Hydroxycarbazepine Distribution in Three Postmortem Cases

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Abstract

This paper presents three cases investigated by the Office of the Chief Medical Examiner where hydroxycarbazepine, the active metabolite of the anticonvulsant prodrug oxcarbazepine, was detected in the biological specimens submitted for toxicological analysis. Hydroxycarbazepine was quantitated using a single-step pH 5 extraction and detection on a DB-5 column by gas chromatography–nitrogen-phosphorus detection. In the three cases, the heart blood concentrations were 34.6, 40.5, and 3.7 mg/L, respectively. In cases 2 and 3, the peripheral blood concentrations were 36 and 4.1 mg/L, respectively. In each case, the medical examiner ruled that the hydroxycarbazepine was an incidental finding to the cause of death.

Introduction

Oxcarbazepine (Trileptal®) is an anticonvulsant drug structurally similar to carbamazepine. It is used to treat generalized tonic-clonic and partial seizures, either alone or in combination with other anticonvulsant drugs. It inhibits seizure activity by blocking voltage dependent sodium channels (1). Side effects of oxcarbazepine are similar to other central nervous system depressants: fatigue, headache, dizziness, ataxia, and nystagmus. One possible significant effect on laboratory results is hyponatraemia (2).

Oxcarbazepine is rapidly and almost completely absorbed following oral ingestion. Whereas carbamazepine is metabolized to an epoxide that is responsible for much of its toxicity, oxcarbazepine is metabolized to 10,11-dihydro-10-hydroxycarbamazepine (hydroxycarbazepine). In fact, oxcarbazepine is a prodrug with hydroxycarbazepine accounting for the anticonvulsant activity of the drug. This metabolism is catalyzed by a cytosolic ketoreductase, not by the microsomal cytochrome P450 (CYP) system. Therefore, oxcarbazepine metabolism is unaffected by co-administered drugs that inhibit or induce this system (3). Oxcarbazepine does, however, induce the CYP3A group that is responsible for the metabolism of dihydropyrimidine calcium antagonist drugs and oral contraceptive drugs (2).

The plasma half-life of parent drug is 1–2.5 h; the plasma half-life of the metabolite is 8–10 h. Therapeutic drug monitoring of oxcarbazepine is usually not performed. However, a therapeutic range of 20–35 mg/L for hydroxycarbazepine has been proposed (3).

This paper presents distribution data of hydroxycarbazepine in three cases investigated by the Office of the Chief Medical Examiner where it was detected in the biological specimens.

Experimental

Materials

Oxcarbazepine and hydroxycarbazepine were obtained from Novartis Pharmaceuticals (Basel, Switzerland). Cyclopall was obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were purchased from J.T. Baker (Phillipsburg, NJ) or Aldrich Chemical Co. (Milwaukee, WI). Solvents were Fisher Scientific (Fair Lawn, NJ) “Optima grade.” Trimethylammonium hydroxide (TMAH), 0.2M in methanol was obtained from Pierce (Rockford, IL) and a working solution of 0.033M was prepared by mixing 10 mL 0.2M TMAH and 50 mL methanol. Phosphate buffer (0.1M, pH 5) was prepared by adding 13.6 g of potassium dihydrogen phosphate to 1 L with distilled water and adjusting to pH 5 with 0.1M sodium hydroxide. Chem-Elut® columns were purchased from Varian Inc. (Harbor City, CA).

Hydroxycarbazepine extraction and analysis

To 1 mL blank, calibrator, and case specimen were added 100 µL of internal standard solution (100 mg/L cyclopall in methanol) and 2 mL of pH 5 phosphate buffer. Tissue specimens were homogenized in distilled water (1 part tissue/4 parts distilled water) prior to the addition of the internal standard and buffer. After vortex mixing, the contents of each tube were poured onto Chem-Elut columns. After 10 min, the drugs were eluted from the columns with 8 mL of methanol. This was repeated after an additional 10 min. The methylene chloride was evaporated to dryness at 50°C. The extracts were reconstituted in 600 µL of methanol and vortex mixed for 5 s. Fifty microliters of 0.033M TMAH and 400 µL of each extract
were transferred to an autosampler vial. Two milliliters were injected into the gas chromatograph (GC).

Hydroxycarbazepine was quantitated from a matrix based calibration curve using 3-6 calibrators of concentrations ranging from 2.5 to 40 mg/L. Appropriate tissue dilutions were made to ensure that area ratios were within the range of the standard curve.

