Disposition of Valproic Acid in a Case of Fatal Intoxication

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Abstract

The case history and toxicological findings of a fatal suicidal valproic acid overdose are presented. Valproic acid concentrations were determined in body tissues and fluids by gas-liquid chromatography (GLC) following both direct extraction and the method of standards addition and quantitative fluorescence polarization immunoassay. The quantitative results obtained by the three procedures were in good agreement. Qualitative identification of valproic acid as its methylated derivative was by ion-trap gas chromatography–mass spectrometry. Toxicological analysis by direct extraction GLC yielded the following valproate concentrations (mg/mL or mg/kg): blood, 1050; bile, 713; brain, 510; heart, 670; kidney, 1580; liver, 985; and vitreous, 516. A total of 15.1 g of valproate was recovered in the stomach contents. These findings far exceed those associated with valproate therapy and are similar to the limited valproate disposition data reported in prior fatal overdoses.

Introduction

Valproic acid (2-propylpentanoic acid) has been successfully used as an anticonvulsant agent for over 30 years. The drug is primarily indicated for the treatment of various forms of epilepsy. The anticonvulsant efficacy of valproic acid is highly variable and dependent upon the type and severity of seizure and an individual’s pharmacokinetic parameters. The minimum effective serum concentration for optimal seizure control is approximately 50 mg/L, and seizure control tends to improve as concentration increases over the range of 50 to 100 mg/L (1,2). Therapeutic doses range from 200 to 2500 mg daily. Adverse reactions associated with valproic acid therapy include mild ataxia, nausea, vomiting, and tremor (3,4). Fatal pancreatitis (5–7) and hepatotoxicity (8–10), particularly in children, have developed during valproic acid therapy.

Acute intoxication attributable to massive valproic acid overdose is uncommon and may be successfully treated when discovered (11–14). Central nervous system toxicity with symptoms varying from drowsiness to coma and cerebral edema is the most frequent effect of overdose. Respiratory failure has been observed in all fatal cases (13). Fatal overdose is rare, and only limited data concerning toxic and fatal blood and tissue concentrations are available in the published literature (15–23).

We report a case of fatal suicidal valproic acid overdose. Valproic acid concentrations were determined in body tissues and fluids by quantitative immunoassay and gas–liquid chromatography (GLC) following both direct extraction and the method of standards addition. Qualitative identification of valproic acid as its methylated derivative was by ion-trap gas chromatography–mass spectrometry (GC–MS). Case findings were compared to previous fatalities.

Case History

The deceased was a 28-year-old Caucasian male found dead in his bedroom. He had a history of epilepsy and had recently experienced several business and personal failures. A prescription for 120 500-mg valproic acid tablets, one to be taken twice daily, had been filled the day prior to his death. Only 17 tablets were recovered at the scene. A suicide note was also found next to the body. With the exception of generalized visceral congestion and acute pulmonary and cerebral edema, gross pathology and histopathological findings were unremarkable. Body fluids and tissues were collected and sent for toxicological analysis.

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Toxicological Analysis

Sample preparation

Blood, bile, and vitreous humor samples were diluted with an equal quantity of distilled water before analysis. One-gram samples of brain, liver, kidney, and heart were diluted with 4.0 mL of distilled water and mixed in a small ground glass homogenizer. Aliquots of these homogenates were then analyzed by the methods described here.

Immunoassay

All reagents necessary for fluorescence polarization immunoassay (FPIA) were purchased from Abbott Laboratories (Abbott Park, IL). The FPIA valproic acid assays were performed in an Abbott TDx analyzer with 3.03 version software. The calibration curve for the assay was constructed with six calibrator solutions supplied with the assay consisting of valproic acid concentrations of 0, 12.5, 25, 50, 100, and 150 mg/L. FPIA controls with target values of 37.5, 72.5, and 124.5 mg/L valproic acid were obtained from Abbott Laboratories. All immunoassay reagents were stored refrigerated at 1°C.

GLC reagents

HCl (1N) was prepared by diluting 86 mL concentrated hydrochloric acid to 1 L with distilled water. Methanol, chloroform, and hydrochloric acid were all ACS grade or better.

