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Postmortem Clomipramine: Therapeutic or Toxic Concentrations?

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ABSTRACT: Postmortem blood and liver concentrations of clomipramine were determined in ten cases by high performance liquid chromatography (HPLC). Blood concentrations ranged from 0.21 to 4.9 mg/L, and liver concentrations from 7.0 to 320 mg/kg. Two cases associated with clomipramine toxicity were clearly differentiated from other cases by the analysis of liver. The concentrations of clomipramine in these two cases were 3.3 and 1.8 mg/L in blood, and 280 and 320 mg/kg in liver. The liver concentrations were 10 to 30 fold greater in the deaths associated with drug toxicity compared with the other cases. One case, where cardiac blood was collected in place of femoral blood, showed a high blood concentration (4.9 mg/L), but an arguably therapeutic liver concentration (13 mg/kg). The analysis of femoral blood together with liver provides the best guide as to the significance of postmortem clomipramine concentrations.

KEYWORDS: toxicology, clomipramine, toxicity, blood, liver, HPLC, postmortem redistribution

Clomipramine is a tricyclic antidepressant drug structurally related to imipramine. Although it is used primarily in the treatment of endogenous depressive illnesses, including manic depression, it is also used in obsessive-compulsive disorders [*1,2*].

Published data of postmortem clomipramine concentrations, or details of fatal clomipramine overdoses are scant. Haqqani and Gutteridge [*3*] described two cases using a nonspecific colorimetric analytical method which was incapable of separating metabolites. A few reports have subsequently presented individual cases of clomipramine toxicity, analytically separating clomipramine and its principal metabolite desmethyl-clomipramine [*4–6*].

In this report, we describe ten cases where routine toxicological analyses of postmortem specimens detected the presence of clomipramine. Distinctions between those concentrations reflecting therapeutic usage from those concentrations in blood and liver reflecting toxic concentrations are described.

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Subjects and Methods

The cases reported here were autopsied and this involved full macroscopic and microscopic examination of all the major organs by specialist pathologists. Blood was collected in tubes containing 1% sodium oxalate/potassium fluoride as preservative and anticoagulant and were stored frozen until use. Police reports were used to obtain information as to the circumstances of death and any other relevant information obtained by way of statements was also made available at the State Coroner's Office.

All cases were subject to a full toxicological examination. This involved a urine screen for methadone, opiates, amphetamines, cannabinoids, cocaine metabolites, benzodiazepines and barbiturates. Blood extracts were also analyzed on a capillary column gas chromatographic screen using nitrogen-phosphorous detection for basic and neutral drugs [*7*]. An additional screen was conducted by gradient elution high performance liquid chromatography (HPLC) using photodiode array detection [*8*]. This system was capable of detecting acidic and neutral compounds. Further tests for alcohol and the chloral hydrate metabolite, trichloroethanol (TCE) were separately conducted. Medications suggested from receipt of exhibits or known to be prescribed to the decedents were specifically excluded in the toxicological testing. Only positive findings of the toxicological testing are described.

Drugs detected, including clomipramine, were quantified by HPLC, except for TCE, which was quantified by gas chromatography.

Materials

Acetonitrile and hexane were of HPLC grade (Mallinckrodt, Melbourne, Australia) and diethylamine was Labgrade (Fluka, Melbourne, Australia). All other chemicals were of Analytical Reagent grade (Ajax, Melbourne, Australia). Clomipramine was provided by the Curator of Standards at the Australian Government Analytical Laboratories (Sydney, Australia). Cianopramine HCI was a gift from Roche Products (Australia).

Reagents and Standards

Drug stock solutions (1 mg/mL) were prepared in methanol, stored at −20°C until use, and proved stable for at least one month. Working standards were prepared from stock solutions in whole-blood to give concentrations from 0.20 to 2.5 mg/L. Whole-blood, used in the preparation of standards, was expired donor blood collected by the Red Cross Blood Bank (South Melbourne, Australia). Standards for liver homogenates were prepared in drug-free liver homogenates to give concentrations from 7.5 to 150 mg/kg. Cases with clomipramine concentrations greater than the range of the standard curve, were diluted and reassayed.

