CASE REPORT

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A Fatality Involving Clothiapine and Clomipramine

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ABSTRACT: A fatality resulting from the suicidal ingestion of clothiapine, clomipramine and biperiden is reported. Clomipramine, its metabolite N-desmethylclomipramine and clothiapine were quantified in blood, liver, kidney and gastric contents by HPLC and GC. Biperiden was detected only in the gastric content. Significant differences of drug levels were found in postmortem blood obtained from brain and from heart. Concentrations of clomipramine and N-desmethylclomipramine ranged from 0.48 to 1.61 mg/L and 0.26 to 1.32 mg/L, respectively, and clothiapine from 0.50 to 2.15 mg/L. This phenomenon may reflect a postmortem drug redistribution.

KEYWORDS: toxicology, clothiapine, clomipramine, chromatographic analysis

Clothiapine, 2-chloro-11-(4-methylpiperazin-1-yl) dibenzo [*b,f*] -(1,4)thiazepine, is a dibenzothiazepine tricyclic antipsychotic agent effective in the treatment of acute and chronic schizophrenia. Only one case of nonfatal clothiapine poisoning and no fatal overdoses have been reported [*1*]. Therapeutic blood levels have not been found in the literature. Fatalities have been attributed to loxapine [*2–4*], a dibenzoxapine structurally similar to clothiapine.

Clomipramine, 3-(3-chloro-10,11-dihydro-5H-dibenz [*b,f*] azepin-5yl)-NN-dimethyl-propylamine, is a tricyclic antidepressant widely used in the treatment of endogenous depressive and manic-depressive illness. The drug is rapidly and completely absorbed after oral administration and undergoes extensive N-demethylation to the major active metabolite N-desmethylclomipramine. Since antidepressant drugs are often prescribed to patients with suicidal tendencies, tricyclic antidepressants are frequently involved in fatal overdose cases [*5–11*].

Biperiden, 2-(1,2-diphenylethoxy)ethyl trimethyl-ammonium bromide, is an anticholinergic agent of low toxicity, consequently no fatal biperiden overdoses have been reported.

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This paper reports a fatality involving clothiapine in combination with clomipramine, which presents interesting features attributable to postmortem redistribution.

Case History

A 31-year-old man with a long history of mental illness and previous suicide attempts, treated with antidepressive, antipsycotic and antiparkinson drugs, was brought to the emergency room of a neighboring hospital. When admitted to hospital, approximately 1 h after the ingestion of drugs, the patient was comatose. A gastric lavage was performed immediately, and large amounts of undigested pills and tablet debris were recovered. A few hours later he was intubated and transferred to an intensive care unit in another city, but he died during transportation, 6 h after ingestion of the drugs. In his bedroom, empty boxes and packages of Anafranil (75 mg clomipramine) and Akineton Retard (4 mg biperiden), together with three empty 10 mL vials of Entumin (10% clothiapine solution) were found.

Autopsy was performed 28 h after death. Postmortem examination showed congestion and edema of the lungs and congestion of the liver, kidneys, brain and spleen. Some undigested Akineton Retard pills in pink fluid (approximately 80 mL) were found in the stomach.

Toxicological Analysis

Standards and Reagents

Clomipramine and N-desmethylclomipramine hydrochloride were obtained from Ciba Geigy (Saronno, Italy). Clothiapine and loxapine were supplied by Sandoz (Milano, Italy) and by Cyanamid (Catania, Italy), respectively. Acetonitrile and water were HPLC grade from Merck. Bicarbonate buffers were prepared as follows: a) pH 8.6, 8.3 g of $NaHCO_3$ were dissolved in 100 mL of water; b) pH 10.2, 5 g each of $NaHCO_3$ and Na_2CO_3 were dissolved in 100 mL of water.

Thin-Layer Chromatography

A gastric content specimen was subjected to a preliminary drug screen by thin-layer chromatography (TLC). Two and one half mL 0.1 M phosphoric acid were added to 0.5 g of gastric content. After filtration, the solution was made basic with sodium carbonate and mixed with chloroform. After centrifugation the organic layer was separated and evaporated under nitrogen. The residue was dissolved in methanol and spotted on silica gel 60 F 254 HPTLC plates (Merck). Four eluent systems (ethyl acetate-methanol-30% ammonia (85:10:5), cyclohexane-toluene-diethylamine (65:25:10), ethyl acetate-chloroform (50:50) and acetone with the plate dipped in potassium hydroxide solution (0.1 M) were used. Drugs were detected by spraying first with 10% sulphuric acid, then with the Dragendorff spray reagent and finally with acidified iodoplatinate solution. The identification of drugs was achieved by principal components analysis of standardized Rf values [*12*].