GLC standards

Stock valproic acid standard, 1000 mg/L, was prepared by dissolving 115.2 mg sodium valproate (propylpentanoic acid, Sigma Chemical Co., St. Louis, MO) in 100 mL methanol. A calibration curve was prepared using 0–200 mg/L concentrations in drug-free serum. Stock Internal standard, 7.0 mg/L, was prepared by diluting 1.0 mL Cyclohexane carboxylic acid (Aldrich Chemical Co., Milwaukee, WI) to 150 mL with methanol. This compound is a semi-solid at room temperature and was placed in a 37°C water bath until melted before diluting. Working internal standard solution was prepared by diluting the stock solution 1:40 with methanol. Control serums with target values of 25 mg/L (Quality Assurance Services, Augusta, GA), 60 mg/L (chemTRAK®-TDM, Medical Analysis Systems, Inc., Carmarillo, CA), and 120 mg/L (Lyphochek®, Bio-Rad Laboratories, Hercules, CA) valproic acid were analyzed with each batch of samples.

Direct extraction procedure

Quantitative GLC analysis of valproic acid was based upon the method of Wohler and Poklis (24). Aliquots of 200 μL working internal standard and 200 μL of calibrator, control, or sample were pipetted into separate 12-mm x 75-mm test tubes and mixed lightly in hand. One-hundred microliters 1N HCl and 500 μL chloroform were added to each tube. The mixture was then vortex mixed for 15 s and centrifuged at 3400 rpm for 5 min. One microliter of the lower chloroform layer was injected into the GC. Valproic acid serum concentrations were calculated from a linear regression of the calibrator responses based on peak-height ratio (peak height of valproic acid to that of the internal standard). The presented method for valproic acid in serum was determined to have a lower limit of detection (LOD) of 5 mg/L and a lower limit of quantitation (LOQ) of 10 mg/L, and it was linear up to 6000 mg/L (24).

Standard addition procedure

To three 200-μL aliquots of sample homogenate were added valproic acid calibrator to produce separate aliquots with additional valproic acid concentrations of 50, 100, and 150 mg/L. The aliquots were vortex mixed, allowed to stand for 4 h, and then extracted as described above under the direct extraction procedure. The valproic acid concentration in the sample was determined by the linear regression equation: y (total concentration determined against internal standard) = x (concentration added to aliquot) + b, where b is the actual concentration in the aliquot. All regression equations for each specimen yielded slopes of 1 and r² values of 1.

GLC instrumentation

Analyses were performed on a Shimadzu GC-14 GC equipped with a flame ionization detector. The detector response was recorded and integrated on a Hewlett Packard 3395 integrator. Chromatography was performed isothermally at 140°C on a “Nukol” poly(ethylene glycol) modified with nitroterephthalic acid bonded phase capillary column (15 m x 0.53-mm i.d., 0.5-μm film thickness, Supelco, Bellefonte, PA). Injection port temperature was 200°C, and detector temperature was 230°C. Gas flow rates were as follows: helium carrier gas, 20 mL/min;
hydrogen, 20 mL/min; and air, 300 mL/min. Under these conditions, the retention times were as follows: valproic acid, 3.0 min and internal standard, 5.4 min. The relative retention time (RRT) of valproic acid to the internal standard was 0.56.

**GC–MS**

Following direct extraction as described, chloroform extracts were evaporated, dissolved in 100 µL of ethyl acetate, methylated with 50 µL of trimethylanilinium hydroxide (Pierce, Rockford, IL), and injected into the GC–MS. The GC was a Varian Star model 3400Cx with a split/splitless injection port connected to a Varian Saturn model 3 ion-trap (IT) MS with Saturn software version 5.2 to control the operation of the GC and IT. The GC oven program was as follows: initial temperature, 60°C; initial time, 1.0 min; rate, 15°C/min; final temperature, 300°C; and final time, 3.0 min. The injection port temperature was 170°C, and the transfer line temperature was 280°C. The column was a DB-5ms capillary column (30 m × 0.25 mm i.d., 0.25-µm film thickness). The IT was operated in the full scan mode. Tissue extract spectra were compared with that of unextracted derivatized valproic acid. The methylvalproate derivative yielded the following major fragments and relative abundances: 87 (100%); 115 (50%); 55 (33%); 57 (30%); and molecular ion, 159 (30%) (Figure 1).