Extraction Procedure

Blood standards, controls or cases (1.0 mL), and 1.0 μg of cianopramine (internal standard) were added to 10 mL silanized glass extraction tubes. One mL of deionized water was added and the tubes vortexed before addition of 1 mL 0.2 M Na_2CO_3. Tubes were again vortexed and 6 mL of hexane/butan-1-ol (95:5 v/v) was added and the tubes gently agitated for 30 min. Centrifugation (3500 rpm) for 5 min was followed by the transfer of the organic layer to a clean set of silanized extraction tubes containing 100 μL of 0.2% phosphoric acid. These were gently agitated for 30 min and then centrifuged for 5 min. The organic layer was aspirated and an aliquot of the aqueous layer (30 μL) was injected into the chromatographic system.

Clomipramine was extracted from liver homogenates with minor alterations to the method mentioned for blood. A liver homogenate was prepared by homogenizing 10 g of freshly minced liver in 10 mL of water. The pH was adjusted to 10 using 1M NaOH, subtilisin (10 mg, Sigma) was added and then the homogenate incubated for 60 min at 55°C. The pH was finally adjusted to 7.0 ± 0.5 with dilute mineral acid. Homogenates were either used immediately or stored at −20°C until analysis. Briefly, 0.5 mL of the liver homogenate was added to 10 mL silanized glass tubes, followed by 10 μg of cianopramine (internal standard), 500 μL 2% sodium tetraborate and 8 mL of hexane/butan-1-ol (95:5 v/v). The tubes were gently agitated then centrifuged for 5 min and the organic layer transferred to clean tubes containing 400 μL 0.2% phosphoric acid. Further agitation was followed by centrifugation and removal of the aqueous layer (30 μL) for injection on to the HPLC.

Chromatographic Conditions

The HPLC system consisted of an LC-6 constant flow pump, a SIL-6B autoinjector and SPD-10A dual wavelength variable spectrophotometric detector which were coupled by a programmable system controller (Shimadzu Oceania, Melbourne, Australia). Data was collected on a multi-functional data processor with built-in thermal plotter and a floppy disk drive storage facility (C-R4A Chromatopac, Shimadzu Oceania).

The detector was operated at wavelengths of 220 nm and 254 nm, and a sensitivity of 0.01 absorbance units full scale (aufs). Chromatographic separation was achieved with a Spheri-5 RP-18 column (100 x 4.6 mm ID; 5 μm) protected by a RP-18 Newguard cartridge (15 x 3.2 mm ID; 7 μm) (Applied Biosystems, Melbourne, Australia).

The mobile phase consisted of 40% acetonitrile in 0.1 M potassium phosphate buffer containing 2.5% diethylamine, pH 8.0. The flow rate was 2.0 mL/min, and the total run time was 40 minutes. Clomipramine eluted at about 32 min with a relative retention time (RRT) of 3.60. The main metabolite (desmethyl clomipramine) was detected at about 10 min with a RRT of 1.13. (An authentic pure substance was not available to permit quantification of desmethyl clomipramine).

Case Summaries and Findings

Case 1

This was a 23-year-old 65 kg white male who was found at home suffering a seizure. He was conveyed to hospital where he died in intensive care on the third day after admission. Empty packets of the antidepressants dothiepin, imipramine, and clomipramine were found in the bedroom of the deceased as well as a suicide note. Autopsy showed congestion of the leptomeninges and the mucosa of the larynx and trachea. The lungs were grossly edematous and together weighed 1900 g. There were bilateral pleural effusions. Histology revealed a mild inflammatory infiltrate of the portal tracts of the liver and early bronchopneumonia in the lungs. Edema and collections of eosinophils were present in the myocardium of both ventricular walls, but there was no obvious necrosis and the coronary arteries were normal. Samples taken at the time of admission but analyzed postmortem showed high concentrations of dothiepin, imipramine, and clomipramine and a therapeutic concentration of TCE in plasma obtained shortly after admission to hospital. Thiopental, pentobarbital, phenytoin and phenobarbital were also detected but were most likely associated with administration by hospital staff. The cause of death was given as mixed drug toxicity.

