K23 Investigation of the Analytical Degradation of Clozapine-n-Oxide to Clozapine in a Postmortem Case

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Attendees should be able to use this information to assist with the interpretation of cases involving clozapine.

The goal of this presentation is to provide forensic toxicologists with data from a recent postmortem case in which clozapine was determined to be the cause of death. While clozapine-n-oxide does convert to clozapine with analysis by gas chromatography, our results indicate that this may be an insignificant phenomenon.

Clozapine is a tricyclic dibenzodiazepine used for the treatment of severe schizophrenia patients that have failed to respond to more standard therapy. Clozapine undergoes extensive metabolism including N-demethylation, N-oxidation, chlorine ring oxidation, chlorine and thiomethly conjugation. Clozapine-n-oxide is a major metabolite found in plasma with little pharmacological activity.

A 40-year-old male suffering from schizophrenia became agitated and self destructive in the assisted living facility where he was a resident. He was taken to the emergency room where he expired after extensive resuscitative efforts. Autopsy results were negative. Blood, vitreous, liver and brain specimens were submitted to the toxicology laboratory for analysis. Routine drug and alcohol screens using gas chromatography and gas chromatography/mass spectrometry were positive for clozapine, bupropion, chlorpheniramine, atropine, and desmethylsertraline. Clozapine was quantitated with gas chromatography with nitrogen phosphorous detection (GC/NPD), gas chromatography with flame ionization detection (GC/FID) and high performance liquid chromatography (HPLC).

For the GC analyses, 1.0 mL blood or 1.0 g of a 1:4 liver homogenate spiked with 2.0 mcg olanzapine (internal standard) were extracted with 7.5 mL 1-chlorobutane following alkalinization with 0.5 mL concentrated ammonium hydroxide. The organic layer was transferred and back extracted into 2.5-mL sulfuric acid. The solvent layer was removed. The aqueous acid layer was made alkaline with 0.5 mL concentrated ammonium hydroxide and extracted with 3.0-mL chloroform. Following centrifugation, the aqueous was removed and the chloroform layer was dried to residue under nitrogen at 40°C. The samples were reconstituted with 50-mcL methanol and 2.0 mcL was injected on an HP 5890 gas chromatograph with nitrogen-phosphorous detection or an HP 6890 gas chromatograph with flame ionization detection. The assay is linear from 0.5 mcg/mL to 8.0 mcg/mL with a limit of detection of 0.5 mcg/mL clozapine.

For HPLC analyses, 1.0 mL blood or 1.0 gram of a 1:4 liver homogenate spiked with 2.0 mcg promazine (internal standard) was extracted with 7.5 mL 3% isopropanol in pentane following alkalinization with carbonate buffer (pH 9.5). The extracts were dried to residue under nitrogen at 40°C. The samples were reconstituted with 200 mcL acetonitrile, filtered with a 0.2 um syringe filter and 20 mcL were injected on the Varian 5500 HPLC equipped with a cyanopropyl column and ultraviolet detection. The samples were analyzed using ultraviolet detection at 257 nm and a flow rate of 1.5 mL/min for the 60:35:5 acetonitrile: ammonium acetate: methanol mobile phase.

Clozapine-n-oxide has been shown to convert to clozapine by thermal degradation by GC analysis, in vivo processes in animals, and base reduction during extraction. We investigated the influence of clozapine-n-oxide on the results obtained by gas chromatography for the present case. Injection of unextracted clozapine-n-oxide on GC/NPD resulted in the detection of clozapine plus other unknown products. Three clozapine and 3 clozapine-n-oxide blood controls (2.0 mcg/mL, each) were spiked and extracted using the alkaline liquid-liquid method above. They were compared to extracted standard curves for each compound ranging from 0.5 to 8.0 mcg/mL. The mean concentration of clozapine (± SD) from the clozapine controls was found to be 2.2 (± 0.1) mcg/mL. The mean concentration of clozapine found when the extracted clozapine-n-oxide samples were compared to the clozapine curve was 1.1 (± 0.07) mcg/mL. When the clozapine-n-oxide extracts were compared to the clozapine-n-oxide standard curve, a mean of 1.8 (± 0.1) mcg/mL was obtained.

Two experiments were performed using HPLC. First, standards were prepared and subjected to the extraction protocol outlined above (blank, negative control, 0.5 mcg clozapine, 0.5 mcg clozapine-n-oxide, 0.5 clozapine + 0.5 mcg clozapine-n-oxide). We found no conversion of clozapine-n-oxide to clozapine. Secondly, a clozapine standard curve (0.5 to 8.0 mcg/mL blood) was extracted and used for comparison to 2 blood controls, 1 containing 1.0 mcg/mL clozapine-n-oxide, the other 1.0 clozapine-n-oxide + 2.0 mcg/mL clozapine. The control containing only clozapine-n-oxide demonstrated no clozapine conversion. The control containing both compounds showed the expected 2.0 mcg/mL clozapine.

These results indicate that clozapine-n-oxide does convert to clozapine during GC analysis and/or under strong alkaline conditions. Our HPLC analysis showed no conversion of clozapine-n-oxide to clozapine. Interestingly, when the blood from the postmortem case was extracted and analyzed by both the GC and HPLC protocols, the same result was obtained: GC, 2.1 mcg/mL; HPLC, 2.0 mcg/mL. These results indicate that the likely presence of clozapine-n-oxide did not cause significant overestimation of clozapine in a long-term user of this drug. **Clozapine, Clozapine-N-Oxide,Chromatography**

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