem blood, brain, and kidney concentrations of 0.023 mg/L, 0.024 mg/kg, and 0.086 mg/kg, respectively.

The present case is unique in that no other reports of tizanidine overdose are available in which quantitative measurements have been included. The postmortem blood concentration is well in excess of peak levels in therapeutic trials and is comparable with the levels in toxic overdoses of similar α2-adrenergic agonists. The medical examiner ruled the cause of death as ethanol and combined drug intoxication and the manner of death as suicide. Because ethanol and diazepam...
clearly played a role in this intoxication case, any comparison of this case with other potential tizanidine intoxication cases must be made with some caution.

References


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