High Performance Liquid Chromatography (HPLC)

Clomipramine and N-desmethylclomipramine were analyzed by liquid chromatography according to the method of Fraser et al. [*9*], using loxapine as internal standard. HPLC chromatography was performed with a Perkin Elmer Series 4 instrument, variable wavelength LC-95 detector, using a 160 by 5 mm ODS-Hypersil column with 5

μm particle size (HP). Detector wavelength was set at 205 nm. Quantification of clomipramine and N-desmethylclomipramine was linear from 0.1 to 3 mg/L. The mobile phase consisting of 0.01 mol/L of potassium dihydrogen phosphate, acetonitrile and n-nonylamine (555/450/0.6), was adjusted to pH 3.2 with phosphoric acid. The flow rate was 1.6 mL/min.

Gas Chromatography

A Dani 3200 gas chromatograph fitted with a Ni 63 ECD and a 12 m × 0.53 mm i.d. fused silica widebore column (SPB 1, Supelco) was used for clothiapine quantification. Nitrogen was used as the carrier gas at a flow rate of 15 mL/min. The analysis was isothermal with a column temperature of 260°C and injector and detector temperatures of 290°C.

Biperiden was detected using a Dani 3600 gas chromatograph with a NPD and a 30 m × 0.32 mm i.d. fused silica capillary column (SPB 1, Supelco). The operating temperatures were as follows: column, 200–280°C (5°C/min); injector 280°C and detector 300°C. The flow rate of carrier gas (helium) was 3 mL/min.

Extraction

Two mL blood sample were mixed with 2 mL of the pH 8.6 buffer, 5 μL of the internal standard solution (loxapine 1 mg/mL in methanol) and 5 mL of hexane: isoamyl alcohol (97:3). The tubes were shaken for 10 minutes and centrifuged. Tissue samples were prepared by homogenizing 10 g of liver or renal cortex with 40 mL of the pH 8.6 buffer and 50 μL of the loxapine standard solution. The extraction procedure was carried out on 5 mL of the homogenized mixture shaken for 10 minutes with 5 mL of hexane: isoamyl alcohol (97:3). After centrifugation, the organic fractions were transferred to screw cap tubes and each extracted with 2 mL of 0.1 mol/L H_3PO_4 on a shaker for 10 minutes. The tubes were centrifuged and the aqueous fractions were transferred into 15 mL conical tubes. The samples were made basic with 1.5 mL of the pH 10.2 buffer and then extracted with 3 mL of hexane:isoamyl alcohol (97:3) by vigorous shaking for 2 min. Following centrifugation the organic layer was aspirated and the solvent evaporated under a nitrogen stream.

The residue was taken up in 50 μL methyl alcohol and a 1 to 2 μL aliquot was injected into the gas chromatograph. Following alcohol evaporation the residue was reconstituted with 100 μL mobile phase and a 10 μL aliquot was injected into the HPLC column. Drug-free specimens were prepared with known quantities of clomipramine, N-desmethylclomipramine and clothiapine to construct calibration curves for each drug. All three drugs showed good linearity over the examined concentration range (0.2 to 5.0 mg/L, $r = 0.974$ clomipramine, $r = 0.963$ N-desmethylclomipramine, $r = 0.992$ clothiapine).

Results

Concentrations of clothiapine, clomipramine and its metabolite, N-desmethylclomipramine, were found in two blood specimens obtained from different sites (heart and brain) and in organ tissues (liver and kidney). These results are shown in Table 1. In the gastric contents (about 80 mL) 4.16 mg of clothiapine, 3.04 mg of clomipramine and 1.2 mg of biperiden were detected. Concentrations of biperiden in blood and tissues were less than 0.1 mg/L.

Gas chromatographic analysis with electron capture detector suggested the presence of both clothiapine and its main metabolite, desmethylclothiapine. Three other peaks,

TABLE 1—*Drug concentrations in blood (mg/L) and in organ tissues (mg/kg).*

Sample	Clothiapine	Clomipramine	Desmethylclomipramine
Blood (Heart)	2.15	1.61	1.32
Blood (Brain)	0.50	0.48	0.26
Liver	13.71	3.95	2.24
Kidney	3.32	2.36	1.68

probably attributable to hydroxyl derivatives, were seen, although no quantitative determination was possible due to the lack of pure reference standards.