**Table I. Comparison of Three Methods to Determine Valproic Acid Concentrations in the Presented Case**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Valproic Acid (mg/L or mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct extraction</td>
</tr>
<tr>
<td>Blood</td>
<td>1050</td>
</tr>
<tr>
<td>Bile</td>
<td>713</td>
</tr>
<tr>
<td>Brain</td>
<td>510</td>
</tr>
<tr>
<td>Heart</td>
<td>670</td>
</tr>
<tr>
<td>Kidney</td>
<td>1580</td>
</tr>
<tr>
<td>Liver</td>
<td>985</td>
</tr>
<tr>
<td>Vitreous</td>
<td>516</td>
</tr>
<tr>
<td>Gastric (total)</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND = Not determined.

**Table II. Comparison of Valproic Acid Disposition in Fatal Overdose Cases versus Therapeutic Administration**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Present case (mg/L or mg/kg)</th>
<th>Overdose case (mg/L or mg/kg)</th>
<th>Therapeutic case (mg/L or mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1050</td>
<td>1753</td>
<td>60</td>
</tr>
<tr>
<td>Bile</td>
<td>713</td>
<td>3057</td>
<td>ND*</td>
</tr>
<tr>
<td>Brain</td>
<td>510</td>
<td>262</td>
<td>15</td>
</tr>
<tr>
<td>Heart</td>
<td>670</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Kidney</td>
<td>1580</td>
<td>1520</td>
<td>36</td>
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<tr>
<td>Liver</td>
<td>985</td>
<td>811</td>
<td>100</td>
</tr>
<tr>
<td>Vitreous</td>
<td>516</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Gastric (total)</td>
<td>15.1 g</td>
<td>1129</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND = Not determined.

**Results and Discussion**

The results of the toxicological analysis by the three different procedures are presented in Table I. The results between the procedures were in good agreement. Both diluted body fluids and tissue homogenates were easily analyzed by the FPIA immunoassay yielding comparable results to gas-liquid chromatography. The agreement between the direct extraction procedure and the method of standard additions demonstrates the lack of matrix effects and ease of direct extraction of valproic acid from tissue matrices.

Only eight cases of fatal valproic acid overdose have been reported in the literature (15–23; references 18 and 19 report the same case). Blood or serum valproic acid concentrations in previous fatal cases range from 520 mg/L (16) to 1970 mg/L (17). Tissue concentrations have been seldom reported; only one case provided tissue disposition data (21), and liver values of 487 (23) and 800 mg/kg (22) have been reported in only two other cases. The findings in the presented case far exceed those observed in a patient receiving therapeutic doses (25) and are similar to those in a prior fatal overdose (22) (Table II). The ratios of the valproic acid concentrations in the solid tissues to those of the blood ranged from 0.48 for the brain to 1.5 for the kidney. This is consistent with the relatively low apparent volume of distribution of valproic acid, 0.1 to 0.5 L/kg, which results from its extensive serum protein binding (> 90%) and ionization at physiological pH (> 90%) (1).

The deceased had available to him a new prescription for 120 500-mg valproic acid tablets of which only 17 were found. Several older empty prescription vials for valproic acid were also recovered at the scene. This indicated ingestion of a dose of at least 51.5 g. As 15.1 g of valproic acid was recovered in the stomach contents, it was assumed that the absorbed dose was at least 36.4 g of valproate. The probable ingested dose was also determined by a pharmacokinetic-based estimate relating the decedent's weight (79 kg at the time of death), the measured valproate blood concentration (1.05 g/L), and the volume of distribution of valproic acid (0.5 L/kg). The calculation yielded an absorbed dose of 41.5 g of valproate. Given that the decedent was routinely ingesting 1.0 g daily by prescription before his death, it can be assumed that he would have had at least several grams of valproate in his body before the overdose. Therefore, the pharmacokinetic estimate is approximately 39.5 g, which is in good agreement with the amount based upon the death scene investigation.

**Conclusion**

Based on the circumstances surrounding the presented death, unremarkable pathology, and the high tissue concentrations and dose of valproic acid ingested, the cause of death was attributed to an overdose of valproic acid. The manner of death was ruled suicide. Because of the relatively low apparent volume of distribution of valproic acid, tissue concentrations do not vary widely as compared with blood values and the drug may be reliably analyzed in tissue homogenates by direct extraction GLC or FPIA.

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References