Case 2

This 29-year-old 87 kg white male was being treated for depression. He had attempted suicide two months earlier. The deceased was found in the grounds of a school holding a bag containing two empty bottles of alprazolam and thirteen empty blister packs of clomipramine (10 x 25 mg tablets), A plastic bottle containing a mixture of soft-drink and alcohol was also found. Autopsy revealed pulmonary congestion and edema. The air passages were injected and contained gastric contents. There was minimal coronary atheroma. The stomach contained bile-stained fluid and the liver, spleen, kidneys, and brain were all congested. Toxicology detected high blood and liver concentrations of clomipramine, 290 mg of clomipramine in the stomach contents, a high blood concentration of alprazolam and a blood alcohol concentration of 0.06 gram/100 mL. The cause of death was given as terminal aspiration of gastric contents as a result of the combined toxicity of clomipramine, alprazolam and alcohol.

Case 3

This 41-year-old 77 kg white female, who lived alone, was being treated for depression with clomipramine. She also had a slight memory impairment and a history of dizziness and light-headedness and had suffered some falls. The deceased was found on the pathway in the garden a few meters from her house, wearing her coat. Her handbag, a flashlight and a small bag of food were found nearby. Autopsy revealed autolytic changes consistent with a period of some days elapsing prior to discovery of the body. No other remarkable findings were reported. The appearances of the deceased indicated that she had suddenly collapsed in a face down position. Toxicology detected benztropine and high concentrations of clomipramine in blood and liver. It was thought that the deceased's memory impairment had led to over medication and that the toxic levels of clomipramine had precipitated the collapse by virtue of an arrhythmia. The cause of death was given as drug (clomipramine) toxicity.

Case 4

This 25-year-old 72 kg white male was a user of illicit drugs and owed money for their purchase. The deceased also used prescribed medication for therapeutic purposes. He was found dead in a public toilet in a shopping center slumped on the seat. Found with the deceased was an unused syringe, a blue and white capsule, a yellow balloon, a small blue balloon containing a capsule, a syringe lid (but no uncapped syringe was found), and an unopened can of lemonade. Empty oxazepam and diazepam containers were found in the bedroom of the deceased. The body was that of a normally nourished young man with the stigmata of chronic intravenous drug abuse together with what appeared to be a recent injection mark with bruising on the front of the left elbow. At autopsy there were no injuries present to suggest that the deceased had been assaulted prior to death. Analysis of the exhibits by gas chromatography-mass spectrometry (GC-MS) detected heroin and hydrolysis products in the empty blue and white capsule and the capsule found in the blue balloon. No drugs or poisons were detected in the yellow balloon. Toxicology detected morphine, oxazepam and clomipramine in blood. The cause of death was given as acute on chronic intravenous drug abuse (heroin, oxazepam, clomipramine).

Case 5

A 29-year-old 80 kg white male with a past history of schizophrenia was shot following a disturbance at a suburban address. At least three separate shotgun injuries to the

face, shoulder, upper arm and pelvis were found at autopsy. No significant natural disease was present. Toxicology detected clomipramine and thioridazine in blood and liver. The cause of death was multiple shotgun injuries.

Case 6

This 29-year-old 74 kg white male had been depressed for the last two years and was receiving psychiatric treatment. The deceased was found seated in his motor vehicle in the driveway of his home address. The motor was running and the doors locked. A hose was connected to the exhaust pipe, which led into the rear window of the driver's side. No vital signs were found on arrival of the ambulance crew. Although the deceased had been prescribed medication, none was found in the home. No significant natural disease or signs of significant injury were present. Toxicology showed a carboxyhaemoglobin saturation of 86% as well as the presence of clomipramine. The cause of death was given as carbon monoxide poisoning.

