TECHNICAL NOTE

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A Study Involving Venlafaxine Overdoses: Comparison of Fatal and Therapeutic Concentrations in Postmortem Specimens

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ABSTRACT: The distribution and redistribution of venlafaxine were investigated in two overdoses and several cases involving the therapeutic use of venlafaxine. Blood, liver, bile vitreous humor, urine and gastric contents were analyzed using high performance liquid chromatography with ultraviolet detection. Blood concentrations of venlafaxine in the two overdose cases were 53 mg/L and 78 mg/L. Comparison of venlafaxine concentrations in blood samples taken at different times after death revealed increases in concentrations over time, suggesting the possible postmortem redistribution of venlafaxine.

KEYWORDS: forensic science, forensic toxicology, venlafaxine, drug distribution, drug redistribution, overdose, postmortem

Venlafaxine hydrochloride (trade name Efexor, Wyeth Australia Pty. Ltd.) is a nonselective serotonin reuptake inhibitor, which is structurally unrelated to the tricyclic or tetracyclic antidepressants, the monoamine oxidase inhibitors, or the selective serotonin reuptake inhibitors (1). Venlafaxine inhibits the reuptake of serotonin, noradrenaline and to a lesser extent dopamine (2). Venlafaxine thus shares its mechanism of action with both the tricyclic antidepressants and the selective serotonin reuptake inhibitors. Venlafaxine was introduced as an alternative to the traditional tricyclic antidepressants which possess sedative, anticholinergic and cardiac arrhythmic activities and which cause several side effects, among them reduced compliance and serious problems when taken in overdose situations. Previous reports have suggested that venlafaxine is relatively safe in overdose, with most overdoses proving asymptomatic, even though the pharmacodynamic profile of venlafaxine suggests that there is a potential for cardiac arrhythmias, hypertension and seizures (2,3).

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The therapeutic plasma/serum concentration of venlafaxine is approximately 0.2 mg/L (4). At this concentration venlafaxine may produce several side effects, including small increases in diastolic blood pressure and heart rate, insomnia, fatigue, nausea, vomiting, nervousness and anxiety (2).

Venlafaxine is well absorbed from the gastrointestinal tract, with mean peak plasma concentrations being reached in approximately 2.4 h (3). Venlafaxine undergoes extensive first-pass metabolism in the liver, the major metabolite being the active compound O-desmethylvenlafaxine. The half-lives of the parent and metabolite are 5 and 11 h, respectively. The primary route of excretion is through the kidneys, with 87% of a single dose recovered in the urine after 48 h. Venlafaxine and its metabolite are less than 35% bound to plasma proteins, and have volumes of distribution of approximately 7 L/kg (2,3,5).

There have been no previous reports in the scientific literature concerning the postmortem redistribution of venlafaxine; however, this is a process that affects many drugs (6–8). Postmortem redistribution is simply defined as the diffusion or release of a drug from a site of high concentration, such as the liver or heart, into neighboring blood vessels (8). Postmortem redistribution is a time-dependent process with a general trend being the longer the postmortem interval, the greater the increase in drug concentration (9). Blood collected at a peripheral site is thought to be the most accurate sample to indicate antemortem drug levels (7,9), due to the distance from the major organs that may have concentrated the drug (10). An understanding of postmortem redistribution is important for accurate interpretation of toxicological data.

Case Histories

Case 1

The deceased was a 41-year-old female with a history of alcoholism who was being treated for severe depression. She was found unconscious by her sister, face down on the front porch of her home. Ambulance officers using cardiac pulmonary resusitation (CPR) obtained a weak heartbeat but her condition continued to deteriorate until she was removed from life support some 12 h later. Two packets of 75 mg venlafaxine tablets were located adjacent to the deceased's bed with 84 of 112 tablets missing.

Postmortem Findings—An autopsy, performed within 48 h of death, included complete macroscopic and microscopic examina-

tion. Histological examination revealed mild to moderate steatosis of the liver.

Toxicological Findings—Preliminary screening was performed using enzyme multiplied immunoassay (EMIT) on bile, in addition to gradient high performance liquid chromatography (HPLC) with photodiode array detection (HPLC-DAD) (11), and capillary gas chromatography (GC-B) followed by mass spectrometry (MS) (12) on blood. The analysis of stomach contents was inhibited by copious amounts of charcoal. Other than venlafaxine, lignocaine was the only other drug detected in the deceased's blood (at a level of approximately 0.5 mg/L) and was administered during the resuscitation attempt. No ethanol was detected.

Case 2

The deceased was a 36-year-old female with a longstanding history of psychiatric illness who was being treated for depression after a serious overdose attempt six months prior to her death. Four weeks after being released from hospital the deceased was found lying dead in her bed. A suicide note was located at the scene as was a plastic bag containing the following medication: venlafaxine tablets 75 mg—empty box of 56, Diamicron tablets 18 mg—70 missing from 200, Diabex tablets 50 mg—95 missing from 200, and Lithicarb tablets 250 mg—800 tablets with very few missing.

