CASE REPORT

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A Fatality Involving Clomipramine

REFERENCE: Fraser, A. D., Isner, A. F., and Moss, M. A., **"A Fatality Involving Clomipramine,"** *Journal of Forensic Sciences*, JFSCA, Vol. 31, No. 2, April 1986, pp. 762-767.

ABSTRACT: A fatality following ingestion of the tricyclic antidepressant clomipramine (Anafranil®), alprazolam (Xanax®), and ethyl alcohol is described. Clomipramine and *N*-desmethylclomipramine were quantitated by high performance liquid chromatography and alprazolam by gas liquid chromatography. Concentrations of clomipramine and *N*-desmethylclomipramine were: in blood—0.84 and 1.4 mg/L; in urine—0.56 and 0.62 mg/L. Alprazolam concentration in blood was 0.069 mg/L. Ethyl alcohol was measured by headspace gas chromatography and found to be 375, 385, and 435 mg/dL in blood, urine, and vitreous humor, respectively. These findings are compared to previous reports of clomipramine related fatalities and alprazolam toxicity combined with ethyl alcohol.

KEYWORDS: toxicology, clomipramine, alprazolam, chromatographic analysis, antidepressants

Clomipramine hydrochloride (Anafranil®, Ciba-Geigy Canada Ltd., Mississauga, Ontario) is a tricyclic antidepressant drug used in the treatment of depressive illness, including manic depressive psychosis and involutional melancholia [*1*]. It is the 3-chloro analogue of imipramine and shares many of the pharmacological properties of imipramine [*2*]. Although this drug has not been approved by the U.S. Food and Drug Administration, it is an established drug with 18 years of clinical use in Europe and available for several years in Canada.

Alprazolam (Xanax®) is a relatively new triazolobenzodiazepine which is known to be anxiolytic in man [*3*] and may be effective in treatment of depression [*4*].

Several recent reports summarize fatal and nonfatal intoxications for the most common tricyclic antidepressants [*5-9*]. However, only two reports of fatalities involving clomipramine have been published [*10,11*]. The first report of two cases described a colorimetric assay which did not differentiate clomipramine from its major metabolites. Meatherall [*11*] reported a case where quantitation of clomipramine and *N*-desmethylclomipramine was performed by gas

Received for publication 26 March 1985; revised manuscript received 9 July 1985; accepted for publication 10 July 1985.

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liquid chromatography. This report documents analytical findings of clomipramine, *N*-desmethylclomipramine, alprazolam, and ethyl alcohol in a single case of death.

Case History

This 51-year-old woman had a long history of alcohol abuse and depression. The husband left for work on 9 November with the intention of staying away from home on a hunting trip for several days. The decedent was in a drunken state when he left and was checked by a daughter later that day and at noon the following day. The daughter noted that her mother was heavily asleep but rousable. Upon returning shortly before midnight on 10 November, she found her mother dead, lying face down beside the bed.

Investigation revealed that the decedent had a history of receiving medication for her depression and had been a patient at a mental hospital. One week before her death, she was taken to the emergency department of a local hospital for treatment of alcohol withdrawal symptoms. On 8 November, she received a prescription for alprazolam (Xanax) and clomipramine (Anafranil). At the time of death, 57 alprazolam tablets (0.5 mg each) and 42 clomipramine tablets (25 mg each) were missing. There were indications that she had consumed a pint of liquor and six bottles of beer before her death.

An autopsy was not performed. Toxicologic analysis on blood, urine, and vitreous humor are described below. External examination revealed a superficial recent abrasion above the right eye measuring 3 cm. A mixed drug overdose was considered the cause of death.

Toxicologic Analysis

Standards and Reagents

Acetonitrile and methanol were HPLC grade and glass distilled (Caledon Laboratories Ltd., Georgetown, Ontario). Doxepin hydrochloride was obtained from Pfizer Canada Inc., Pointe Claire-Dorval, Quebec. Protriptyline hydrochloride was obtained from Merck Frosst Canada, Inc., Pointe Claire-Dorval, Quebec. Clomipramine hydrochloride was supplied by Ciba-Geigy, Mississauga, Ontario and *N*-desmethylclomipramine hydrochloride by Ciba-Geigy, Basle, Switzerland. The Upjohn Company of Canada, Don Mills, Ontario, provided the alprazolam standard, and the flurazepam hydrochloride was a gift from Hoffman LaRoche, Ltd., Vaudreuil, Quebec.

Thin-Layer Chromatography

A urine specimen was subjected to a general drug screen by thin-layer chromatography (TLC). The urine aliquot was made basic with sodium bicarbonate (pH 8.5) and mixed with chloroform. The chloroform layer was dried under nitrogen. After drying, the residue was dissolved in chloroform:methanol (1:1) and spotted on a silica gel 60 plate (E. Merck and Co., Darmstadt, W. Germany). After developing in a tank saturated with ethyl acetate:methyl alcohol:ammonium hydroxide (170:20:10), the plate was sprayed was iodoplatinate reagent [*12,13*].

