K39 Postmortem Tissue Distribution of Atomoxetine in Fatal and Non-Fatal Dosing Scenarios – Three Case Reports

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After attending this presentation, attendees will learn at what tissue concentrations to expect to find atomoxetine, and at what blood and tissue levels atomoxetine can be considered non-toxic. This data will be useful, since the presence of atomoxetine is already becoming prevalent in medical examiner cases, as the drug is an increasingly prescribed alternative to traditional stimulant therapy for Attention-Deficit/Hyperactivity Disorder (ADHD).

This presentation will impact the forensic community and/or humanity by educating forensic pathologists and toxicologists in the evaluation of atomoxetine levels in various postmortem fluids and tissues. This knowledge will be helpful, since atomoxetine is being used increasingly by prescribing physicians as a non-stimulant alternative to traditional drug treatment for Attention-Deficit/Hyperactivity Disorder

Atomoxetine (Strattera®, Lilly) is a selective norepinephrine reuptake inhibitor prescribed for the treatment of Attention-Deficit/Hyperactivity Disorder (ADHD) in children, adolescents and adults. It is the first nonstimulant drug-therapy option for ADHD. Three case reports are presented in which atomoxetine was detected in two individuals who died from causes unrelated to the drug and a third from the intentional ingestion of atomoxetine and other drugs. Postmortem blood levels of atomoxetine in the discussed cases ranged from 0.1 to 8.3 mg/L while the postmortem liver levels ranged from less than 0.44 to 29 mg/L.

Postmortem Fluid and Tissue Distribution of Atomoxetine

	Atomoxetine (mg/L or *mg/kg)						
	Aorta Blood	Femoral Blood	Vitreous	Bile	Urine	Liver*	Gastric*
Case 1	0.65	0.33	0.1	1.0	NA	3.9	0.54
Case 2	8.3	5.4	0.96	33	NA	29	860
Case 3	< 0.1	< 0.1†	NA	NA	< 0.1	< 0.44	NA

Routine organic base drug screening detected presence of atomoxetine in the central blood of two cases presented. Screening analyses were performed on aliquots of blood using a liquid-liquid extraction. After the addition of alphaprodine (1 mg/L) as the internal standard, specimen aliquots were made basic by the addition of ammonium hydroxide (0.5 mL). Extraction was affected by the addition of *n*-butyl chloride:ether (4:1; 7 mL). The organic solvent was subjected to a back-extraction with 1 M sulfuric acid (2.5 mL). The aqueous phase was separated from the organic layer and was washed with hexane (2 mL). Removal of the hexane layer and further addition of ammonium hydroxide to the aqueous phase allowed the basic drugs to be drawn into *n*-butyl acetate (100 µL). The *n*-butyl acetate extracts were transferred to autosampler vials for analysis by gas chromatography (GC), equipped with a nitrogen-phosphorous detector (NPD), followed by definitive identification by full scan mass spectrometry (MS). Quantification of the original blood specimen and analysis of additional specimens collected in each case on the appropriate amount of specimen to fall with in the linear range of the assay followed the same extraction procedure with the addition of a standard curve and analyte-specific quality control, utilizing GC/NPD. Working methanolic spiking solutions of both standards (10, 100 µg/mL) and controls (10 µg/mL) were prepared by serial dilution from a 1 mg/mL stock solution prepared from the powder supplied from the pharmaceutical company. Calibration curves and quality control samples were created by spiking aliquots of drug-free blood with the working spiking solutions at the appropriate concentrations. Confirmation analyses utilized a 5-point calibration curve at concentration levels of 0.2, 0.5, 1.0, 2.0 and 4.0 mg/L, with a positive control sample at 0.5 mg/L. An aliquot of the drug-free blood was analyzed concurrently with each batch as a negative control. The assay was linear from 0.2 – 10 mg/L with a least squares linear regression analysis correlation coefficient (r^2) of 0.998 or better. The limits of quantitation and detection were 0.1 mg/L and 0.05 mg/L, respectively. Accuracy and precision studies conducted with a control spiked at 0.5 mg/L (3 x n=5) gave a mean within 8% of the target value (CV= 7%). GC/NPD was then performed; injections of 1 µL in the splitless mode were made with an inlet temperature of 275°C. Helium was the carrier gas at a flow rate of 6.7 mL/minute with an initial oven temperature of 120°C. This was followed by an increase of 15°C/min until 300°C, holding for 3

 ** Presenting Author*

minutes. Drug confirmation by GC/MS was performed Split injections (3:1) of 1 µL were made with an inlet temperature of 275°C. Helium was the carrier gas at a flow rate of 1.4 mL/minute with an initial oven temperature of 70°C for 2 minutes. The oven temperature was then ramped at a rate of 15°C/minute until 300°C was reached where it was held for 7 minutes. Electron impact ionization was utilized in the scan mode, monitoring ions from 40-550 *m/z* ratio.

Atomoxetine is well absorbed after oral administration, with a bioavailabilty of 63% and is highly plasma protein bound (98% to albumin). Maximal plasma concentrations of atomoxetine occur 1-2 hours after dosing and its half-life is approximately 5 hours. Atomoxetine has a low volume of distribution (Vd) (0.85 L/kg) suggesting little tissue sequestration. Atomoxetine is metabolized primarily by cytochrome P450 (CYP) 2D6 to yield 4-hydroxyatomoxetine, which is subsequently glucuronidated. A second metabolite, *N*-desmethylatomoxetine is formed by the action of CYP2C19. 4-hydroxyatomoxetine has equipotent SNRI pharmacological activity to the parent drug but is only present in the plasma at very low concentrations. *N*-desmethylatomoxetine not only has less pharmacological activity than atomoxetine, but is also present in plasma at lower concentrations. The elimination half-life of the two metabolites is 6-8 hours. Greater than 80% of the dose of atomoxetine is excreted in the urine as 4hydroxyatomoxetine-*O*-glucuronide with NA – not available; † - vena cava blood less than 17% of the dose appearing in the feces. Poor CYP2D6 metabolizers will display altered pharmacokinetic data.

Atomoxetine can be considered non-toxic at whole blood and liver concentrations below 1.3 mg/L and 5 mg/kg, respectively. Although the drug has a low volume of distribution, it appears to undergo postmortem redistribution with a mean central to peripheral ratio of 2.7 (range: 1.5 – 5.6).

Although this is a preliminary study, little is known through clinical trials or reports of the toxicity of atomoxetine in overdose; even less is known about postmortem toxicology. The presence of atomoxetine was considered an incidental finding in two of the cases presented while the third involved an intentional overdose. The corresponding postmortem fluid and tissue distribution of atomoxetine in addition to the significant anatomic findings autopsy are reported.

Atomoxetine, Postmortem, Forensic Science

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