A Fatality Due To Alprazolam Intoxication

Amanda J. Jenkins, Barry Levine*, J. Taron Locke, and John E. Smialek
Office of the Chief Medical Examiner, State of Maryland, Baltimore, Maryland

Case Report

Alprazolam has a short duration of action with an average plasma half-life of 11 h (1). It is extensively metabolized by oxidation and conjugation with only 20% of the parent drug appearing unchanged in urine (5). The major metabolites are α-hydroxylalprazolam, 4-hydroxylalprazolam, α,4-dihydroxylalprazolam, and 3-hydroxy-5-methyltriazolyl chlorobenzophenone (HMTBP). α-Hydroxylalprazolam and 4-hydroxylalprazolam are pharmacologically active with approximately 66 and 19% of the potency of alprazolam, respectively (5). Smith and Kroboth (6) reported that unconjugated forms of these two metabolites were detected at plasma concentrations of less than 10% of the parent drug concentration even after chronic dosing, and, therefore, their contribution to pharmacological effects may be minimal in therapeutic cases.

Adverse reactions to alprazolam are typically observed at the beginning of therapy and diminish under continued treatment. The most common effects reported are drowsiness and fatigue. Other adverse reactions include confusion, headache, nausea and vomiting, tachycardia, hypotension, and blurred vision (2). Abrupt cessation of chronic alprazolam therapy has resulted in withdrawal symptoms, the most important being seizure activity (2). However, alprazolam is generally considered a safe and effective drug with a high therapeutic index. Few overdoses attributable to alprazolam have been reported, and even fewer fatalities due to alprazolam intoxication without the combination of other drugs are documented. In this study, we report the tissue distribution of alprazolam and α-hydroxyalprazolam in a death due solely to the ingestion of alprazolam.

Case History

The deceased was a 44-year-old white female with a history of psychiatric problems. She was found dead in bed at her residence. Her current medications, which were found at the scene, included alprazolam, fluoxetine, phenytoin, lorazepam, and venlafaxine. Although there was a history of suicidal ideation, there was no suicide note.

At autopsy, external examination showed a well-nourished,
well-developed female. Internal examination was unremarkable except for 60 mL of a white granular substance and 40 mL of a green fluid in the stomach. Microscopic findings demonstrated mild emphysematous changes in the lungs with intra-alveolar hemorrhages and focal mild pulmonary edema. Specimens collected for toxicological analysis were stored in sterile polypropylene containers without preservative and were frozen at -20°C until assayed.

Methods

Materials
Alprazolam and α-hydroxyalprazolam were purchased from Sigma Chemical (St. Louis, MO). Chlordiazepoxide was obtained from Alltech Applied Science Labs (State College, PA). Analytical-grade sodium borate was purchased from Mallinckrodt (St. Louis, MO), and a saturated solution was prepared for use as a buffer. Reagent-grade methylene chloride, methanol, and diethylamine were obtained from Fisher Scientific (Fair Lawn, NJ). Solid-phase extraction columns (Chem ElutTM) were purchased from Varian (Harbor City, CA).

Procedure
Alprazolam and α-hydroxyalprazolam analytical standards were prepared in methanol at a concentration of 100 mg/L. Chlordiazepoxide (100 mg/L), the internal standard, was also prepared in methanol. Internal standard (50 μL) was added to 2 mL of blank blood or case specimen and 2 mL of buffer solution. Standard curves for alprazolam and α-hydroxyalprazolam were prepared at the following concentrations: 0, 0.5, 1.0, 2.5, and 5 mg/L. Homogenates of tissue samples were prepared by adding 1 g tissue to 4 mL distilled water. Samples were vortex mixed and applied to solid-phase extraction columns. The tubes were rinsed with 1 mL buffer, which was then applied to the columns. Drugs were eluted from the column with 2 x 7 mL methylene chloride. The eluent was evaporated to dryness, and the residue was reconstituted with 400 μL methanol. An aliquot was injected onto the high-performance liquid chromatograph (HPLC) for quantitative analysis. The assay was linear from 0.2 to 5.0 mg/L with a limit of sensitivity of 0.2 mg/L. Drug identification was achieved by full-scan gas chromatographic–mass spectrometric (GC–MS) analysis and comparison with an authentic standard.

HPLC instrument conditions
A Waters (Milford, MA) 501 HPLC pump interfaced with a Waters 715 Ultra Wisp sample processor, a Waters 490E programmable multiwavelength detector, and a Hewlett-Packard (Palo Alto, CA) integrator model 3394A were used for the quantitative determination of alprazolam and α-hydroxyalprazolam. A Beckman (San Ramon, CA) ODS column (15 cm x 4.6-mm i.d., 5-μm particle size) was heated to 30°C. The detector wavelength was 240 nm. The mobile phase consisted of methanol and water (60:40) with 1% diethylamine (pH 6) and a flow rate of 1.5 mL/min.

