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Postmortem Concentrations of Citalopram

ABSTRACT: The postmortem concentrations of citalopram in blood, bile, liver, and vitreous humour were investigated in 14 cases using a specially developed high performance liquid chromatography assay. Concentrations from drug and non-drug related deaths were categorized to determine a postmortem therapeutic and toxic range. Therapeutic citalopram concentrations for blood, bile, liver, and vitreous humour ranged to 0.4 mg/L, 2.1 mg/l, 6.6 mg/kg, and 0.2 mg/L, respectively. In one potentially fatal response to citalopram, concentrations were 0.8 mg/L, 6.0 mg/L, 0.3 mg/L for blood, bile and vitreous humour, respectively.

KEYWORDS: forensic science, forensic toxicology, citalopram, postmortem, toxicity, and serotonin reuptake inhibitor.

Citalopram is an antidepressant classed as a selective serotonin reuptake inhibitor (SSRI) with a chemical structure unrelated to that of other SSRIs or other available antidepressant agents (1). The selective serotonin re-uptake inhibitors (SSRI) are considered to be less toxic than the tricyclic antidepressants (2–4). The mechanism of action of SSRIs is related to the potent inhibition of serotonin re-uptake in the presynaptic neurons (5,6). On the basis of *in vitro* studies, citalopram is the most selective SSRI developed to date, with minimal or no effects on noradrenaline, dopamine, and gamma-aminobutyric acid reuptake (1).

Citalopram is generally administered orally as a single daily dose, with the starting dose being 20 mg/day. It can be increased in increments of 10 mg until a satisfactory clinical response is achieved, the maximum daily dosage being 60 mg (7). Following a single oral dose, maximum plasma levels are reached within 4 h of administration. The oral bioavailability of citalopram is about 80%. The volume of distribution is about 12–17 L/kg (8–10).

Citalopram is metabolized to the less active desmethylcitalopram, didesmethylcitalopram, citalopram-N-oxide and an inactive deaminated acid derivative (11). The terminal elimination half life of citalopram is about 1.5 days.

Therapeutic concentrations of citalopram have been reported to range to 0.6 mg/kg in post mortem blood (8). Currently little evidence in the literature exists to suggest that citalopram overdoses are fatal by themselves, but when combined with monoamine oxidase inhibitors (MAO-I), can lead to a potentially fatal "Serotonin Syndrome."

This is believed to occur by combined inhibition of the reuptake of serotonin into nerve cells and by the reduction of the rate of breakdown of serotonin by moclobemide. The two therefore appear to act synergistically to substantially increase serotonin levels. Symptoms of this syndrome include hypertension, tachycardia, labile blood pressure, muscle rigidity, and cardiac arrest (7,12,13).

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Reports of citalopram concentrations in samples collected in living patients and at autopsies are few (8). Consequently, there is little information to assist in the interpretation of citalopram concentrations in postmortem specimens. This paper describes postmortem blood, bile, liver, and vitreous humour contents for 14 cases involving citalopram in an attempt to provide more evidence of the postmortem therapeutic and toxic range.

Materials and Methods

Drug Screening

Toxicological analyses were conducted on blood and urine using a combination enzyme-multiplied immunoassay (EMIT) for drugs of abuse, gradient high performance liquid chromatography (HPLC) with photodiode array for detection of acid/neutral substances (14), capillary gas chromatography (GC) followed by mass-spectrometric confirmation for basic/neutral substances (15), and an alcohol analysis.

Specimen Collection

All specimens were collected by forensic technicians at the Victorian Institute of Forensic Medicine (VIFM) under the supervision of forensic pathologists or under contract at a regional center, according to standard mortuary procedures. Specimens collected included blood, liver, bile, vitreous humour, urine, and gastric contents. Blood was taken from the femoral region whenever possible, however the site of sampling was recorded. In all cases, blood was stored in commercially prepared preservative tubes containing 1% (w/v) sodium fluoride/potassium oxalate (Biolab, Australia) and kept at −20°C until analysis. Bile, vitreous humor, and urine were stored without preservatives at −20°C; a portion of liver was stored in a plastic specimen pot at −60°C.