Gas Liquid Chromatography

Alprazolam was analyzed by gas chromatography based on the method of Moffatt [*14*] with some modifications. An aqueous solution of alprazolam was prepared at 1000 μg/dL. Drug free blood standards were prepared from the aqueous standard to obtain standards of 1, 2, 5, and 10 μg/dL of alprazolam. To a 0.5-mL sample or standard were added 0.5 mL of 0.05*M* potassium carbonate buffer (pH 10), 0.1 mL of flurazepam internal standard (20 μg/dL), and

5.0 mL of 1-chlorobutane. Tubes were mixed for 5 min, 4.5 mL of organic layer removed, and evaporated under nitrogen. Residues were taken up in methyl alcohol for injection onto the gas chromatograph.

An HP 5730A gas chromatograph fitted with an electron capture detector (Hewlett Packard Canada Ltd., Montreal, Quebec) was used for alprazolam quantitation. A 1.2-m by 2-mm inner diameter glass column packed with 3% SP 2250 on 80–100 mesh Supelcoport (Supelco Inc., Bellefonte, PA) was operated at 280°C. The injector and detector temperatures were set at 300°C.

Ethyl alcohol was quantitated in blood, urine, and vitreous humor by headspace analysis [*15*] on a Carle 9500 gas chromatograph (obtained from Technical Marketing Associates, Halifax, Nova Scotia). The column was packed with 5% Hallcomid M-18 and 0.5% Carbowax 600 on 40–60 mesh Teflon 6 (Technical Marketing Associates, Halifax, Nova Scotia).

Peak height ratios relative to the internal standards were used for quantitation.

High Performance Liquid Chromatograph (HPLC)

Liquid chromatography was performed on a model 740 solvent delivery system by Spectra Physics, SF 770 variable wavelength detector by Schoeffel Instruments, and an Omniscribe recorder by Houston Instruments (all obtained from Technical Marketing Associates, Halifax, Nova Scotia). Analysis was performed at ambient temperature using a 250- by 4.6-mm RP-8 column with 5-μm particle size (Brownlee Labs, Santa Clara, CA). Detector wavelength was set at 205 nm. Quantitation of clomipramine and *N*-desmethylclomipramine was linear from 0.025 to 1.6 mg/L. Absolute recovery ranged from 90 to 95%.

A buffer solution consisting of 0.01 mol/L of potassium dihydrogen phosphate was mixed with acetonitrile and *n*-nonylamine to form the mobile phase (555/450/0.6). This mixture was adjusted to pH 3.2 with phosphoric acid. The flow rate was 1.6 mL/min.

Doxepin and protriptyline were used as internal standards for clomipramine/*N*-desmethylclomipramine analysis. Stock drug solutions are 100 μg/mL (as free bases) were prepared in methyl alcohol. A drug free serum was added to give stock standards of clomipramine and *N*-desmethylclomipramine at 500 ng/mL. To a 2.0 mL of sample (standard and unknown) was added 5 mL of hexane:isoamyl alcohol (97:3) containing the two internal standards (50 ng/mL) and 0.1 mL of saturated sodium carbonate. The tubes were mixed for 20 min and centrifuged. The organic extract was transferred to an 8-mL screw cap tube with a plastic pasteur pipette. A 0.1-mL mixture of mobile phase:0.1*M* phosphoric acid (1:1) was added followed by vortexing and then centrifugation. The organic layer was aspirated to waste and the aqueous phase injected onto the HPLC column. The range was set at 0.02 absorbance units full scale and 30 μL of aqueous phase was injected.

Peak height ratios were used to calculate drug concentrations using the average factor obtained from the two internal standards.

The HPLC procedure provides better resolution of clomipramine from *N*-desmethylclomipramine than a previously reported gas chromatography procedure [*11*].

Results

Qualitative analysis of the urine gave a TLC pattern consistent with a tricyclic antidepressant and a trace of a benzodiazepine with the Bratton Marshall reagent.

A summary of the quantitative results appears in Table 1.

A chromatogram of the blood extract for clomipramine is found in Fig. 1.

Discussion

Therapeutic serum concentrations for clomipramine and *N*-desmethylclomipramine have been reported to range from 24 to 200 μg/L and 36 to 300 μg/L, respectively [*2,16*].

