Case Report

Two Cases Involving Clomipramine Intoxication

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Abstract

Clomipramine and its active metabolite norclomipramine were identified and quantitated in multiple tissues recovered from two postmortem cases using liquid chromatography–mass spectrometry. In both cases clomipramine toxicity was assessed primarily upon levels determined from brain samples. This communication supplements the database on clomipramine and norclomipramine levels in general and a total absence of documented brain levels. In patients who have undergone long-term tricyclic antidepressant (TCA) therapy, blood and liver analysis alone may not be sufficient to establish toxicity. Such patients can sequester substantial amounts in liver, a concern because the TCAs are subject to significant postmortem redistribution. When conducting postmortem investigations, the inclusion of brain determination provides valuable information in assessing the magnitude of toxicity in cases involving clomipramine and its active metabolite norclomipramine.

Introduction

The tricyclic antidepressant clomipramine (Anafranil®) has been available since 1961 (1). Although clomipramine’s usage has declined as other therapeutic agents have been developed, it is still occasionally prescribed for the treatment of depression and obsessive-compulsive disorder (OCD) (2). Like other tricyclic antidepressants, clomipramine blocks the reuptake of the catecholamines norepinephrine and dopamine as well as serotonin by the presynaptic nerve terminal, thereby increasing the concentrations of the monoamines in the synaptic cleft. During chronic drug administration, clomipramine is N-demethylated to norclomipramine, an active metabolite that accumulates in plasma. Over 90% of the drug is eliminated from the body within two weeks of administration, primarily in urine (60%) and feces (30%) (1).

Plasma concentrations in 8 patients receiving 150 mg clomipramine daily ranged from 0.082 to 0.236 mg/L for clomipramine and 0.083 to 0.316 mg/L for norclomipramine (3). Other researchers have reported higher steady state plasma levels using the same dosing regimen of 150 mg. Broadhurst et al. (4) reported concentrations of 0.040–0.282 mg/L for clomipramine and 0.144–1.053 mg/L of norclomipramine in 14 patients.

In five fatal cases, postmortem blood levels ranged from 0.54 to 3.3 mg/L for clomipramine and 0.58 to 1.4 mg/L for norclomipramine (1,5,6). Because of a degree of overlap between the reported therapeutic and toxic concentration ranges and the fact that clomipramine may exhibit postmortem redistribution (7), the interpretation of plasma levels can be complicated and blood levels alone may not be the best indicators of clomipramine toxicity. McIntyre et al. (8) suggested that examining blood (especially peripheral specimens) and liver concentrations together would serve as a better indicator when attempting to assess clomipramine toxicity. In the cases detailed by McIntyre et al. (8), it was reported that in therapeutic regimens, liver clomipramine levels ranged from 7 to 20 mg/kg (norclomipramine levels were not reported).

Tricyclic antidepressants (TCAs) are sequestered in the liver as reflected in their large volumes of distribution (Vd). For example, the Vd of clomipramine is 17 L/kg (1). Patients undergoing chronic TCA treatment for depression or OCD can accumulate substantial amounts of parent drug and metabolite hepatically. Consequently, postmortem clomipramine levels may be difficult to assess when complicated by postmortem redistribution (9,10) and a difference between therapeutic and toxic levels may be difficult to establish using liver and blood samples alone especially if peripheral blood samples are unavailable.

In this communication, we report two cases involving clomipramine toxicity where both the parent drug and the active metabolite, norclomipramine, were determined in multiple biological samples. Quantitative values are reported in blood, liver, brain, urine, and gastric contents.

Case Histories

Case #1

The decedent, a 23-year-old female with a history of depression, presented to her attending pulmonologist with a respiratory tract infection. She was diagnosed with pneumonia upon admission to the hospital. The patient was placed on antibiotics and oxygen and appeared stable. Within 12 h, the patient became...
confused and anxious. She began to have difficulty breathing and subsequently coded and expired despite resuscitative efforts. An autopsy was conducted, and heart blood, brain, liver, urine, and gastric contents were submitted for toxicological analysis.

**Case #2**

The decedent was a 56-year-old female noteworthy for a history of depression and anxiety/panic attacks. She had previously attempted suicide with prescription painkillers. The evening prior to her death, the decedent had no complaints and retired for the night. The following morning she was found dead in her bed. Autopsy revealed evidence of medication around her mouth and in her gastric contents. No significant natural disease was found, and toxicology was requested to assess the possibility of an overdose.

