J Forensic Sci, May 2005, Vol. 50, No. 3
Paper ID JFS2004067
Available online at: www.astm.org

CASE REPORT

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Postmortem Tissue Distribution of Olanzapine and Citalopram in a Drug Intoxication*

ABSTRACT: A 40-year-old white male was found dead in bed in a group home for mentally ill adults. The decedent had been diagnosed a paranoid schizophrenic. An autopsy was performed at the Office of the Cuyahoga County Coroner in Cleveland, Ohio. Toxicological testing detected olanzapine and citalopram in post mortem specimens. Multiple fluids and tissues were assayed by liquid-liquid extraction followed by gas chromatography with nitrogen phosphorus detection, and qualitative confirmation by electron impact gas chromatography/mass spectrometry. Drug concentrations [olanzapine : citalopram; mg/L or mg/Kg] determined in this case are the highest reported to date involving these drugs- 1.38:3.35 heart blood, 1.11:1.65 femoral blood, 60.24:32.43 urine, 6.47:10:71 liver, and 38.36:49.16 lung, respectively. Drug concentrations in tissues were found to be the highest in lung for both drugs and lowest in the heart. Citalopram but not olanzapine was detected in bone. The cause of death was ruled acute intoxication by the combined effects of olanzapine and citalopram and the manner, accident.

KEYWORDS: forensic science, olanzapine, citalopram, postmortem

Olanzapine is a thienobenzodiazepine derivative, structurally similar to clozapine. It is classified as an antipsychotic, prescribed for the treatment of schizophrenia. Olanzapine interacts with multiple receptors including dopamine-d_2, serotonin 5-HT 2A and antagonizes dopamine-d_1, d_4, serotonin 5-HT 2C, 5-HT3, alpha-1-adrenergic, H_1 histaminergic and muscarinic receptors. It is available as tablets in doses of 2.5, 5, 7.5 and 10 mg and the resulting plasma levels in clinical samples are generally less than 0.1 mg/L (1) .

Citalopram is an antidepressant that inhibits central nervous system neuronal serotonin reuptake. It is structurally unrelated to other SSRI's and is available as a racemate. It is dispensed as 20 and 40 mg tablets which result in plasma concentrations in clinical samples of less than 0.3 mg/L (1).

This report details the disposition of olanzapine and citalopram in a fatal intoxication in multiple specimens including tissues.

Case History

A 40-year-old white male was found dead in bed in a group home for mentally ill adults. The decedent had been diagnosed a paranoid schizophrenic. At the scene several medications were observed including olanzapine, citalopram, risperidone, clonazepam, famotidine and aspirin. Olanzapine (10 mg), citalopram (20 mg), risperidone (3 mg), and clonazepam (0.5 mg) were prescribed to the decedent. The prescriptions found at the scene were filled 6–18 days prior to death, depending on the drug. The body was transported to the Office of the Cuyahoga County Coroner in Cleveland, Ohio, for autopsy. At autopsy the following specimens were collected for toxicological analysis- heart and femoral blood, urine, vitreous humor, cerebrospinal fluid, stomach contents, liver, spleen, brain, kidney, heart, lung and iliac bone [pelvic crest]. Blood, urine and vitreous humor specimens were refrigerated until testing. All other specimens were stored frozen and thawed before analysis.

Materials and Methods

The heart blood and urine specimens from this case were subjected to comprehensive toxicological screening [Note: the authors' laboratory does not test for risperidone and famotidine and no request was received from the case pathologist for such testing to be conducted]. This included volatile analysis by headspace gas chromatography; enzyme immunoassay of the urine for amphetamines, cocaine metabolite, opiates, phencyclidine, cannabinoids, and benzodiazepines; modified enzyme immunoassay of the blood for opiates; acidic/neutral drug screen by liquid-liquid extraction followed by gas chromatography with flame ionization detection; basic drug screen by liquid-liquid extraction followed by dual-column gas chromatography with nitrogen phosphorous detection (GC-NPD); and presumptive colorimetric testing for salicylate, ethchlorvynol, and acetaminophen. In addition, the vitreous humor was subjected to clinical testing [electrolytes, glucose, creatinine]. The only positive drug findings were olanzapine and citalopram. These drugs were detected and quantitated as follows:

An olanzapine standard was obtained from Eli Lilly and Co. (Indianapolis, IN). A citalopram standard was obtained from Forest Pharmaceuticals, Inc., (St Louis MI).

