Comprehensive Duloxetine Analysis in a Fatal Overdose

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Abstract

Duloxetine is a second-generation selective serotonin and norepinephrine reuptake inhibitor used primarily for the treatment of depression. Relatively few fatalities have been reported in association with its use. Similarly, there are no known reports that provide a comprehensive analysis of blood, fluid and tissue samples in an overdose setting. Herein we present a fatal case of duloxetine toxicity with both the highest reported post-mortem blood concentration and a comprehensive toxicological analysis of duloxetine in femoral blood, vitreous humor, liver tissue, urine and gastric contents. In doing so, we hope to provide data that can assist both toxicologists and forensic pathologists with assessing duloxetine toxicity in the future.

Introduction

Duloxetine (Cymbalta®) is a potent and selective serotonin and norepinephrine reuptake inhibitor (SNRI), available in delayed-release capsule form. Its chemical designation is (+)-(S)-N-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride (1) (Figure 1). The empirical formula is C18H19NOS·HCl, which corresponds to a molecular weight of 333.88 g/mol (2).

It has regulatory approval for the management of a number of central nervous system (CNS) conditions, including as an analgesic for diabetic peripheral neuropathic pain, fibromyalgia and chronic musculoskeletal pain. This second-generation drug is also used in the treatment of major depressive disorder, generalized anxiety disorder and stress urinary incontinence in women (1). This expanding scope of usage along with several known prescription drug–drug interactions may lead to new concerns in post-mortem toxicology. Duloxetine has been associated with serotonin toxicity in combination with certain drugs, but there are still few known cases of toxicity only due to duloxetine overdose (3). Like the other second-generation SNRIs, duloxetine had fewer side effects than tricyclic antidepressants (TCA) and are less likely to result in fatality following an overdose (3). Duloxetine overdose symptoms can include somnolence, coma, serotonin syndrome, seizures, syncope, tachycardia, hypotension, hypertension and vomiting (2).

Duloxetine hydrochloride is prescribed at daily doses of 60–120 mg with doses for the treatment of anxiety and/or depression typically ranging from 40 to 60 mg, either once daily or in two divided doses [2], http://www.pdr.net/drug-summary/cymbalta (accessed July 10, 2015)]. The maximum plasma concentration occurs after 6 h, and steady state is reached within 3 days. Duloxetine is highly bound (>90%) to proteins in human plasma, with the apparent volume of distribution averaging ~1,640 L (1). Metabolism occurs predominantly in the liver with biotransformation involving the cytochrome P450 system with 80–90% through the CYP1A2 and CYP2D6 iso-enzymes to numerous nonactive metabolites (4). The kinetics exhibit a linear fashion within the recommended dosage levels, beyond which saturation is evident with a nonlinear kinetic rate. The mean elimination half-life (t1/2) is approximately 12 h, ranging from 8 to 17 h in clinical trials (4).

The American Association of Poison Control Centers (AAPCC) reported 3,428 poisoning cases involving duloxetine in 2013 (5). In addition, they report that duloxetine-related fatality numbers have increased from 1 case in 2004 to 14 in 2007 (4). Despite this fact, relatively few cases of fatal duloxetine toxicity have been described in the literature. In a report from Australia, between 2009 and 2012 there were 19 mixed drug fatalities where duloxetine was present. The highest reported duloxetine concentration in femoral blood in this
A 70-year-old Caucasian female with a medical history significant for valvular heart disease with remote valve replacement, hypertension, chronic back pain with multiple surgeries and prior medication overdose and remote alcohol abuse was found lying on the floor in her residence when a neighbor performed a well-being status check. She was awake, but confused, and complained of a dry mouth and also voiced that she had been on the floor since earlier that morning. An empty vodka bottle was found near her, and she was incontinent of urine. She was taken to a nearby hospital where she was found to be afebrile (37.2°C), normotensive (130/101 mmHg), tachycardic (104 beats per minute) and tachypneic (22 breaths per minute). Initial labs showed an elevated white blood cell count with mildly elevated blood urea nitrogen (BUN) and creatinine levels and an anion gap metabolic acidosis. A chest X-ray showed suspected right lung pneumonia and a CT scan of the head showed no trauma. Shortly after her return from radiology testing, she sustained a cardio-pulmonary arrest and was unable to be successfully resuscitated.