Postmortem Findings—The deceased was in the early stages of progressive decomposition; no anatomical cause of death was obvious after the routine macro and microscopic postmortem examination.

Toxicological Findings—Initial drug screening was performed as for Case 1; venlafaxine was the only drug detected. Atomic absorption spectrometry was used to test for lithium; however, none was detected. Alcohol was detected in the blood at a concentration of 0.01 g/100 mL and can be most likely attributed to decomposition processes. No alcohol was detected in the vitreous humor.

Materials and Methods

Specimen Collection

All specimens were collected by qualified forensic technicians at the Victorian Institute of Forensic Medicine (VIFM) or affiliated country center, according to standard mortuary procedures. Samples collected were blood, liver, bile, vitreous humor, urine and gastric contents. Blood was taken from the femoral region whenever possible; alternative collection sites included the heart chambers when femoral blood was unobtainable. Blood samples were 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 preservative at -20°C; a portion of the deceased's liver was stored in a plastic specimen pot at -80°C.

Chemicals and Reagents

Venlafaxine hydrochloride and acepromazine maleate (internal standard) were obtained from the Division of Analytical Laboratories, Health Department, Sydney Australia (DAL) and Australian Government Analytical Laboratories (AGAL), 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, whereas methanol and acetronitrile (HPLC grade) were purchased from Mallinckrodt Australia. Orthophosphoric acid and sodium tetraborate were from Ajax Chemicals.

Liver Homogenization

The liver homogenate, used for toxicological analysis, was prepared by finely dicing a 10 g portion of liver that was placed, along with 10 mL deionized water, in plastic homogenizing bags and then placed in a stomacher (Lab-Blender 80, Seward Medical) 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) before adjusting the pH 10 with 10 M NaOH. Ten milligrams of Subtilisin (Sigma Chemical Company, USA) 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. All liver concentrations were multiplied by a correction factor of 1.5 to obtain a result in mg/kg.

Venlafaxine Extraction

Venlafaxine from each case was extracted from the blood, bile, vitreous humor, urine and liver using the following extraction method: One milliliter of each matrix (standard or sample) was added to a 10 mL polyproplyene extraction tube and to all tubes 10 μL of 100 mg/L dilution of the internal standard acepromazine was added. To each tube 0.5 mL of 2% sodium tetraborate (10 g in 500 mL water, pH 9.15 to 9.2) was added and vortexed before the addition of 8 mL butyl chloride extraction solvent. All tubes were placed on a rotating wheel for 30 min to extract—prior to centrifuging for 5 min at 3000 rpm and placing in an alcohol bath (-30°C) to freeze—the dense 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 to allow for back-extraction into the acid; this was 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 was injected into a Hewlett-Packard (HP) 1050 series HPLC which was linked to a HP ChemServer 4900 series (Hewlett-Packard, Australia). The column used was a Novapak Phenyl, 3.9 × 150 mm, 5 μL particle size (Waters Australia, #10656) with a Novapak phenyl (Waters Australia, #020795) precolumn. The mobile phase of 55% acetonitrile (HPLC grade, Mallinckrodt Australia)/45% 10 mM potassium phosphate buffer pH 3.0 was pumped at 1.5 mL/min, with a sample run time of 15 min. Ultraviolet detection was used, with wavelengths of 214 nm (quantify) and 230 nm (qualify). All data analyses were performed using Target3 software designed for HP Unix-based software.

Redistribution

The redistribution study involved testing blood samples taken over a time frame of up to 80 h. Blood was initially taken from

the cadaver immediately upon admission to the mortuary; this sample was always taken from the femoral vein or artery. Blood was again collected at autopsy, from the femoral region whenever possible. If an admission blood sample was available and the autopsy blood was collected from the femoral region, then this case was included in the study.

Results

The method was sensitive and specific for the quantification of venlafaxine. The between-assay percentage coefficient of variation (%CV) at a concentration of 1 mg/L (blood) was 9.0%, after ten determinations. The within-assay CV% was less than 5%. The sensitivity of this assay (± 3 times baseline noise) was determined to be 0.05 mg/L (for blood). Recovery of venlafaxine from the blood matrix was approximately 100%.

Calibration curves were produced individually for blood, bile, liver, vitreous humor and urine. The calibration range was from 1 mg/L to 10 mg/L (the liver curve included an extra point at 20 mg/kg), with all curves being linear up to at least the concentration reported for the established curves.

Discussion

The two overdose cases presented are unique for not only their high venlafaxine concentrations but also for the absence of other drugs, including alcohol (Table 1). From these cases it is clear that venlafaxine, not in combination with any other drug, can prove fatal in overdose.

