A Fatal Case of Serotonin Syndrome after Combined Moclobemide-Citalopram Intoxication

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Abstract

We present a case involving a fatality due to the combined ingestion of two different types of antidepressants. A 41-year-old Caucasian male, with a history of depression and suicide attempts, was found deceased at home. Multiple containers of medication, the MAO-inhibitor moclobemide (Aurorix), the SSRI citalopram (Cipramil), and the benzodiazepine lorazepam (Noctamid) as active substance, as well as a bottle of whiskey were present at the scene. The autopsy findings were unremarkable, but systematic toxicological analysis (EMIT, radioimmunoassay, high-performance liquid chromatography–diode-array detection [HPLC–DAD], gas chromatography–nitrogen-phosphorus detection, and gas chromatography–mass spectrometry) revealed the following: ethanol (0.23 g/L blood, 0.67 g/L urine), lorazepam (1.65 µg/mL urine), cotinine (0.63 µg/mL blood, 5.08 µg/mL urine), caffeine (1.20 µg/mL urine), moclobemide (and metabolites), and citalopram (and metabolite). Thereupon, we developed a new liquid chromatographic separation with optimized DAD, preceded by an automated solid-phase extraction, for the quantitation of the previously mentioned antidepressive drugs. The results obtained for blood and urine, respectively, were as follows: Ro 12-5637 (moclobemide N'-oxide) not detected and 424 µg/mL; Ro 12-8095 (3-keto-moclobemide) 2.26 µg/mL and 49.7 µg/mL; moclobemide 5.62 µg/mL and 204 µg/mL; desmethylcitalopram 0.42 µg/mL and 1.22 µg/mL; and citalopram 4.47 µg/mL and 19.7 µg/mL. The cause of death was attributed to the synergistic toxicity of moclobemide and citalopram, both antidepressants, which, by intentional or accidental combined ingestion, can produce a potentially lethal hyperserotonergic state. Based on the history of the case and pharmacology of the drugs involved, the forensic pathologists ruled that the cause of death was multiple drug intoxication, resulting in a fatal "serotonin syndrome," and that the manner of death was suicide.

Introduction

During the last 10 years, a new generation of antidepressive drugs, including selective serotonin reuptake inhibitors (SSRIs) and selective monoamine oxidase inhibitors (MAO-Is), has been introduced in Europe. These new antidepressants have been claimed to be as effective as tricyclic antidepressants (TCAs) but better tolerated and less toxic (1).

One of the most selective serotonin reuptake inhibitors is citalopram, a bicyclic phthalate (Figure 1). It has an elimination half-life of approximately 1.5 days (2), and its main metabolite, desmethylcitalopram, is itself active as a serotonin reuptake inhibitor, although two to four times weaker. Normal citalopram daily dosage ranges from 20 to a maximum of 60 mg, with reduced dosage to elderly and persons with impaired liver and kidney function (3). Blood concentrations associated with therapeutic use, range from 0.4 to 3.0 µg/mL (4).

Moclobemide, structurally a benzamide, is one of the newer selective and reversible inhibitors of monoamine oxidase type-A (MAO-A) (Figure 1) (5). With a terminal elimination half-life from 1.5 to 4 h, it undergoes a rapid and extensive metabolization in the liver with the formation of numerous metabolites, most of which are also pharmacologically active. Recommended doses are 300 to 600 mg per day in divided doses. Blood levels, following therapeutic use, range from 0.4 to 3.0 µg/mL (6,7).

Figure 1. Structure of moclobemide, citalopram, the internal standards (Ro 11-9900 and Lu 10-202), and the main degradation products.
Both antidepressive drug types are considered to be less cardiotoxic than TCAs, and thus relatively safe with regard to lethal overdose. Therefore, death attributed primarily to these drugs individually is extremely rare. However, co-ingestion of MAO-Is and SSRIs, intentionally or by accident, even in therapeutic use, may cause a hyperserotonergic state, that is, a “serotonin syndrome,” a condition with a high mortality rate (8). Symptoms include slurring of speech, hyperthermia, tachycardia, labile blood pressure, diaphoresis, muscular rigidity, convulsions, and finally full cardiac arrest (6).

This report presents a fatal case of combined intoxication with moclobemide and citalopram. It documents the analytical findings of both components and their major metabolites in blood and urine.