Case 7

This 59-year-old 70 kg man had a history of depression since suffering a heart attack about two years earlier. This caused him to give up work. The deceased was found hanging in the garage at the rear of his house. Apart from the ligature mark around the neck, the only significant finding at autopsy was a heart that weighed 770 g and showed marked ventricular hypertrophy and dilatation. There was little coronary artery atheroma and no evidence of valvular disease. Toxicology detected clomipramine and mianserin in blood and liver. The cause of death was given as hanging.

Case 8

This 67-year-old woman had a long history of mental illness for which she was receiving medication. She was found dead in bed one morning. At autopsy the heart weighed 330 g. The right ventricle was dilated but the left ventricle showed no obvious pathology. The coronary arteries showed mild patchy atheroma. The lungs together weighed in excess of 1450 g and were congested and edematous. Toxicology detected the antidepressants clomipramine and fluoxetine. The cause of death was given as left ventricular failure with pulmonary congestion and edema.

Case 9

This was a 19-year-old man who suffered from an obsessive compulsive disorder. He had contemplated suicide in the past, but there was no indication of such intention on the day of his death. The deceased was a poor swimmer and was walking along a rugged rocky beach along slippery rocks. He was found in the water close to the shore. Autopsy showed a young man of normal build exhibiting no external signs of injury. Both lungs were slightly overexpanded and exuded moderate quantities of frothy fluid. The bronchi contained froth. The remainder of the autopsy was unremarkable. Toxicology detected clomipramine in blood and liver. The cause of death was given as drowning.

Case 10

This 25-year-old 73 kg white male was an epileptic and was mentally and physically retarded. The deceased was with other handicapped people in a mini van. He removed his seat belt, moved to the center door of the bus, opened the door and fell onto the

roadway. At the time the bus was traveling at approximately 60 k.p.h. He died in the hospital some 10 days later by choking on an apple. Autopsy showed multiple injuries mainly abrasions, and a fractured pelvis. Histological examination revealed acute pyelonephritis. His brain was small, weighing in total 1015 g (cerebellum 125 g) with evidence of old inflammation affecting the ependyma of the ventricular system. Toxicology detected clomipramine, chlorpromazine and diazepam in blood. The cause of death was given as aspiration of pieces of apple in an epileptic retarded man with multiple injuries.

Results and Discussion

A summary of the toxicological findings for ten cases from our laboratory, where clomipramine was detected in the course of routine screening, are shown in Table 1. These cases include four drug-related deaths (Cases 1 to 4). Three suicidal deaths in which the cause of death was not drug-related (Cases 5 to 7), and three deaths that involved either natural causes or were accidental but in which drugs did not directly contribute to death (Cases 8 to 10). Blood concentrations in all cases ranged from 0.21 to 4.9 mg/L. Peripheral blood concentrations in the four cases in which the cause of death was drug-related ranged from 0.2 to 3.3 mg/L. Liver concentrations ranged from 7 to 320 mg/kg. Peripheral blood concentrations in the remaining six cases ranged from 0.2 mg/L to 0.9 mg/L. Liver concentrations ranged from 7 to 20 mg/kg. In one instance,

TABLE 1—*Summary of toxicological results.*

		Clomipramine Concentrations			
Case No.	Age/Sex	Femoral Blood (mg/L)	Liver (mg/kg)	Stomach Contents (mg)	Other Drugs Detected in Femoral Blood (mg/L)
Drug-related deaths					
1.	M 23	0.7	15	N.R.	Dothiepin 2.7 Imipramine 1.3 Trichloroethanol 4.5 Thiopental 41[a] Pentobarbital 6.5[a] Phenytoin 9.3[a] Phenobarbital 3.2[a]
2.	M 29	1.8	320	290	Alprazolam 0.10 Ethanol 600
3.	F 41	3.3	280	N.D.	Benztropine 0.20
4.	M 25	0.2	7.0	N.R.	Morphine 0.28 Oxazepam 2.3
Non drug-related deaths					
5.	M 29	0.2	12	N.R.	Thioridazine 0.15 Mesoridazine 0.11 Sulforidazine 0.03
6.	M 29	0.3	7.0	N.D.	Carbon monoxide 86%
7.	M 59	0.4	20	N.D.	Mianserin 0.28
8.	F 67	4.9[b]	13	2	Fluoxetine 0.14
9.	M 19	0.9	9.0	N.D.	No other drugs detected
10.	M 25	0.3	N.D.	N.R.	Chlorpromazine 0.27 Diazepam 0.10 Nordiazepam 0.10

NOTE: N.R.-No Result, N.D.-Not Detected.
[a]Associated with hospital treatment.
[b]Heart blood.