Discussion

The above toxicological results indicated a fatal overdose associated with clothiapine and clomipramine. The prompt gastric lavage eliminated most undigested biperiden pills, while significant amounts of clothiapine syrup and clomipramine tablets, more easily digested, were already absorbed. The finding of undigested biperiden pills in the gastric content would explain the detection of significant amounts of biperiden in the gastric content, in contrast with its absence in the examined blood and tissues samples.

The toxicity of clothiapine is not well documented. Therapeutic blood levels and concentrations in overdosed patients have not been found in the literature. Baldi et al. [*1*] report that serum concentrations greater than 0.3 mg/L are associated with central nervous system depression, claiming that "In a severe intoxication case, due to ingestion of 2.4 g of clothiapine, the clinical course was dominated by coma and pronounced hypotension, and 25 hours after ingestion a serum level of 384 ng/mL was detected."

In fatalities attributed to loxapine, a dibenzoxapine similar to clothiapine, loxapine blood levels ranged from 1.22 to 7.7 mg/L [*2,4*].

Deaths related to clomipramine have been reported in the literature. Two cases have been reported in 1974 [*5*], but the colorimetric method employed did not allow adequate quantitation.

In a fatal case attributed to self-administration of toxic amounts of clomipramine, analysis of post mortem blood revealed low concentrations of clomipramine (0.54 mg/L) and of its active metabolite N-desmethyl-clomipramine (0.58 mg/L) [*8*].

In a fatality following ingestion of clomipramine, alprazolam and ethyl alcohol, blood concentrations of clomipramine and N-desmethylclomipramine were respectively 0.84 and 1.4 mg/L; moreover alprazolam and ethyl alcohol concentration in blood were 0.069 mg/L and 3.75 g/L respectively [*9*].

In one case of non-lethal acute poisoning, a patient with a 1 mg/L blood concentration of clomipramine who promptly underwent continuous cardiac monitor and intensive diuresis did not die [*10*].

To illustrate the phenomenon of postmortem drug redistribution, Pounder and Jones [*11*] reported a suicidal overdose of chloral hydrate, clomipramine and flurazepam. Clomipramine and its metabolite desmethyl-clomipramine were present in mixed cardiac blood in concentrations of 15.3 mg/L and 4.1 mg/L respectively. Concentrations in other blood samples ranged from 4.0 to 21.5 mg/L and 1.7 to 8.1 mg/L respectively. Higher concentrations were found in pulmonary arterial and venous blood, lower concentrations in the femoral venous blood.

Most of the above literature cases report deaths as a consequence of clomipramine associated with other substances or with ethyl alcohol. Although in the present case the death can be ascribed to the combined effect of two substances (clothiapine and clomipramine), it is not clear if a single substance, at the same concentration, would

be lethal or which one has been more important in the determination of the cause the fatality.

Another interesting feature regards the difference in the concentrations of both drugs in the heart and brain blood samples. Although this phenomenon may reflect a redistribution of drug occurring in the body in the time interval between death and autopsy sampling, it is not clear whether either value can be assumed as similar to the blood level when death occurred.

Pounder and Jones [*11*] point out the dramatic extent of the phenomenon of postmortem redistribution, suggesting the hypothesis of a diffusion of drugs along a concentration gradient, from sites of high concentration in solid organ into the blood, with resultant artifactual elevation of drug levels in blood.

The lower cerebral blood concentration evidenced in the present case, instead, could be ascribed to a postmortem absorption of both highly lipophilic drugs by the central nervous system lipids. This finding is in agreement with the following in vitro experiment on the interactions of drug mixtures with blood samples to which brain tissues were added. Twenty mL of blood containing 2 μg/mL of clothiapine and clomipramine, respectively, were placed in a silanized beaker with 20 g of brain fragments. After three days a 50% decrease of both drug concentrations was noted, while after one week the decrease was 75%.

These results suggest that, in case of poisoning from highly lipophilic substances, blood samples should be taken from various sites (that is, arterial and venous blood from heart, brain, lungs and from inferior vena cava, subclavian and femoral veins, etc.) and the relative drug concentrations compared with those from organ tissues. Drug determination in the cerebrospinal fluid is also recommended for useful comparisons.

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