Case History

A 41-year-old man was found deceased at home in the afternoon by a relative. The last time the man was seen alive was the previous evening. The naked body sat in a basket. The police were informed because of suspicion of an unnatural death. Three empty containers of medication, Noctamid 2 mg (lormetazepam, 30 tablets), Aurorix 150 mg (moclobemide, 30 tablets), and Cipramil 20 mg (citalopram, 28 tablets), and a nearly empty bottle of whiskey were present at the scene. Several farewell letters were also found in the house. Further police investigation revealed no suspicious circumstances. The police had a history of several suicide attempts by self-intoxication and tentative cuts on the wrist.

The body was brought to a morgue and the next day, in the morning, an external examination of the body was performed. The deceased was of average height and weight. Rigor mortis was present, and hypostasis was already fixed. Some froth (edema) was observed around the mouth and nostrils. A few minor abrasions and ecchymoses were present at the arms, legs, and back. Old scars were seen at both wrists (hesitation cuts). There was abundant defecation. Blood (subclavia) and urine were collected for toxicological analysis.

Experimental

Materials

Moclobemide, its metabolites (Ro 12-5637 and Ro 12-8095), and the internal standard (Ro 11-9900) were a gift from Hoffmann-La Roche (Basel, Switzerland), and citalopram.HBr, desmethylcitalopram.HCl, and the internal standard (Lu 10-202), diluted with 4.7 mL of acetonitrile/25mM pH 11.5 phosphate buffer (5:95, v/v), and placed in the sample rack. In case of the blood samples, the dilution step was set to be followed by 5 min of centrifugation at 1121 x g, after which the supernatant was separated from the precipitate, and then placed in the sample rack. The extraction columns were placed in the SPE column turret, and the automated procedure, described in Table I, was started. The total analysis time for one sample was 26 min. After elution, the organic layer was transferred into a conical tube and evaporated under a gentle stream of nitrogen at 40°C (TurboVap LV Evaporator, Zymark). The dry residue was finally dissolved in 100 µL of solvent A from the chromatographic system, and a 50-µL aliquot was injected into the HPLC.

Drug screening

Postmortem samples were analyzed following a previously described comprehensive screening using EMIT®, RIA (9), and chromatographic techniques such as HPLC with diode-array detection (HPLC-DAD) preceded by alkaline extraction (10) and gas chromatography with nitrogen-phosphorus detection (GC-NPD) and mass spectrometric detection (GC-MS) (9).

Quantitation

Automated extraction procedure. Automated SPE was performed on a RapidTrace® SPE Workstation (Zymark, Hopkinton, MA). The extraction procedure used was based on an optimized SPE for citalopram and its metabolites, previously described by Carlsson and Norlander (11). One milliliter of postmortem fluid (blood or urine) was spiked with 100 mL of a 50-mg/mL internal standard solution containing both internal standards (Ro 11-9900 and Lu 10-202), diluted with 4.7 mL of acetonitrile/25mM pH 11.5 phosphate buffer (5:95, v/v), and placed in the sample rack. In the case of the blood samples, the dilution step was followed by 5 min of centrifugation at 1121 x g, after which the upper layer was separated from the precipitate, and then placed in the sample rack. The extraction columns were placed in the SPE column turret, and the automated procedure, described in Table I, was started. The total analysis time for one sample was 26 min. After elution, the organic layer was transferred into a conical tube and evaporated under a gentle stream of nitrogen at 40°C (TurboVap® LV Evaporator, Zymark). The dry residue was finally dissolved in 100 µL of solvent A from the chromatographic system, and a 50-µL aliquot was injected into the HPLC.

Chromatographic conditions. The gradient HPLC system consisted of a model 168 DAD and a type 210A manual injector fitted with a 50-µL sample loop from Beckmann (Analys,
Gent, Belgium). The photodiode-array detector was operated in a 4-nm band-pass mode, monitoring light from 225 to 350 nm. The display wavelength was 237 nm. Chromatographic separation was achieved on a Kromasil 100 C-18 column (150.0 × 4.6 mm i.d., 5-µm particle size), which was a gift from Varian Chrompack International (Middelburg, The Netherlands). The mobile phase was a mixture of 0.0125 M NaOH in methanol/water (90:10, v/v) (solvent B) and 0.0125 M NaOH in water/methanol (90:10, v/v) (solvent A, pHapp ≥ 9.5) as described earlier (10). Before use, both solvents were filtered through Nylon 66 (0.2-µm pore size) filters (Alltech, Deerfield, IL). The following new gradient conditions were used: a linear gradient starting from 60% B and going to 75% B (in 5 min), followed by a hold at 75% B (8 min), and another linear gradient to 100% B (in 10 min). After an isocratic period (100% B) of 5 min, the pump was programmed to regain the initial conditions (60% B/40% A) over a 3-min interval. A 5-min reconditioning time was allowed before the next injection. The total analysis time was 36 min at a flow rate of 1.00 mL/min.