**Experimental**

**Toxicological analysis**

In both cases a full toxicological analysis was performed. The volatile analysis of blood was done using headspace gas chromatography (GC). Immunoassay analysis was performed on urine using EMIT II Plus® for drugs of abuse. For the basic drugs, liver was macerated, and liquid–liquid extraction was conducted. Extracts were submitted to gas chromatography–mass spectrometry (GC–MS) for analysis. Finally, blood was extracted and analyzed by GC–MS for weak acids and neutrals.

**Materials**

Clomipramine and norclomipramine (1 mg/mL in methanol) calibrator and controls were purchased from Cerilliant and Altech. Protriptyline (internal standard, 1 mg/mL in methanol) was purchased from Cerilliant. Clomipramine and norclomipramine mixed working calibrator and control solutions (5.0 and 4.0 mg/L, respectively) were prepared by dilution in methanol. A working internal standard solution (4.0 mg/L) was prepared by dilution in methanol.

**Reagents**

All solvents and chemicals were ACS reagent grade or better. Ammonium hydroxide, hexane, iso-amyl alcohol, sulfuric acid, sodium carbonate, ammonium acetate, tolune, acetonitrile, and methanol were purchased from Fisher Scientific. Trifluoroacetic acid (TFA) was purchased from J.T. Baker. Nanopure water was obtained using a Barnstead/Termolyne Nanopure system.

**Extraction**

The method consisted of a liquid–liquid extraction using protriptyline as the IS followed by analysis by liquid chromatography–mass spectrometry (LC–MS). Protriptyline was selected as the internal standard (IS) because deuterated clomipramine did not yield unique qualifier ions and deuterated norclomipramine was not available. Samples of autopsy blood (femoral, heart, or pulmonary), liver, brain, urine, and gastric were analyzed. Tissues were homogenized in distilled water using a Waring blender. For example, 10 g of tissue was macerated with 40 mL of distilled water to yield a 1:5 dilution. Each 2-mL aliquot of blood or tissue homogenate was enriched with 0.100 mg/L of protriptyline. The samples were alkalinized to pH 9.5 using 0.5 mL of ammonium hydroxide and extracted into 8 mL of 95:5 hexane/iso-amyl alcohol. Following centrifugation, the top organic layer was transferred to a conical tube and back-extracted into 2.5 mL of 2N sulfuric acid. The organic layer was aspirated to waste and the aqueous portion was re-alkalinized to pH 9.5 using 0.5 mL of ammonium hydroxide and 2.5 mL of 95:5 carbonate buffer. Final extraction was into 0.5 mL of 85:15 tolune/iso-amyl alcohol. Following a brief centrifugation, the bottom aqueous layer was aspirated to waste and the remaining organic layer was transferred to a disposable tube and dried at 55°C under a stream of nitrogen. The dried extract was reconstituted in 0.5 mL of 85:15 (A:B) mobile phase, transferred to an auto-sampler vial, and submitted to LC–MS analysis.

**Instrumental analysis**

Samples were analyzed using an Agilent 1100 series LC–MS equipped with a G1312A binary pump and a G1313A ALS. Chromatographic separation was performed using a Zorbax® SB C18 2.1 x 150-mm x 5-μm column with flow set to 0.5 mL/min.

The mobile phase consisted of a gradient mixture of A (2mM ammonium acetate in nanopure water with 0.01% TFA) and B (2mM ammonium acetate in acetonitrile with 0.01% TFA). The gradient was programmed as follows: 70:30 A:B at time 0 increasing to 65:35 at 1 min, increasing to 62:38 at 9 min and 60:40 at 10.5 min.

The ionization mode was API-electrospray with a 350°C gas temperature, 13 mL/min drying gas, and a nebulizer set at 35 psig.

**Analysis**

Effective analysis of compounds by electrospray positive ion SIM (single ion monitoring) mass spectrometry requires that each compound have a predetermined fragmentation voltage. During method development, each intended analyte undergoes a flow injection analysis (FIA) to determine what fragments to monitor and what voltage to apply to each fragment in order to maximize ion abundance.