Sigma Chemical Co. (St. Louis, MO) was the supplier of promazine (the internal standard of the analysis), and Trizma base. Sodium chloride, sodium hydroxide, ammonium chloride,

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[*] Presented in part at the American Academy of Forensic Sciences annual meeting, Atlanta, GA, February 2002.
Received 22 Feb. 2004; and in revised form 16 July and 6 Nov. 2004; accepted 6 Nov. 2004; published 6 April 2005.

ammonium hydroxide, sulfuric acid, and hydrochloric acid all of ACS-grade were obtained from Mallinckrodt Chemical Co. (Paris, KY). HPLC-grade hexane, ethyl acetate, isopropanol, and methanol were provided by American Burdick and Jackson Laboratories, Inc. (Muskegon, MI).

Acidic and basic reagents including buffers were prepared with deionized water. Tris buffer (1.2 M, pH 9.2) was prepared by dissolving 145.3 g of Trizma base in 1L of water then adjusting the pH to 9.2 with concentrated hydrochloric acid. A hydrochloric acid/methanol solution of 0.1% was made by diluting 100 μL of concentrated hydrochloric acid into 100 mL of methanol. Ammonium chloride was saturated in 1 L of water, then the pH adjusted to 9.2 with ammonium hydroxide to be used as buffer. Sodium hydroxide was prepared as a 6 N solution by dissolving 240 g of the base in 1 L of water. The internal standard, promazine, was diluted in water to 5 mg/L from a 1000 mg/L methanol stock promazine solution.

Tissues to be analyzed were prepared as homogenates by adding 6 g tissue to 30 mL deionized water with the aid of a Waring® commercial blender (Waring Products Division, New Hartford, CT).

Bone Preparation-Since little work has been published on the concentration of drugs in bone the authors investigated two preparation techniques- incubating 1g samples of bone in 2 mL of water or methanol for 24 h at room temperature; and incubating 1g of bone which had been cut into small slivers with surgical scissors in 2 mL water or methanol for 24 h at room temperature. An aliquot of each of the solvents was then assayed.

Typically, a five mL aliquot of biological fluid, or tissue homogenate (or 2 mL solvent for the bone) was combined with 1 mL of 5 mg/L promazine (internal standard), 2 mL of saturated sodium chloride, 100 μL of 6 N sodium hydroxide, 2 mL of ammonium chloride buffer (pH 9.2), and 10 mL of ethyl acetate. The samples were extracted by 20 min rotation and then centrifuged at 3000 rpm. The organic layer was removed, 2 mL of 0.5 N sulfuric acid was added, and the samples were rotated for 20 minutes, then centrifuged. A 4 mL volume of tris buffer (1.2 M, pH 9.2) and 5 mL of hexane/isopropanol (9:1) were added to the acid phase. The samples were rotated for 20 min, centrifuged, the organic layer removed, 2 drops of 0.1% hydrochloric acid in methanol added, and evaporated to dryness (Zymark TurboVap® LV Evaporator, Hopkinton, MA) with nitrogen at 40°C. Extracts were reconstituted with 100 μL of methanol and 1 μL was injected onto the GC.