**Results and Discussion**

In accordance with the laboratory's operating procedures, a general screening of the blood and urine was performed with EMIT and RIA. This revealed the presence of ethanol (0.23 and 0.67 g/L in blood and urine, respectively), caffeine (not detected and 1.20 µg/mL in blood and urine, respectively), and cotinine (0.63 and 5.08 µg/mL in blood and urine, respectively). As a result of the initial HPLC screening on an alumina-based packing material, three and four prominent peaks were observed in the respective chromatograms of the extract of blood and urine. Based on retention and their typical UV-spectra (Figure 2B), the identities of these compounds were disclosed as moclobemide, preceded by its metabolites, and citalopram, which was also preceded by its main metabolite desmethylcitalopram in urine. After hydrolysis of the urine, lorzepam was also demonstrated with HPLC–DAD. The presence of moclobemide and citalopram were also confirmed in blood and urine by routine GC–MS analysis (9) (Figure 2C). No other drugs were found with either GC–NPD or GC–MS.

During routine HPLC–DAD analysis of the antidepressive drugs on the alumina-based HPLC column (10), we were confronted with two major problems: a very low recovery after liquid–liquid extraction (< 50%) and a poor resolution of the parent compounds from their metabolites. Thus, for the accurate quantitation of these compounds in the postmortem fluids, a specific extraction in combination with optimized chromatographic conditions, and preferably a suitable internal standard, was required.

With the standard solvent composition (10) but modifications in the gradient steepness, no gain in resolution could be obtained. Therefore, it was decided to work with another stationary phase, a Kromasil 100 C18 packing.
Although a silica-based column, this particular packing shows a high stability to hydrolysis at low pH and, of major interest for this application, a silica stability over a pH of 7, actually between pH 2.0 to 9.5, due to derivatization and extensive endcapping of the silica particles (12). This column with appropriately modified solvent conditions did allow adequate separation of all compounds involved, including internal standards. In previous simultaneous quantitations of SSRIs and MAO-Is, protriptyline (13), imipramine (2), and methadone (8) have all been used as the internal standard. Because of structural differences between moclobemide and citalopram, we preferred to use two separate internal standards, Ro 11-9900 and Lu 10-202. Although already used individually for the same purpose (5,11), a combination of both compounds was expected to result in a more accurate quantitation for this application. The parent compounds, the metabolites, and the two internal standards chromatographed baseline separated (Figure 2A) under the new chromatographic conditions (as described in the Experimental section). Retention times were 2.90 min (Ro 12-5637), 3.33 min (Ro 12-8095), 4.55 min (moclobemide), 5.63 min (Ro 11-9900), 7.79 min (desmethylcitalopram), 10.35 min (citalopram), and 14.46 min (Lu 10-202).

The extraction step was also optimized because the routine liquid–liquid extraction method applied in the initial sample screening showed a recovery of less than 50% for citalopram, moclobemide, and their metabolites. After comparison with modified liquid–liquid extractions, a solid-phase procedure based on the work of Carlsson and Norlander (11), was selected. Because the method was developed especially for citalopram, the washing step had to be adjusted to retain more polar compounds such as moclobemide and its metabolites. The resulting simple solid-phase conditions were adapted on a Rapid-Trace SPE Workstation, which resulted in high and reproducible recoveries for all compounds in the biological samples (Table II). Recoveries were not concentration dependent (data not presented).

The limit of detection (S/N ≥ 3) for all compounds was 15 to 20 ng, and the quantitation limit (S/N ≥ 10) was approximately 50 ng injected on the column (corresponding to a level of 100 ng/mL sample). Calibration graphs were prepared in blank whole blood and urine. Weighted linear regression was performed in an effort to account for data heteroscedasticity. All obtained calibration curves were linear in the selected range (0.1–10 µg/mL) with correlation coefficients (r) ranging from 0.994 to 0.999. The coefficient of variation for within-day precision (n = 3) was below 10% for the lowest calibration point and below 5% for the rest of the calibration points for all compounds.