Chemicals and Reagents

Citalopram HBr and imipramine (internal standard) were obtained from the Division of Analytical Laboratories of the New South Wales Health Department, Australia and Sigma Melbourne, Australia respectively. Stock solutions of 1 mg/mL were prepared

fresh monthly in methanol, and dilutions were made in deionized water to give 100 mg/L and 10 mg/L dilutions. Butyl chloride (HPLC grade) was obtained from Prolabo Melbourne, Australia, whereas methanol and acetonitrile (HPLC grade) were purchased from Mallinckrodt Australia. Orthophosphoric acid and sodium tetraborate were obtained from AJAX Chemicals, Australia.

Liver Homogenization

The liver homogenates used for toxicological analyses were prepared by finely dicing a 10 g portion of liver and blending with 10 mL deionized water, in a stomacher (Lab-Blender 80, Seward Medica, Australia) for approximately 5 min. The mixture was then transferred to a plastic pot and homogenized using a tissue homogenizer (Janke and Kunkel, IKA-Labortechnik Ultra-Turrax T25, Germany) before adjusting the pH to 10 with 10 M NaOH. Ten milligrams of Subtilisin (Sigma, U.S.) was added and the homogenate incubated at 55°C for 1 h. Following the incubation, the pH was readjusted to 7.0 using 1 M HCl and the specimen stored at −20°C until analyzed.

Extraction

Citalopram was extracted from blood, bile, vitreous humor, and liver using the following extraction method: 1 mL of each matrix (standard or sample) was added to a 10 mL polypropylene extraction tube. Internal standard (100 ng of imipramine) was added to each tube followed by 1.0 mL of 2% sodium tetraborate (10 g in 500 mL water, pH 9.15 to 9.2) and vortexed before the addition of 8 mL butyl chloride extraction solvent. All tubes were placed on a rotating wheel for 30 min.

The tubes were then centrifuged for 5 min at 3000 rpm and then placed in an alcohol bath (−30°C) to freeze the aqueous layer. When the aqueous layer was frozen, the solvent layer was decanted into a fresh extraction tube and 200 μL of 0.2% orthophosphoric acid was added. The tubes were placed on the rotating wheel for 30 min followed by centrifuging for 5 min at 3000 rpm. The aqueous layer was again frozen in the alcohol bath and the solvent layer was aspirated and discarded. The acid plug was allowed to thaw before being transferred into an autosampler vial.

Analysis

Fifty μL of each sample were injected into a Agilent 1050 series HPLC, which was linked to a ChemServer 4900 series (Agilent, Australia). The column used was a Novapak Phenyl, 3.9 × 150 mm, 5 μm particle size (Waters Australia) with a Novapak phenyl precolumn (Waters, Australia). The mobile phase consisting of 55% acetonitrile/45% 10 mM potassium phosphate buffer, pH 3.0 was pumped at 1.5 mL/min. The sample run time was 10 min. A multichannel ultraviolet spectrophotometric detection was used at wavelengths of 214 nm (quantifier) and 230 nm and 255nm (qualifiers). All data analyses were performed using Target 3 software designed for HP Unix-based software.

Case Report

The deceased (35 year old female) was last seen alive by her defacto in the kitchen of their house (Case 1 in Table 1). The pair had gone to bed in separate rooms that night and the deceased was seen by the defacto the following morning, lying face down in the entry of the toilet door. Paramedics attempted resuscitation but to no avail. The deceased had antibodies to Hepatitis C and smoked approximately 15 cigarettes a day. The death was unexpected. Microscopic and macroscopic findings were both nonspecific and unremarkable.

The toxicology is shown in Table 1. The pathologist gave the cause of death as citalopram toxicity but considered that positional asphyxia may have contributed to the death as the deceased person had pronounced conjunctival petechial haemorrhages and was found in a prone position.

The deceased had, in the past, been treated for alcohol and benzodiazepine abuse and had been attending a rehabilitation center. The deceased had been prescribed Citalopram by her doctor two months prior to her death following the termination of an unwanted pregnancy. One week prior to her death, the dosage of Citalopram was increased due to an exacerbation of her depression. The deceased had also been using marijuana on a daily basis.

The Coroner's finding concluded that the deceased contributed to her of death and Citalopram was the cause of death.