Olanzapine calibrators were prepared in drug free blood at the following [mg/L] concentrations: 0.1, 0.3, 0.5, 0.8 and 1.00 mg/L. Citalopram calibrators were prepared in drug free blood at the following mg/L concentrations: 0.5, 1.0, 1.5, and 2.00 mg/L. A negative control consisting of drug free blood and a positive control targeted at 0.6 mg/L (Acceptability = +/−20% of target) was assayed for each drug. Concentrations of drug in the case and controls were determined by linear regression ($r^2 > 0.99$) of the calibrator responses based on the peak-area ratio (Drug/Internal Standard). Appropriate calculation adjustments were made to account for dilution factors (e.g., for tissues) and differences in aliquot amounts (e.g., bone). Previously published work demonstrated the following assay parameters (2,3):

Drug	Limit of Detection	Limit of Quantitation	Upper Limit of Linearity mg/L
Olanzapine	0.05	0.1	2.00
Citalopram	0.01	0.02	2.00

Samples were diluted when necessary to ensure quantitation within the range of linearity for each drug. After extraction samples were assayed by GC-NPD for quantitation [cross linked RTx-50 fused silica capillary column, 30 M × 0.32 mm I.D. and 0.5 μm film thickness] and qualitatively identified by gas chromatography/mass spectrometry (GC/MS) using a HP 6890 GC with a HP 5973 mass selective detector and a DB-5 capillary column (30 M × 0.32 mm I.D. and 0.5 μm film thickness) and full scan electron impact ionization.

Results and Discussion

The autopsy did not reveal an anatomical cause of death. Findings included moderate macrovesicular hepatic steatosis, chronic bronchitis, cardiomegaly (weight = 415 g) and obesity (weight 187 lb, body length 65 in.). The stomach contents contained approximately 80 mL of thick brown paste-like material with vegetables.

Table 1 provides the quantitative results for olanzapine and citalopram in this case (reported to 2 decimal places). The heart blood concentrations for these drugs were 1.38 and 3.35 mg/L, respectively. The concentration of olanzapine was several times higher than published postmortem therapeutic levels and the concentration of citalopram at least double. Postmortem redistribution appeared to be minimal for both drugs when considering the difference between the heart and femoral blood concentrations. The heart/femoral blood ratios were 1.24 and 2.03 for olanzapine and citalopram, respectively. This agrees with previous reports in which heart/femoral blood ratios were 0.75–1.98, N = 14, in a series of cases of non-citalopram related deaths (2), and 1.20, and 1.48 (carotid) in two olanzapine-related fatalities (4,5).

Levine et al. (6) reported a therapeutic range for olanzapine of 0.04–0.27 and 0.19–0.50 mg/L in heart blood and urine from 5 cases. In addition, Anderson et al. (5) reported a range of 0.025–0.34 mg/L from 27 cases. Obviously, the olanzapine concentration determined in this case in the heart blood, femoral blood and urine was higher than these previous studies have reported. Similarly, Jenkins and Gubanich (2) reported a series of 22 cases in which citalopram was detected postmortem. None of the cases were ruled citalopram overdoses. The reported concentration range in heart blood was 0.09–1.64 mg/L. In this case the heart blood concentration was more than twice the highest concentration reported by Jenkins and Gubanich (2).

TABLE 1—*Postmortem specimen concentrations of olanzapine and citalopram.*

DRUG CONCENTRATIONS (mg/L or mg/kg)		
Specimens	Olanzapine	Citalopram
Heart Blood	1.38	3.35
Femoral Blood	1.11	1.65
Urine	60.24	32.43
Vitreous Humor	0.45	0.84
CSF	ND	0.33
Spleen	2.78	11.12
Liver	6.47	10.71
Brain	2.13	7.41
Kidney	2.39	7.00
Heart	1.72	4.59
Lung	38.36	49.16
Gastric Contents*	4.47	56.48
Bone	ND	Positive

ND = not detected.
CSF = cerebrospinal fluid.
Gastric Contents*/100 mL 0.44 mg 5.64 mg.

High concentrations of both drugs were detected in the urine, which suggests that urine would be an adequate screening specimen.