The quantitative results for the forensic sample are summarized in Table III, and because both antidepressants show no postmortem redistribution (2,8), they can be interpreted as such. For moclobemide and citalopram, high concentrations consistent with overdose were demonstrated in the blood. The metabolite concentrations in blood, however, were much lower. In accordance with a study performed by Geschke et al. (7), we could also identify Ro 12-8095 (blood and urine) and Ro 12-5637 (urine only) as the main metabolites of moclobemide in blood and urine, respectively. In a previous study it had been suggested that the parent-drug-to-metabolite ratio (P/M) may be a helpful tool in diagnosis (2). Because the P/M in blood is larger than 2 for both antidepressants, an acute overdose can be suggested as the cause of death. Because of the massive amounts of parent compounds and metabolites present in the urine, we also suspect chronic, concurrent use of moclobemide and citalopram. Although this is generally discouraged, some have suggested possible advantages of co-prescription (14,15).

Death attributed solely to overdose of SSRIs or MAO-As is very rare. However, co-ingestion of both types of antidepressants can lead to a “serotonin syndrome,” which has been described in the literature (5,6,16). Citalopram, one of the most potent SSRIs, inhibits the presynaptic serotonin reuptake, which leads to enhanced serotonergic transmission. Moclobemide is a specific and reversible inhibitor of MAO-A. However, at high concentrations, it is probable that the amount of moclobemide is sufficient to saturate the MAO-A sites, thus negating its normal MAO-A reversibility (17). In our case, this is of great importance as these are the sites at which serotonin oxidation takes place. In the absence of monoamine oxidase to destroy the excess, serotonin will accumulate in the synapses, leading to a hyperserotonergic state. In this case, the co-ingestion of very high amounts of both antidepressants very likely produced a serotonin syndrome that led to death.

**Table II. Mean Recoveries and Coefficients of Variation (%)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Blood (n = 8)</th>
<th>Urine (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ro 12-5637</td>
<td>*</td>
<td>87.7 (1.7)</td>
</tr>
<tr>
<td>Ro 12-8095</td>
<td>82.0 (3.6)</td>
<td>88.5 (2.7)</td>
</tr>
<tr>
<td>Moclobemide</td>
<td>94.1 (4.3)</td>
<td>98.7 (3.1)</td>
</tr>
<tr>
<td>Ro 11-9900 (IS)</td>
<td>97.3 (4.3)</td>
<td>80.4 (5.4)</td>
</tr>
<tr>
<td>Desmethylcitalopram</td>
<td>93.1 (3.3)</td>
<td>100.4 (1.3)</td>
</tr>
<tr>
<td>Citalopram</td>
<td>100.2 (2.0)</td>
<td>101.6 (0.6)</td>
</tr>
<tr>
<td>Lu 10-202 (IS)</td>
<td>92.0 (4.1)</td>
<td>91.5 (1.3)</td>
</tr>
</tbody>
</table>

* not present in blood.

**Table III. Summary of the Quantitative Results**

<table>
<thead>
<tr>
<th>Analyzed compounds</th>
<th>Blood (µg/mL)</th>
<th>Urine (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ro 12-5637</td>
<td>–</td>
<td>424</td>
</tr>
<tr>
<td>Ro 12-8095</td>
<td>2.26</td>
<td>49.7</td>
</tr>
<tr>
<td>Moclobemide</td>
<td>5.62</td>
<td>204</td>
</tr>
<tr>
<td>therapeutic range</td>
<td>0.36–3.00</td>
<td>–</td>
</tr>
<tr>
<td>Desmethylcitalopram</td>
<td>0.42</td>
<td>1.22</td>
</tr>
<tr>
<td>Citalopram</td>
<td>4.47</td>
<td>19.7</td>
</tr>
<tr>
<td>therapeutic range</td>
<td>0.03–0.20</td>
<td>–</td>
</tr>
</tbody>
</table>

Conclusions

In summary, these data clearly demonstrate that the described fatality was due to a presumably intentional co-ingestion of high amounts of moclobemide and citalopram by a
probable chronic abuser of these antidepressants. The case history and circumstances at the scene of the crime support this hypothesis.

The synergistic toxicity between SSRIs and MAO-A inhibitors, in particular moclobemide and citalopram, and the simultaneous quantitation of these two types of antidepressants have been previously described (18). However, to the best of our knowledge, the use of a double internal standard and an automated SPE combined with HPLC-DAD analysis for these two components and their metabolites in different biological matrices has not yet been published. Thus, it can also be concluded that the newly developed method can be successfully applied for specific case work.

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References


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