Autopsy examination was performed the following day and showed an obese female weighing 168 pounds and 61 inches in height (BMI = 31.7 kg/m²) with evidence of prior bio-prosthetic aortic valve replacement showing no gross or microscopic vegetations. No microorganisms were isolated from post-mortem blood cultures. There was mild systemic atherosclerosis and hypertension with an enlarged and dilated heart (450 g). The gastric contents consisted of 40 cc of thick, brown-yellow liquid with no foreign objects or detectable pills. No injuries were present with the exception of cardiopulmonary resuscitation-related anterior rib fractures. No pneumonia was seen grossly or microscopically.

A femoral blood sample of 50 mL was collected during intake of the body at the medical examiner’s office (~3 h after death) via a transcatheter draw. Additional toxicology samples taken during autopsy examination (~20 h after death) were as follows: vitreous humor, gastric contents, liver tissue and urine. No central blood was collected. Medications/containers collected by emergency medical personnel at the scene are listed in Table I.

### Table I. Medications Collected by Emergency Medical Personnel

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dosage</th>
<th>Rate</th>
<th>Days since issued</th>
<th>Total issued</th>
<th>Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buspirone HCl</td>
<td>10 mg tab</td>
<td>1 TID</td>
<td>70</td>
<td>270</td>
<td>16</td>
</tr>
<tr>
<td>Cyclobenzaprine</td>
<td>10 mg tab</td>
<td>1 TID, pm</td>
<td>10</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>60 mg cap</td>
<td>1 QD</td>
<td>33</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>Furosemide</td>
<td>20 mg tab</td>
<td>1 QD</td>
<td>4</td>
<td>30</td>
<td>38</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>400 mg cap</td>
<td>1 QD</td>
<td>69</td>
<td>360</td>
<td>17</td>
</tr>
<tr>
<td>Hydroxyzine</td>
<td>25 mg cap</td>
<td>1 TID, pm</td>
<td>118</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>K-chlor</td>
<td>8 meq tab</td>
<td>1 QD</td>
<td>38</td>
<td>85</td>
<td>61</td>
</tr>
<tr>
<td>Levothyroxine</td>
<td>100 mcg tab</td>
<td>1 QD</td>
<td>4</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>100 mg tab</td>
<td>1 QD</td>
<td>83</td>
<td>90</td>
<td>25</td>
</tr>
</tbody>
</table>

series was 1.42 mg/L (3). Anderson et al. reviewed post-mortem levels of duloxetine in 12 fatalities with peripheral blood concentrations ranging from 0.0 to 0.26 mg/L (6). Similarly, Vey and Kovelman reported on six deaths in which duloxetine was detected, with the highest post-mortem central blood level of 2.5 mg/L (4). While these isolated reports describe blood levels, there are no known data on the tissue and body fluid levels of duloxetine in the overdose/toxicity setting. Therefore, we report a case of a mixed drug fatality where duloxetine was measured in femoral blood, vitreous fluid, liver tissue, urine and gastric contents.

### Case report

A 70-year-old Caucasian female with a medical history significant for valvular heart disease with remote valve replacement, hypertension, chronic back pain with multiple surgeries and prior medication overdose and remote alcohol abuse was found lying on the floor in her residence when a neighbor performed a well-being status check. She was awake, but confused, and complained of a dry mouth and also voiced that she had been on the floor since earlier that morning. An empty vodka bottle was found near her, and she was incontinent of urine. She was taken to a nearby hospital where she was found to be afebrile (37.2°C), normotensive (130/101 mmHg), tachycardic (104 beats per minute) and tachypneic (22 breaths per minute). Initial labs showed an elevated white blood cell count with mildly elevated blood urea nitrogen (BUN) and creatinine levels and an anion gap metabolic acidosis. A chest X-ray showed suspected right lung pneumonia and a CT scan of the head showed no trauma. Shortly after her return from radiology testing, she sustained a cardio-pulmonary arrest and was unable to be successfully resuscitated.