**Instrumentation**

Hydroxycarbazepine analysis was performed on a Hewlett-Packard 5890 GC equipped with a nitrogen-phosphorus detector (NPD). The column was a J&W DB-5 5% phenylmethyl-silicone fused capillary column (15 m x 0.25-mm i.d., 0.25-µm film thickness). The oven temperature began at 100°C for 1 min, increased by 25°C/min to 260°C, and held for 6 min. Total analysis time was 12 min.

Drug confirmation was performed using a Hewlett-Packard 5890 series 2 GC equipped with a 5972 mass selective detector. The column used was a cross-linked HP-5 fused silica capillary column (25 m x 0.32-mm i.d., 0.17-µm film thickness). Helium was the carrier gas flowing at 1 mL/min. The oven temperature began at 100°C for 1 min, increased at 30°C/min to 200°C, increased at 10°C/min to 260°C, and increased at 20°C/min to 300°C, holding for 8 min. Splitless injection mode was utilized. The mass spectrometer (MS) was operated in the scan electron ionization mode.

**Results and Discussion**

Specimens from each case were tested for volatiles, therapeutic, and abused drugs. This included volatile testing for methanol, ethanol, acetone, and isopropanol by headspace GC; acid/neutral drug testing by GC-NPD; alkaline drug testing by GC-NPD; morphine by radioimmunoassay; and acetaminophen, ethchlorvynol, and salicylate by color test. No ethanol or other volatile substances were detected in the three cases. Table I lists the drugs identified and quantitated in the heart blood of each case. Each drug was identified by GC-NPD or radioimmunoassay and confirmed by GC-MS. Table II provides the distribution of hydroxycarbazepine in all of the specimens received with each case. Parent drug was not detected in any of the presented cases at a limit of quantitation of 2.5 mg/L. Oxcarbazepine is detectable using the same extraction and chromatographic system. It elutes approximately 0.3 min. after hydroxycarbazepine under the chromatographic system used.

Hydroxycarbazepine was originally identified in the basic drug screen. The acid/neutral screen used in this laboratory is designed to identify specific drugs. Conversely, attempts are made to identify all peaks on the base screen chromatogram. On a DB-5 column, hydroxycarbazepine elutes after the common antidepressants, antihistamines, and carbamazepine. On an acid screen, it also elutes after the common barbiturates, but prior to phenytoin. The analytical procedure used is identical to the one used in this office to screen for acid/neutral drugs. This includes a flash methylation step using TMAH; hydroxycarbazepine was methylated. Like carbamazepine, it has an electron ionization mass spectral base peak at m/z 193. It also has prominent ions at m/z 180, 210, and 254. Quantitation was achieved using the same analytical procedure routinely used in this laboratory for quantitating barbiturates and anticonvulsants. For the purposes of this study, the limit of quantitation was 2.5 mg/L and the upper limit of linearity was 40 mg/L.

The causes and manners of death in the reported cases are given in Table III. In each case, the presence of hydroxycarbazepine was ruled to be an incidental finding to the ultimate cause of death. In case 1, although the hydroxycarbazepine was at the top of the reported therapeutic range, the bupropion concentration in this case was well above the therapeutic range. In case 2, the decedent had chest injuries, the presence of morphine in the blood and heart disease. In case 3, hydroxycarbazepine was present in very low concentrations.

There were two cases where both the heart blood and peripheral blood (from the subclavian vein) were analyzed. In
both cases, the concentrations were within approximately 10% of each other. This suggests that postmortem redistribution is not a significant factor in the interpretation of hydroxycarbazepine concentrations. This is consistent with previously published work on carbamazepine. Prouty and Anderson (4) reported a heart blood to femoral blood ratio of between 0.89 and 1.4 in four cases of carbamazepine use. Dalpe-Scott et al. (5) found an average cardiac to peripheral blood ratio of 0.9 ± 0.2 in 12 cases of carbamazepine ingestion.

The blood hydroxycarbazepine concentrations in the presented cases were higher than the liver and kidney concentrations. Klys et al. (6) published 16 fatal cases in which carbamazepine, its epoxide, and dihydroxy metabolites were detected. There were variable liver to blood and kidney to blood concentration ratios for both drug and metabolites. The bile and urine also contained a significant amount of hydroxycarbazepine in relation to the blood. Therefore, either specimen could be used for screening purposes.

References