This case is particularly noteworthy as the authors were able to determine drug concentrations in multiple tissues. Lung tissue contained the highest concentration of both drugs in solid specimens with heart tissue containing the least. Citalopram but not olanzapine was detected in iliac bone. The results demonstrated that the technique, which detected drug at the highest concentration, was soaking pieces or slivers of bone in methanol. The least effective method appeared to be incubating whole bone in water. Since the % recovery of drug in bone using this preparatory procedure is unknown, only qualitative results are presented.

In this case, the concentration of olanzapine and citalopram in brain (frontal cortex) was 2.13 and 7.41 mg/kg, respectively. Merrick et al. (4) reported the distribution of olanzapine in brain specimens in a fatality. The highest concentration was reported in the midbrain followed by the caudate/putamen. The lowest concentration was found in the amygdala. The results for the frontal cortex differed depending on whether the specimen was collected from the left or right- a higher concentration of olanzapine was measured in the right frontal cortex. The cerebellum was negative.

The possibility of a drug-drug interaction must be considered in this case. The formation of the oxidative metabolites of olanzapine is achieved by the cytochrome P450 (CYP) isozymes CYP1A2 and CYP2D6 (7). Olanzapine does not appear to inhibit the isozymes CYP3A, CYP2C9 and CYP2C19. It is known that olanzapine may enhance the effects of antihypertensive drugs. No drug-drug interaction has been demonstrated between olanzapine and lithium. Olesen and Linnet (8) reported that patients comedicated with potential inhibitors of CYP2D6 (amitriptyline, clomipramine, methotrimeprazine, nortriptyline, perphenazine and zuclopenthixol) and also drugs not known to interfere with this isozyme activity (benzodiazepines, citalopram, chlorprothixene, clozapine, disulfuram, orphenadrine, oxytetracycline, sertraline and valproic acid), displayed a median concentration to dose ratio approximately 40% higher than patients taking olanzapine only. According to the 2004 Physicians Desk Reference (9), caution should be exercised when olanzapine is ingested with other centrally acting drugs (e.g., diazepam) and alcohol. These two compounds were observed to potentiate the orthostatic hypotension which may occur with olanzapine. Weigmann (10) et al. reported that fluvoxamine, an SSRI, inhibits the metabolism of olanzapine. Patients ($N = 10$) ingesting fluvoxamine and olanzapine exhibited a mean concentration/dose ratio 2.3 times higher than patients receiving olanzapine only. The authors suggested inhibition of metabolism occurred at CYP 1A2. This isozyme accounts for 15% of the total CYP P450 liver content (11).

Citalopram is metabolized by CYP2C19 and CYP3A4 to desmethylcitalopram and further demethylated by CYP2D6 (12). Citalopram is known as a weak inhibitor of CYP2D6, but does not appear to inhibit other CYP P450 isozymes. It is available as a racemate but only the S-isomer is thought to exhibit significant antidepressant activity (13). Indeed, a recently marketed drug, Lexapro® (Forest Pharmaceuticals, St Louis, MO), contains only the S-enantiomer (9). Reported drug interactions include coadministration with monoamine oxidase inhibitors. For example, Dams et al. (14) reported a fatality due to combined ingestion of citalopram and moclobemide. Based upon the above, it is therefore, reasonable to conclude that any potential pharmacokinetic drug interaction in this case was most likely not inhibition but competition by both drugs for the CYP isozymes.

Adverse effects of both drugs include hypotension and tachycardia (9). Therefore, the potential for a pharmacodynamic interaction could not be excluded.

In summary, this paper has described the analysis and detection of two commonly prescribed medications in an intoxication. The drug concentrations detected in this case, to the authors' knowledge, are the highest reported to date. Further, the disposition of these drugs was delineated in multiple specimens, including tissues, and a sample preparation procedure described for the detection of drug in bone. The Coroner determined that the cause of death in this case was acute intoxication due to the combined effects of olanzapine and citalopram, and the manner, accident.

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