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A femoral blood sample of 50 mL was collected during intake of the body at the medical examiner’s office (~3 h after death) via a transcatheter draw. Additional toxicology samples taken during autopsy examination (~20 h after death) were as follows: vitreous humor, gastric contents, liver tissue and urine. No central blood was collected. Medications/containers collected by emergency medical personnel at the scene are listed in Table I.

### Experimental

#### Post-mortem specimen collection

Femoral blood was collected ~3 h after death as described above. Vitreous, urine, liver and gastric contents were collected at the time of the autopsy ~20 h after death. All samples were refrigerated until analyzed.

#### Toxicology analysis

The post-mortem femoral blood was screened for alcohol and volatile compounds (GC-FID headspace), carbon monoxide (UV-Vis), ethylene glycol (GC-MS), common drugs of abuse by ELISA (amphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine/metabolite, fentanyl, methamphetamine, methadone, opiates, oxycodone/oxymorphine and phencyclidine) (Immunoanalysis Inc., Pomona, CA), a standard acid/neutral drug screen following a liquid–liquid extraction similar to that published by Lo et al. (7) and a standard alkaline drug screen by GC-MS following a liquid–liquid extraction similar to that published by Foerster and Mason (8). Positive results were confirmed and quantified by subsequent and specific techniques.

#### Materials

Solvents; methanol was obtained from EMD Chemicals (Germany), OmniSolv grade was purchased through VWR International (Randor, PA) and JT Baker through Fisher Scientific (Hampton, NH), acetonitrile (20%) in water from EMD (Germany) was obtained from Fisher Scientific (Hampton, NH), n-butyl chloride was from EMD Chemicals (Germany), OmniSolv grade was purchased through VWR International (Randor, PA), 80:20 water–acetonitrile, 5 mM ammonium bicarbonate (Fluka) and 0.2% acetic acid in acetonitrile (Sigma) were purchased from Sigma (St. Louis, MO). Concentrated ammonium hydroxide (NH₄OH), concentrated hydrochloric acid (HCl) and 0.5 M sulfuric acid (H₂SO₄) were purchased from JT Baker through Fisher Scientific (Hampton, NH) and (1% v/v) methanolic HCL was prepared in-house.

**Positive calibrators/control:** diphenhydramine was obtained from Cerilliant (Round Rock, TX). Second source diphenhydramine control obtained from Lipomed (Cambridge, MA), prepared in methanol. **Internal standard:** diphenhydramine-d₃ obtained from Cerilliant (Round Rock, TX).

**Positive calibrators/control:** cyclobenzaprine obtained from Cerilliant (Round Rock, TX). Second source control obtained from Lipomed (Cambridge, MA), prepared in methanol. **Internal standard:** cyclobenzaprine-d₃ obtained from Toronto Research (North York, ON, Canada) in methanol.
Methods

Alkaline drug screen

Using mepivacaine as the internal standard and a modified liquid-liquid extraction similar to that published by Foerster and Mason (8), the case sample and the corresponding controls were analyzed using GC–MS in full scan mode. Diphenhydramine, cyclobenzaprine and duloxetine were identified based on the relative retention time and full scan mass spectra.

Presumptive identification of analytes by MS full scan mode includes a retention time (RRT) match compared with a reference standard and a library match containing at least the three most abundant ions in appropriate proportions.

Diphenhydramine and cyclobenzaprine were quantified in-house. The femoral blood, vitreous, liver, urine and gastric were sent to NMS Laboratories to quantitate duloxetine.