Results and Discussion
A comprehensive toxicological screen was performed on the heart blood and urine specimens obtained in this case. This included ethanol and volatile analysis by headspace GC, radioimmunoassay for morphine, a liquid–liquid extraction followed by GC–nitrogen-phosphorus detection (NPD) for basic drugs, and solid-phase extraction with GC–NPD for acidic or neutral drugs. In addition, color tests were performed for acetaminophen, aspirin, and ethchlorvynol. The urine was positive for alprazolam, chlorpheniramine, dextromethorphan, and doxylamine. The heart blood was positive for alprazolam only. Using the normal base extraction procedure therapeutic concentrations of alprazolam are usually undetected because the limit of detection is 0.1 mg/L. However, in this case, alprazolam was easily detected by GC–NPD after base extraction was performed and a full-scan electron impact mass spectrum was obtained.

Quantitation of alprazolam and α-hydroxyalprazolam in biological specimens was performed by reversed-phase HPLC. This procedure is commonly used for the analysis of benzodiazepines (7–9). In this assay, the order of elution was α-hydroxyalprazolam, alprazolam, and the internal standard. The relative retention times with respect to internal standard were 0.70 and 0.84 min for α-hydroxyalprazolam and alprazolam, respectively. Chlordiazepoxide was chosen as the internal standard to enable baseline resolution of the three drugs while optimizing analysis time.

The quantitative results obtained in this case are shown in Table I. Alprazolam was detected in all specimens analyzed. Concentrations of alprazolam >2 mg/L were measured in both heart and subclavian blood specimens. There was good agreement in the alprazolam concentration between the two blood specimens. The alprazolam concentration in liver was approximately 2.4 times the concentration in kidney. The concentration of parent drug measured in bile was approximately three times the concentration determined in urine, which suggested that this specimen would be useful for an initial screening for those assays that are not targeted to drug metabolites. This case also showed a readily detectable concentration of alprazolam in the vitreous humor. Given the relative protection of the vit-
tissue in the absence of other appropriate specimens. The metabolite α-hydroxylazepam was only detected in the subclavian blood, urine, bile, and liver specimens. α-Hydroxylazepam was present at 5.2% of the concentration of parent drug in the blood, which agrees with the work of Smith and Kroboth (6). Therefore, even at fatal concentrations, the relative contribution of α-hydroxylazepam to toxicity may be minimal.

The tissue distribution of alprazolam described in this report differs from that of other benzodiazepines noted in fatalities that are due to intoxication. Cardauns and Iffland (10) described the tissue distribution of diazepam and its active metabolite, nordiazepam, in an intoxication. The concentration of the parent drug was 10 times greater in blood than urine compared with two times greater concentration in the alprazolam case described in this report. Furthermore, the concentration of diazepam in the kidney was only 2.4 times less than its concentration in the liver. In this case, the concentration of alprazolam in the kidney was only 2.4 times less than its concentration in the liver. Cardauns and Iffland (10) found relatively low concentrations of nordiazepam in the tissues analyzed; the highest concentration was reported in the urine. This was similar to the distribution of α-hydroxylazepam reported in this study.

In a fatality attributed solely to chlordiazepoxide intoxication (1), the concentration of the parent drug in the blood was about three times the concentration in urine. Concentrations in the liver and kidney were approximately equivalent, and the highest concentration of drug in the specimens analyzed was detected in the bile. Metabolite concentrations were not reported in this case.

There are only two reports of fatal intoxications that were due to the ingestion of alprazolam documented in the literature. Edinboro and Backer (7) reported a blood alprazolam concentration of 0.177 mg/L in an autemor mortem hospital admission specimen from a depressed and suicidal woman. In another study, Stafford et al. (11) reported a postmortem blood alprazolam concentration of 0.122 mg/L in an acute alprazolam intoxication with concomitant ingestion of ethanol (postmortem blood alcohol concentration = 0.15 g/dL). If the therapeutic range is considered to be 0.025–0.102 mg/L, depending upon low- or high-dose therapy, the alprazolam concentrations determined in both of the previous fatalities were 1.2–7.1 times therapeutic levels. In comparison, the mean blood concentration determined in this case was 22–90 times greater than the therapeutic range. These concentrations are the highest reported in the literature to date.

Conclusion

Following the completion of the investigation and the evaluation of the autopsy and toxicology findings, the medical examiner ruled the cause of death as acute alprazolam intoxication. Given the past psychiatric history of suicidal ideation and the alprazolam concentration in the blood, the manner of death was ruled suicide.

References


Manuscript received July 29, 1996; revision received October 25, 1996.