FIA involves multiple analyses of a pure standard after injection into a flowing stream without chromatographic separation. Each injection is made 0.8 min apart. The fragmentation voltage is increased incrementally with each injection. The high concentration of organic solvent (50%) in the mobile phase ensures short elution time of the compound being optimized. The generation of the M+1 ion as well as two qualifier ions makes SIM analysis in the API/ES mode an effective tool for the identification and quantitation of compounds that may not be amenable to GC–MS analysis.

Ions and fragmentation voltages are given in Table I.

**Quantitation**

Data analysis is performed using the stand-alone GC–MS software (G1701DA version D 00.00.38) installed on the LC–MS with a customized macro that allows for the transfer of up to four discrete MS signals from the LC–MS Chemstation to the 'stand-alone' data analysis. Data are automatically transferred from the LC–MS Chemstation to the data.net directory by the macro at the end of each acquisition run. Processing of data is
then done by editing retention times and updating each calibration level for response and ion ratio range.

Because of variations in recovery, our method utilized matrix matched calibrators. Since the method does not employ deuterated internal standards, matrix matching minimized variations in recovery during extraction from different tissues that otherwise would have resulted in errors in quantitation. Calibration and quantitation was accomplished using four-point calibration curves of 0.125, 0.250, 0.500, and 1.250 mg/L (see Figure 1 for TIC). Positive and negative matrix controls were analyzed with each batch. Linearity for each of the matrix matched calibrations was > 0.990. Qualifier ion ratios (see Figure 2 for ion chromatograms) were stable and well within ±20% of the established value. The controls were within 20% of the target concentration.

Results

A summary of qualitative and quantitative results is listed in Table II.

Case #1

Analysis of the decedent’s blood for volatiles was negative. Urine immunoassay results were positive for acetaminophen with a slightly elevated result for propoxyphene. The laboratory defines elevated results as those whose change in absorbance is above the negative control but below the calibrator cutoff. A subsequent analysis of blood by immunoassay generated a slightly elevated result for acetaminophen. Consequently, further analysis for acetaminophen was considered unnecessary. Analysis of liver revealed the presence of clomipramine, norclomipramine, propoxyphene, and diphenhydramine. No acidic or neutral drugs were detected. Based upon the screening results, quantitative analysis for clomipramine, norclomipramine, propoxyphene, and norpropoxyphene was carried out using LC-MS. Diphenhydramine was quantitated by GC-MS.

Case #2

Analysis of the decedent’s blood for volatiles was negative. Immunoassay analysis indicated acetaminophen in the urine, but

<table>
<thead>
<tr>
<th>Table I. Ions and Fragmentation Voltages</th>
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<tbody>
<tr>
<td>Ion Fragment (m/z)</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Protriptyline</td>
</tr>
<tr>
<td>264 (M+H)</td>
</tr>
<tr>
<td>233</td>
</tr>
<tr>
<td>Clomipramine</td>
</tr>
<tr>
<td>315 (M+H)</td>
</tr>
<tr>
<td>242</td>
</tr>
<tr>
<td>227</td>
</tr>
<tr>
<td>Norclomipramine</td>
</tr>
<tr>
<td>301 (M+H)</td>
</tr>
<tr>
<td>242</td>
</tr>
<tr>
<td>227</td>
</tr>
</tbody>
</table>

Figure 1. Total ion chromatogram. Blood extract of protriptyline: 7.44 min, 0.100 mg/L; norclomipramine: 11.32 min, 0.125 mg/L; and clomipramine: 12.33 min, 0.125 mg/L.

Figure 2. Relative ion responses of protriptyline 0.100 mg/L (A), norclomipramine 0.125 mg/L (B), and clomipramine 0.125 mg/L (C).
a subsequent analysis of blood by immunoassay was negative. Opiates were indicated in both blood and urine by immunoassay. Analysis by GC–MS for opiates (codeine, hydrocodone, hydromorphone, and morphine) detected hydrocodone in blood (Table II). Analysis for oxycodone and oxymorphone by LC–MS was negative. Weak acid neutral analysis of pulmonary arterial blood detected the presence of meperidine. The liver was positive for benzotropine and diltiazem. Both compounds were determined to be present in trace amounts and did not warrant quantitation. GC–MS screening of liver detected clomipramine and norclomipramine. Confirmation/quantification for clomipramine and norclomipramine was performed by LC–MS.