Diphenhydramine quantitation analysis (GC–MS SIM)

Diphenhydramine was measured using a deuterated internal standard and a modified Foerster (8) extraction procedure. Calibrators from Cerilliant (Round Rock, TX) and controls from Lipomed (Cambridge, MA) were prepared in methanol from 1.0 mg/mL standards. Diphenhydramine-d3 was purchased from Cerilliant (Round Rock, TX). The analysis was performed by an Agilent Technologies 7890A gas chromatograph and Agilent Technologies 5975C inert XL MSD. The analytical column was a HP-1 MS (15 m, 0.25 mm diameter and 0.25 µm thickness) with helium as a carrier gas (0.9 mL/min). The oven was programmed to an initial temperature of 100°C for 1 min, ramped 20°C/min to 320°C. The ions monitored for diphenhydramine were 58.1 m/z (quantitation ion) and 73.0 m/z for diphenhydramine-d3, 61 m/z (quantitation ion) and 76 m/z. A six-point calibration was prepared from the stock standards ranging from 20 to 750 ng/mL with a 400 ng/mL control.

Cyclobenzaprine quantitation analysis (LC–MS-MS dynamic multiple reaction monitoring, DMRM)

Cyclobenzaprine was measured using a deuterated internal standard and a modified Foerster (8) extraction procedure. Calibrators from Cerilliant (Round Rock, TX) and controls from Lipomed (Cambridge, MA) were prepared in methanol from 1.0 mg/mL standards. Cyclobenzaprine-d3 was purchased from Toronto Research (North York, ON, Canada). The analysis was performed by LC–tandem mass spectrometry (MS–MS) consisting of an Agilent 6410 LC triple quadrupole mass spectrometer (Santa Clara, CA) operated in electro-spray ionization (ESI) in positive mode, and an Agilent 1290 Infinity HPLC System. Chromatography was performed using a Waters X-Bridge BEH Phenyl column (2.1 x 50 mm x 2.5 µm) with gradient elution. The mobile phases consisted of 5 mM ammonium bicarbonate (pH 10) in DI water (mobile phase A) and 0.2% acetic acid in LC–MS grade acetonitrile (mobile phase B). The flow rate is 0.6 mL/min. The mobile phase gradient was 0.0–9.0 min, mobile phase B increased from 25 to 50%; 9.0–9.1 mobile phase B increased to 75% and held isocratic till 10.5 min, followed by a 2-min post-injection equilibration period. The injection volume was 4 µL, and the column temperature was maintained at 35°C. The mass spectrometer in ESI mode was operated with nitrogen gas under the following conditions: temperature, 350°C; gas flow rate, 8 L/min; nebulizer gas pressure, 45 psi; capillary interface voltage, 1,500 V; delta EMV +20; the polarity was positive. The ions monitored for cyclobenzaprine were 276.2 m/z > 84.1 m/z (quantitation ion), 276.2 m/z > 58.2 m/z and 276.2 m/z > 231.1; for cyclobenzaprine-d3 279.2 m/z > 87.1 m/z (quantitation ion) and 279.2 m/z > 61.2 m/z. A six-point calibration was prepared from the stock standards ranging from 20 to 1,500 ng/mL with three control levels.

Table II. Toxicology Data

<table>
<thead>
<tr>
<th>Drug</th>
<th>Femoral blood (mg/L)</th>
<th>Gastric fluid (mg/L)</th>
<th>Vitreous fluid (mg/L)</th>
<th>Liver tissue (mg/g)</th>
<th>Urine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclobenzaprine</td>
<td>0.039</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>0.049</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duloxetine</td>
<td>6.1</td>
<td>95</td>
<td>0.59</td>
<td>360</td>
<td>43</td>
</tr>
</tbody>
</table>

279.2 m/z > 61.2 m/z. A six-point calibration was prepared from the stock standards ranging from 20 to 1,500 ng/mL with three control levels.