TABLE 1—*Summary of toxicological analysis.*

Drug	Specimen Concentrations		
	Blood	Urine	Vitreous Humor
Clomipramine, mg/L	0.84	0.56	...
N-desmethylclomipramine, mg/L	1.4	0.62	...
Alprazolam, mg/L	0.069	...	...
Ethyl alcohol, mg/dL	375	380	435

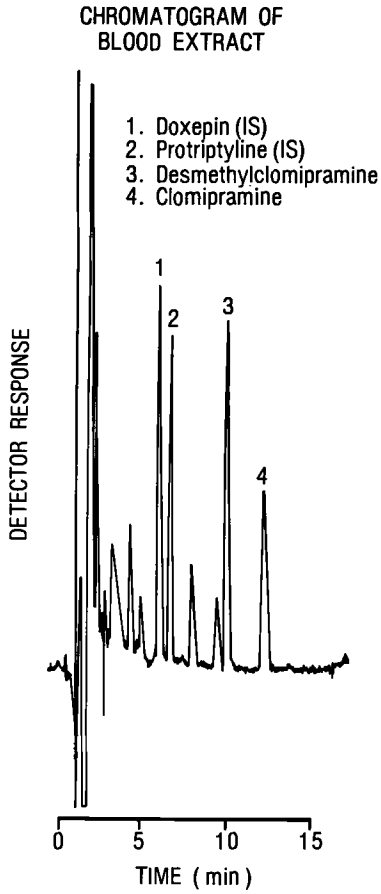

FIG. 1—*High performance liquid chromatogram of blood extract:* (1) *doxepin internal standard,* (2) *protriptyline internal standard,* (3) N-*desmethylclomipramine 1.4 mg/L, and* (4) *clomipramine 0.84 mg/L.*

Montgomery et al [*17*] reported that clinical response in the treatment of depression showed no correlation with plasma clomipramine concentrations, although poor responders tended to be associated with low concentrations of parent drug and high concentrations of metabolite. They stated that severity of depression and side effects worsened three to six weeks after starting therapy and felt that this was due to an increasing concentration of *N*-desmethylclomipramine. In another study, Traskman [*2*] reported a modest correlation between *N*-desmethyl-

clomipramine plasma concentration with therapeutic effect in depression but no correlation with the parent drug. Miller [*18*] felt that plasma concentration of parent and metabolite showed no correlation with clinical response, but poor responders clinically had a tendency toward higher plasma concentration than "good responders."

At steady state, serum concentration ratios of clomipramine to *N*-desmethylclomipramine have ranged from 0.2 to 0.9 [*2, 19*]. At this hospital, the mean ratio is 0.44 with a range of 0.20 to 0.62. The ratio in whole blood in the previously reported fatality [*11*] was 0.93. The present case had a ratio of 0.6. In a large study of tricyclic associated fatalities [*5*], the ratio of parent to metabolite had a mean value of 4.7 with combined concentrations greater than 1.0 mg/L considered clearly toxic and generally associated with fatalities. Bailey et al [*6*] reported an average ratio of 1.43 in blood in fatal overdose cases. In their experience, high ratios were only seen when a short time interval separated ingestion from death. The ratio of 0.6, in this case, is consistent with the investigational impression of a prolonged interval between drug ingestion and death.

The high ethyl alcohol concentrations, in this case, are "borderline on being regularly fatal" [*20*]. The actual contribution of ethyl alcohol to this death was difficult to ascertain because of the decedent's known tolerance to alcohol, after years of abuse.

Alprazolam concentrations, at steady state, ranged from 0.025 to 0.055 mg/L in patients taking 1.5 to 6.0 mg/day [*21*] and 0.02 to 0.03 mg/L in patients receiving 2 to 5.0 mg/day [*22*].

The Upjohn Company provided information on three clinical cases involving alprazolam-ethyl alcohol toxicity. One patient receiving 12 mg of alprazolam daily took an overdose of 70 mg plus an unknown quantity of alcohol resulting in a blood alcohol of 205 mg/dL. The individual was mildly somnolent, but recovered [*23*].

Moffatt [*14*] reported on a combined alprazolam/ethyl alcohol fatality after an automobile accident. Blood concentrations were 150 mg/dL of alcohol and 0.01 mg/L of alprazolam.

Alprazolam possesses pharmacologic activity characteristic of benzodiazepines and is much more potent than diazepam [*24*]. It is not known with certainly how alprazolam interacts with alcohol or whether this interaction is additive or potentiating. Based on the pill count at the scene, in this case, and the slightly greater than therapeutic blood concentration of alprazolam (0.069 mg/L), one could not implicate alprazolam as a major contributor to this multidrug fatality.

The sum of the blood clomipramine and *N*-desmethylclomipramine was 2.24 mg/L. Cardiac and respiratory toxicity leading to death have been associated with combined blood concentrations greater than 1.0 mg/L for the common tricyclic antidepressants [*5*].

In summary, it is felt that the major factor in this fatality is clomipramine and *N*-desmethylclomipramine with an uncertain contribution by alprazolam/ethyl alcohol.

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