**Discussion**

Little data are available regarding the distribution of clomipramine and its pharmacologically active metabolite norclomipramine in postmortem cases. Reports that included solid tissue results (i.e., liver) did not provide results for the metabolite. A review of the literature found no references that documented clomipramine and norclomipramine in brain in either toxic or therapeutic situations.

The absence of data on norclomipramine is significant. The effects and toxicity of this metabolite necessitates that the analysis be capable of obtaining accurate results for both compounds. Additionally, accurate metabolite results are useful in distinguishing between acute and/or chronic use.

Norclomipramine, the more polar demethylated product of clomipramine, often displays poor chromatographic characteristics when analyzed by capillary GC. Consequently, it may be difficult to judge the relative amounts of the metabolite present when screening using GC. The analysis by liquid chromatography (Figures 1 and 2) provides superior chromatography which translates into more accurate quantitation. Molecular ion identification and quantitation by positive ion API-ES coupled with consistent LC provided results that were highly specific and accurate for both the parent drug and its metabolite.

The distribution of drugs throughout the body refers to movement of the drugs from blood to other organs and tissues such as brain, liver, kidney, and adipose tissue. Depending on the chemistry of the compound and the blood supply, each tissue will accumulate varying amounts of a given drug/metabolite. The protection of the blood-brain barrier limits entry of many compounds into the brain. Nevertheless, as the target organ, the brain is a unique and desirable tissue for interpreting centrally acting drug toxicity. The location of the brain and its insulated properties make the likelihood of postmortem redistribution or contamination during autopsy low, thereby providing a reliable postmortem tissue that accurately reflects toxicity at the time of death.

The authors believe that the determination of both blood and brain levels is an excellent way of assessing impairment and toxicity. Because of postmortem redistribution or decomposition, high blood levels have been found (even trauma has resulted in artificially high central blood levels) in cases where the investigation and autopsy did not indicate a drug overdose (9,10). If chronic or recent drug administration results in high liver and/or gastric concentrations, the potential for postmortem redistribution is increased and inflated blood levels may be encountered. In cases such as these the brain may be the preferred tissue to assess toxicity.

In case #1, only central blood was available. The possibility that the heart blood clomipramine concentration was elevated by postmortem redistribution had to be considered. Although this may have been the case, the amount of clomipramine and metabolite detected in the brain supports the assessment that the levels are toxic. It is possible that the decedent’s respiratory difficulties resulted from clomipramine toxicity. Overdose of TCAs has been reported to cause respiratory depression (9,11). Whether the decedent’s respiratory distress was due to or exacerbated by this toxic response to clomipramine is unknown, but it is a distinct possibility.

In case #2, both central and peripheral blood sites were analyzed for clomipramine and norclomipramine making this case a new and useful addition to the existing literature. Not unexpectedly, the results from the pulmonary artery blood are elevated relative to the peripheral femoral blood. The reported blood concentrations are within the range of earlier published reports (1,5,6) where clomipramine was implicated in the cause of death. Though the blood levels measured are consistent with possible toxicity, the effect of postmortem redistribution, especially in the pulmonary artery blood should always be considered. In some overdose cases the findings in liver are unmistakable (i.e., clomipramine concentrations exceeding 200 mg/kg after acute poisoning (8)). In case #2, the liver did not provide enough insight to the