Case 8, where only heart blood was available, the clomipramine concentration was 4.9 mg/L, and the corresponding liver concentration was 13 mg/kg.

Clinical studies show that steady-state plasma concentrations of clomipramine (taken prior to the morning dose) range from 0.08 to 0.24 mg/L (average 0.13 mg/L) following chronic oral daily administration of 150 mg [*9*]. Similar concentrations have been reported for daily doses of 200 to 300 mg [*10*], while clomipramine concentrations up to 0.45 mg/L were reported in some individuals receiving 2 mg/kg body weight [*11*].

In contrast, peripheral blood concentrations of clomipramine in postmortem cases in which overadministration or overdose were not suggested by the circumstances (Cases 5 to 10) ranged up to 0.9 mg/L with a mean of 0.4 mg/L. The higher concentrations in postmortem blood are probably due to a combination of postmortem redistribution [*6,12*], and the measurement of blood versus plasma (or serum) due to nonequivalence of blood and plasma clomipramine concentrations [*13*]. The problem of postmortem redistribution is further highlighted by Case 8, which was regarded by the pathologist who performed the autopsy as a natural death, in which the heart blood concentration of clomipramine was 4.9 mg/L, whilst the liver concentration was well within the range expected following normal therapeutic use in the other cases reported here (5, 6, 7, 9) and by others. However, in view of the autopsy findings (which failed to indicate the mechanism of death), the possibility that death in Case 8 was a consequence of drug toxicity cannot be excluded.

Published case reports of deaths associated with the use of clomipramine have reported blood concentrations of 0.54 mg/L, 0.84 mg/L and 4.1 mg/L [*4–6*]. Pounder and Jones [*6*] also reported a liver concentration of 298 mg/kg in a woman who died from combined toxicity to clomipramine, chloral hydrate, and flurazepam.

In the cases reported here in which clomipramine toxicity was associated with death other drugs were also detected. Benztropine (Case 3), dothiepin, imipramine and trichloroethanol (Case 1), alprazolam and alcohol (Case 2), and morphine and oxazepam (Case 4) are all arguably in concentrations that could either cause death in their own right (Cases 1 and 4) or significantly increase the toxicity of clomipramine by further suppression of the central nervous system (Cases 2 and 3). The relatively low concentrations of clomipramine in Cases 1 and 4 and the circumstances in Case 4 suggest that the other drugs detected in these cases were the predominant toxic agents. In contrast, the high liver clomipramine concentrations in Cases 2 and 3 were also associated with high peripheral blood clomipramine. This suggests that clomipramine toxicity was a major contributory factor in these two cases.

Clomipramine was detected in gastric contents in significant amounts in Case 2 (equivalent to almost 12 25 mg tablets) suggestive of a suicidal overdose. The very high alprazolam femoral blood concentration further confirmed this observation. In contrast, there was no evidence of a suicidal component to the death of the woman in Case 3. The absence of evidence of an ingestion of a large amount of drug and the information obtained at a coroner's inquest suggested she had not committed suicide. The most likely reasons for the fatal concentrations of clomipramine in this death are an accumulation of drug with time as a result of a reduced clearance or unintentional overmedication of clomipramine. It is of interest that in this case the circumstances were those of a sudden collapse, probably precipitated by a cardiac arrhythmia, and that death was not the result of CNS or respiratory depression.

In summary, this review of 10 cases in which clomipramine was detected postmortem suggests that femoral blood and liver concentrations up to 0.9 mg/L and 20 mg/kg respectively, may reflect therapeutic usage. Fatalities attributed by us to clomipramine show femoral blood and liver concentrations greater than 1 mg/L and 200 mg/kg, respectively.

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