TABLE 1—*Summary of toxicology findings in 14 deaths.*

Case No.	Femoral Blood (mg/L)	Bile (mg/L)	Liver (mg/kg)	Vitreous Humour (mg/L)	Cause of Death	Other Drugs/Poisons Detected in Blood, mg/L Unless Otherwise Indicated
1	0.8	6.0	N/A	0.3	Citalopram toxicity	Cannabinoids (urine)
2	0.1	N/A	1.3	N/A	Mixed drug toxicity	Alcohol 2.2 g/L, Codeine 0.3, Salicylate 10, Diazepam trace detected
3	0.7	4.3	5.4	0.4	Mixed drug toxicity	Morphine 0.2
4	0.2	3.2	N/A	0.2	Mixed drug toxicity	Morphine (urine) 18, 6-monoacetyl morphine (urine), Codeine (urine) 3, diazepam 0.1, nordiazepam 0.1
5	0.3	0.8	3.7	0.1	Mixed drug toxicity	7-Aminonitrazepam 0.15, paracetamol 3, frusemide 0.6
6	0.7	N/A	N/A	N/A	Mixed drug toxicity	Alcohol 0.19 g/L, propranolol 5.4, diazepam 0.04 methamphetamine trace detected, cannabinoids (urine)
7	1.3	N/A	N/A	N/A	Mixed drug toxicity	Promethazine 0.6, morphine (free) 0.03, phenytoin 5, salicylate 30, diazepam 0.2
8	0.9	N/A	18	N/A	CO poisoning	Cannabinoids detected, CO 73% saturation
9	0.4	N/A	4.2	N/A	Drowning	Temazepam 1.1
10	0.4	N/A	2.5	0.1	Electrocution	Temazepam 0.5, alprazolam 0.04
11	0.1*	N/A	2.8	0.2	Traffic crash (passenger)	No other substances detected
12	0.4	1.5	2.3	0.1	Suicidal hanging	Cannabinoids (urine)
13	0.3	1.3	6.6	0.1	Plastic bag asphyxia	No other substances detected
14	0.2	2.1	2.4	0.2	Traffic crash (driver)	Thioridazine 0.2, lithium 0.7 mM, 7-aminoclonazepam 0.09, 7-aminonitrazepam 0.1

* Cavity blood analysis. N/A—specimen not available.

Results and Discussion

Calibration curves were produced individually for blood, bile, liver, and vitreous humor. The calibration range for blood was from 0.1–10 mg/L, 10–100 mg/kg for liver, 0.05–8.0 mg/L for bile, and 0.07–8.0 mg/L for vitreous humour. All calibration curves were linear, with correlation coefficients (r^2) better than 0.99.

The within-assay percentage coefficient of variation (%CV) at a citalopram concentration of 0.5 mg/L (in blood) was less than 5% after eighteen replicates. Recovery was ~70% and the accuracy was 88%. There was no interference noted in any of the cases from other co-administered drugs.

Several gas chromatographic or gas chromatographic-mass spectrometric (4,6,7) and high performance liquid chromatographic methods (4,7,16,17) have been described for the determination of citalopram in blood and plasma. A secondary confirmation test can also be done by thin layer chromatography (4). Sample preparation is usually by liquid-liquid extraction (4,7) or solid phase extraction (6). Citalopram is detected by fluorimetric (6,17) or spectrophotometric detection (6,16)

A summary of the toxicological results of the 14 cases that were investigated is presented in Table 1.

The femoral blood concentration in all cases ranged from 0.1–1.3 mg/L. Bile concentrations ranged from 0.8–6.0 mg/L, liver concentrations ranged from 1.3–18 mg/kg, and vitreous humour concentrations ranged from 0.1–0.4 mg/L.

The relationship of citalopram between various postmortem specimens were examined. On average, the citalopram concentration in bile was approximately eight times to that of blood. The citalopram ratio of liver to blood was approximately fourteen. The relatively high volume of distribution (12–17 L/kg) would predispose citalopram to be found in high concentrations in solid tissues.

The citalopram blood to vitreous humour relationship was approximately two. Vitreous humour can be an important analytical specimen for use in postmortem toxicology testing particularly in cases of decomposition.

Citalopram was regarded as the sole cause of death in one case (Case 1). There were seven mixed drug related deaths (Cases 2–8), and six cases that were not considered to be drug related (Cases 9–14). For the mixed drug related cases, the blood citalopram concentrations ranged from 0.1–1.3 mg/L. The cause of death in these cases included alcohol, potassium chloride toxicity, and carbon monoxide poisoning. The manner of death was either suicide or accident. These cases serve to demonstrate the difficulty in ascribing toxicity to a particular postmortem blood concentration when multiple drugs are identified.