### Table II. Toxicological Results

<table>
<thead>
<tr>
<th>Case #1</th>
<th>Clomipramine (mg/L)</th>
<th>Norclomipramine (mg/L)</th>
<th>Diphenhydramine (mg/kg)</th>
<th>Propoxyphene</th>
<th>Norpropoxyphene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart blood</td>
<td>1.39</td>
<td>2.64</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>8.02</td>
<td>26.74</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>14.25</td>
<td>41.12</td>
<td>Trace</td>
<td></td>
<td>Trace</td>
</tr>
<tr>
<td>Urine</td>
<td>0.48</td>
<td>1.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric</td>
<td>3.99 mg/total</td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case #2</th>
<th>Clomipramine (mg/L)</th>
<th>Norclomipramine (mg/L)</th>
<th>Meprobamate (mg/kg)</th>
<th>Hydrocodone (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral blood</td>
<td>0.70</td>
<td>0.66</td>
<td>0.13</td>
<td>8.97</td>
</tr>
<tr>
<td>Pulmonary artery blood*</td>
<td>1.00</td>
<td>1.11</td>
<td><code>&lt; 5.0</code></td>
<td>0.13</td>
</tr>
<tr>
<td>Brain</td>
<td>4.86</td>
<td>7.43</td>
<td>11.68</td>
<td>21.20 mg/total</td>
</tr>
<tr>
<td>Liver</td>
<td>21.20 mg/total</td>
<td>13.4</td>
<td>0.10</td>
<td>8.97</td>
</tr>
<tr>
<td>Small intestinal contents</td>
<td>Not detected</td>
<td>0.10</td>
<td>8.97</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>Not analyzed</td>
<td>Not analyzed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Carisoprodol not detected in pulmonary artery blood.
* Liver: diltiazem and benzotropine present.
degree of clomipramine toxicity and could not alleviate the concern that the blood had been altered by redistribution of clomipramine. The blood levels however are consistent with the high concentrations of clomipramine and norclomipramine found in the brain (4.86 and 7.43 mg/kg respectively), a tissue not as susceptible to postmortem changes as blood. In comparison, brain concentrations reported in amitriptyline fatalities range from 2.6 to 18 and 0 to 7.7 mg/kg for amitriptyline and nortriptyline, respectively (1). The agreement between the elevated brain and blood levels alleviated the concern of postmortem redistribution and the toxic blood levels were confidently compared to previously published fatalities involving clomipramine. The presence of hydrocodone (Table II) is likely to have contributed to respiratory depression induced by clomipramine since respiratory depression is also a major symptom of opiate toxicity (12). Based upon the toxicological findings and the case history, it was concluded that the decedent’s death was due to mixed drug intoxication.

In both of these cases the brain results give credibility to the toxic blood levels. The authors believe these cases demonstrate the usefulness of brain levels when assessing toxicity and the effect of postmortem redistribution while avoiding the potentially misleading conclusions that might have been reached based on liver levels alone. For example, Bailey and Shaw (13) reported liver amitriptyline and nortriptyline concentrations ranging from 0.4 to 17 and 0.3 to 28 mg/kg, respectively, in cases where the patients death was not attributed to drug overdose. In cases where amitriptyline overdose was considered the cause of death, liver amitriptyline and metabolite levels ranged from 13 to 317 and 7.5 to 64 mg/kg, respectively (1). If liver concentrations fall within both the therapeutic and toxic ranges the overlap can confound interpretation. In such cases, analysis of the brain will enhance the ability to accurately assess toxicity. The analysis of peripheral blood also attenuates concerns stemming from postmortem redistribution, but the availability of peripheral samples varies.

In summary, the use of an accurate and specific method of analysis combined with a complete distribution of the parent drug and its pharmacologically active metabolite proved valuable in assessing the role of clomipramine in these fatal overdose cases. As the intended site of action for centrally acting drugs, the brain is a unique tissue valuable in assessing toxicity. Tissue distributions involving centrally active compounds that include accurate brain determinations are less likely to be clouded by issues arising from postmortem redistribution. Additionally, not all references distinguish between concentrations determined from peripheral and central blood sites. This causes ambiguity because the data are often reported only as blood. Brain data are more reliable because they do not suffer from this potential bias.

Conclusions

The use of clomipramine continues for the treatment of depression and OCD (2) despite the availability of the newer selective serotonin reuptake inhibitors. In cases where TCA and active metabolite concentration exceeds 1.0 mg/L, toxicity and fatality are probable (14). However, blood results from post-mortem toxicologic analyses involving TCAs can be subject to misinterpretation. This is especially true in cases involving long-term therapy where the potential for postmortem redistribution is high. Data from our cases enhances the available literature’s database and will help investigators assess clomipramine’s role in toxicity.

The authors concluded that to fully assess toxicity, complete tissue quantitations were necessary. In cases detailed here, brain levels were considered critical findings in determining the extent of clomipramine toxicity at the time of death without complication arising from postmortem redistribution. Pharmacologically active metabolites often accumulate in high concentrations during chronic drug therapy. The ability to accurately analyze the pharmacologically active metabolite, norclomipramine, was foremost in both cases where the metabolite levels greatly exceeded the levels of the parent compound in many tissues. The LC-MS method that was employed using matrix matched calibrators proved to be highly specific and accurate for the quantitation of clomipramine and norclomipramine in all tissues.

References