Duloxetine quantitation analysis (LC–MS-MS) at NMS labs

Duloxetine was measured using a deuterated internal standard with protein precipitation by means of a zinc sulfate solution. Calibrators and control solutions were prepared in methanol from 1.0 mg/mL standards purchased from Cerilliant (Round Rock, TX). Duloxetine-d4 was obtained from Eli Lilly & Co. (Indianapolis, IN). The analysis was performed by a Waters TQD Tandem Mass Spectrometer (Milford, MA) with a Waters Acquity Ultra Performance LC system. The column used was a Phenomenex Synergi Hydro-RP (2.0 x 50 mm, 2.5 µm) (Torrance, CA), with a Thermo Aquasil C18, 2.1 x 10 mm, 5.0 µm pre-column guard cartridge (Bellefonte, PA). The ions monitored for duloxetine-d4 were 302.1 m/z > 46.9 m/z (quantitation ion) and 302.1 m/z > 158 m/z and for duloxetine 298 m/z > 43.8 m/z (quantitation ion) and 298 m/z > 154 m/z. A six-point calibration was prepared from the stock standard solution ranging from 3.0 to 300 ng/mL. The determination of duloxetine in urine, liver tissue homogenate, gastric contents fluid and vitreous humor was performed using two levels of standard addition for each specimen.

Results

The alkaline drug screen revealed the presence of diphenhydramine, cyclobenzaprine and duloxetine. Table II lists the quantitative results.

Discussion

The cause of death in this case was determined to be a mixed drug toxicity of duloxetine, cyclobenzaprine and diphenhydramine. The most significant component of the toxicity was from duloxetine. Interestingly, the decedent’s initial complaint of ‘dry mouth’, while nonspecific, has been described as a side effect of duloxetine therapy (9), likely through its noradrenergic effects.

Duloxetine is a relatively new drug, and limited reports of related overdose and fatalities exist in the literature. In a 2010 review, Vey and Kovelman provided post-mortem data on six fatalities involving duloxetine, either alone or in combination with other medications (4). The levels reported in these cases were all obtained from central blood, with the highest duloxetine concentration (2.5 mg/L) less than one-half of the concentration found in peripheral blood in the current case (6.1 mg/L). Vey’s work also addresses the issue of post-mortem redistribution and cites a central-to-femoral blood duloxetine ratio of 1.98 as obtained by Anderson et al. (6). Given this, it is possible the concentration found in our case is nearly six-times greater than the highest level reported. Unfortunately, no central blood sample was collected for testing in the current case.
In the present case, a number of other fluids and tissue samples were also analyzed. Although the samples that were tested (liver tissue, gastric contents, urine and vitreous humor) were similar to those examined in the 2006 work by Anderson, et al. (6), our report illustrates concentrations in various tissues in an overdose/toxicity setting. In contrast, Anderson described 12 cases in which duloxetine was identified in post-mortem samples, and not ‘implicated as the sole cause of death’. Similar findings were reported by Pilgrim et al. in 2014 in a review of the prevalence of duloxetine in medico-legal death investigations (3). They report duloxetine levels in 34 deaths with post-mortem femoral blood ranging between 0.01 and 1.42 mg/L with an average of 0.14 mg/L. Nineteen of these deaths were attributed to the drug toxicity, but not specifically to duloxetine. In a case report of a duloxetine-related fatality, a urine level of 670 µg/L (0.67 mg/L) was found in association with a post-mortem femoral blood concentration of 910 µg/L (0.91 mg/L) (10). With these and our current report, the range of therapeutic and toxic levels of duloxetine is becoming increasingly more defined.

Conclusion
Presented here is a post-mortem case study with the highest duloxetine concentrations reported to date, in a variety of source samples. These results correlate well with the high volume of distribution that duloxetine is known to exhibit. Despite obtaining duloxetine levels from numerous sources, the complex pharmaco-toxicological profile of duloxetine remains not fully understood. With this and other post-mortem cases emerging, it is likely that duloxetine will be a more significant factor to consider in establishing cause of death. Therefore, it will be important for the toxicologist and forensic pathologist to have a better understanding of the range of duloxetine levels in blood and other toxicology samples.

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References