In the six cases where citalopram was considered an incidental finding and misuse of drug was not suspected, the femoral blood concentrations ranged from 0.1–0.4 mg/L. These cases included drowning, suicidal electrocution, multiple injuries (passenger in motor vehicle accident), suicidal hanging, plastic bag asphyxia and ischemic heart disease. This concentration is consistent with the therapeutic range previously reported for citalopram as up to 0.6 mg/L (8).

In one case (see Case Report), the pathologist determined the sole cause of death as citalopram toxicity. The femoral blood concentration of citalopram was 0.8 mg/L, the bile concentration was 6.0 mg/L, and the vitreous humour concentration was 0.3 mg/L. These concentrations were not substantially elevated above those normally associated with therapeutic use.

Worm et al. (8) reported deaths associated with the use of citalopram. Where citalopram was the main cause of death, the minimum fatal blood concentration was 2 mg/kg. Liver concentrations associated with non drug related deaths ranged up to 6.6 mg/kg and corresponding bile and vitreous humour concentrations ranged up to 2.1 mg/L and 0.2 mg/L, respectively.

In summary, in six cases the citalopram concentrations consistent with therapeutic use for blood, bile, liver, and vitreous humour values were 0.4 mg/L, 2.1 mg/L, 6.6 mg/kg, and 0.2 mg/L respectively. In one case in which death was attributed to citalopram toxicity and in the absence of other contributing factors the concentrations were 0.8, 6.0, and 0.3 mg/L for blood, bile, and vitreous humor, respectively.

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References

1. Celexa prescribing information. St. Louis, MO: Forest Pharmaceuticals, Inc., Subsidiary of Forest Laboratories, Inc, 1999.
2. Ostrom M, Eriksson A, Thorson J, Spigset O. Fatal overdose with Citalopram. 1996;348:339–40.
3. Grundemar L, Wohlfart B, Lagerstedt C, Bengtsson F, Eklundh G. Symptoms and signs of severe citalopram overdoses. Lancet 1997;349:1602.
4. Glassman AH. Citalopram toxicity. Lancet 1997;350:818.
5. Personne M, Sjoberg G, Persson H. Citalopram overdose—review of cases treated in Swedish hospitals. Clin Toxicol 1997;35(3):237–40.
6. Macek J, Ptacek P, Klima J. Rapid determination of citalopram in human plasma by high performance liquid chromatography. J Chromatogr 2001;755:279–85.
7. Dams R, Benijts THP, Lambert WE, Van Bocxlaer JF, Van Varenbergh D, Van Peteghem C, et al. A fatal case of serotonin syndrome after combined moclobemide-citalopram intoxication. J Anal Toxicol 2001;25:147–51.
8. Worm K, Dragsholt C, Simonsen K, Kringsholm B. Citalopram concentrations in samples from autopsies and living persons. Int J Legal Med 1998;111:188–90.
9. Goodnick PJ. Pharmacokinetic optimisation of therapy with newer antidepressants. Clin Pharmacokinet 1994;27(4):307–30.
10. Musshoff F, Schmidt P, Madea B. Fatality caused by a combined trimipramine-citalopram intoxication. Forensic Sci Int 1999;106:125–31.
11. Baumann P. Pharmacokinetic-pharmacodynamic relations of the SSRI. Clin Pharmacokinet 1996;31(6):444–69.
12. Fisher P. Serotonin syndrome in the elderly after antidepressive monotherapy. J Clin Psychopharmacol 1995;15(6):440–2.
13. McIntyre IM, King CV, Staikos V, Gall J, Drummer OH. A fatality involving moclobemide, sertraline and pimozide. J Forensic Sci 1997;42 (5):951–3.
14. Drummer OH, Kotsos A, McIntyre IM. A class-independent drug screen in forensic toxicology using a photodiode array detector. J Anal Toxicol 1993;17:225–9.
15. Drummer OH, Horomidis S, Kourtis S, Syrjanen ML, Tippett P. Capillary gas chromatographic drug screen for use in forensic toxicology. J Anal Toxicol 1994;18:134–8.
16. Olesen OV, Linnet K. Simplified high performance liquid chromatographic method for the determination of citalopram and desmethylcitalopram in serum without interference from commonly used psychotropic drugs and their metabolites. J Chromatogr 1996;675:83–8.
17. Carlsson B, Norlander B. Solid-phase extraction with endcapped C_2 columns for the routine measurement of racemic citalopram and metabolites in plasma by high-performance liquid chromatography. J Chromatogr B 1997;702:234–9.

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