Analysis of several cases involving therapeutic levels of venlafaxine showed that an average therapeutic concentration of venlafaxine of approximately 0.33 mg/L ($n = 3$, range 0.13 to 0.65 mg/L) in the blood can be associated with liver and bile drug concentrations of approximately 1.1 mg/kg ($n = 3$, range 0.47 to 1.8 mg/kg) and 0.47 mg/L ($n = 2$, 0.4 and 0.53 mg/L) respectively. The therapeutic concentration of venlafaxine in the vitreous humor was found to be approximately 0.4 mg/L ($n = 3$, range 0.13 to 0.78 mg/L), which is equivalent to the venlafaxine concentrations found in the blood. Results from the two presented overdose cases show drug concentrations well above the therapeutic concentrations for all specimens tested.

Venlafaxine is known to undergo extensive metabolism in the liver, and the relatively high volume of distribution (6 to 7 L/kg) would also predispose venlafaxine to be found in high concentrations in this organ. Biliary excretion is a common route of drug elimination for drugs with high molecular weights (13); such drugs include the tricyclic antidepressants imipramine and desipramine, with molecular weights of approximately 280 and 266, respectively. Venlafaxine, molecular weight 277.41, was shown by its high concentration in the bile to also undergo biliary excretion.

Vitreous humor drug concentrations are also notable as the relatively high levels, suggesting death did not occur immediately after the overdose, as adequate time was available for the drug to be distributed throughout the body, including into the vitreous humor. The low percentage of venlafaxine bound to plasma proteins may in part explain its ability to readily enter into the vitreous humor, since only unbound drug participates in transport into extravascular sites (14). There also exists a blood-vitreal barrier with properties similar to the blood-brain barrier; drug permeation through this barrier is dependent upon such factors as molecular size and lipid solubility. Blood concentrations of venlafaxine are essentially equivalent to those found in the vitreous humor. However, when extremely large doses of venlafaxine have been taken, as can be noted in the two presented cases and several other reported cases (1,5), the vitreous humor venlafaxine concentration can be as low as half the blood concentration. Vitreous humor is therefore an important analytical sample for use in toxicological testing on cases with suspected venlafaxine involvement, and could prove especially significant in cases where extensive decomposition has occurred.

In comparison with eight previously reported venlafaxine related deaths (1,4,5,15), mainly multiple drug overdoses, the two cases reported here stand out for their relatively low liver drug concentrations compared with the blood drug concentration. These differences may be dependent on the extraction of venlafaxine from the liver. In the present case report, a liver calibration curve was used to calculate the concentration of venlafaxine in the liver samples. It is unclear whether or not previous reports of venlafaxine overdoses (1,5,14) utilized the appropriate liver matrix calibration curves. Variation in recovery of venlafaxine in different postmortem specimens is possible, and hence the use of an inappropriate matrix for the calibration curve could dramatically affect the results. A similar situation arises when water is used to dilute samples (4). The ideal diluent is a blank specimen of the same matrix as the sample, as was used in the present assays.

The toxicological results from Case 2 require added interpretation, because the blood sample was taken from the heart and could therefore have been affected by postmortem redistribution (7–9). Results from the redistribution study clearly show an increase in drug concentration over time (Table 2). Increases in drug concentration of this magnitude in femoral blood suggest that there is possibly extensive redistribution occurring from the central organs. However, in this situation it seems unlikely that postmortem redistribution would have affected the blood drug concentration due to the high levels of venlafaxine found throughout the body, as shown by the analysis of other postmortem specimens (Table 1).

In summary, this report has described two cases of venlafaxine overdose where no other drug or alcohol was found during toxicological analyses; both deaths were attributed to venlafaxine poisoning. Venlafaxine concentrations in femoral blood were also shown

TABLE 1—*Tissue distribution of venlafaxine.*

	Case 1	Case 2
Blood	53 mg/L	78 mg/L
Liver	81 mg/kg	110 mg/kg
Bile	90 mg/L	200 mg/L
Vitreous humor	22 mg/L	58 mg/L
Urine	9 mg/L	NA*
Gastric contents	NA	400 mg

* NA not analyzed.

TABLE 2—*Redistribution of venlafaxine in postmortem femoral blood.*

Case	Admission	Autopsy	Time Interval	Cause of Death
1	0.05	0.13	46 h 50 min	M.D.Tox*
2	0.15	0.21	19 h 53 min	Suicide
3	0.48	0.65	80 h 30 min	M.D.Tox
4	0.51	1.9	64 h 35 min	M.D.OD†
5	1.9	3.2	52 h 50 min	Suicide

* M.D.Tox = multiple drug toxicity.
† M.D.OD = multiple drug overdose.

to increase with time, suggesting that venlafaxine is subject to postmortem redistribution.

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