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Olanzapine Concentrations in Clinical Serum and Postmortem Blood Specimens—When Does Therapeutic Become Toxic?

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ABSTRACT: The concentration of olanzapine (Zyprexa®) was determined in 1653 clinical serum specimens during routine drug monitoring, and in 58 postmortem whole blood specimens as part of routine toxicological analysis. The analysis of olanzapine was performed by the solid-phase extraction of 1.0 mL of buffered serum or blood, followed by gas chromatography separation with nitrogen-phosphorus detection. The analysis of the clinical serum samples showed that 86% of positive serum values were within the range of 5 to 75 ng/mL, with a mean and median of 36 and 26 ng/mL, respectively. These data suggest that the concentrations of olanzapine expected during therapy may be higher than those previously reported. In 58 postmortem whole blood specimens the mean olanzapine concentration was 358 ng/mL with a range of 10 to 5200 ng/mL. Further, investigation of deaths involving olanzapine suggest that potential toxicity *should be considered* at concentrations above 100 ng/mL. Although the majority of the olanzapine-related deaths were associated with many other drugs, death primarily *due* to olanzapine toxicity was determined at concentrations in postmortem blood as low as 160 ng/mL.

KEYWORDS: forensic science, forensic toxicology, Zyprexa, olanzapine, antipsychotic, therapeutic, clinical toxicology, postmortem

Olanzapine (Zyprexa®), a new drug for the treatment of schizophrenia, has been reported to provide favorable anti-psychotic efficacy with minimal adverse side effects. Olanzapine is a thienobenzodiazepine analog that is structurally and functionally similar to clozapine (1).

Olanzapine is a selective monoaminergic antagonist with high affinity for a large number of receptors in the central nervous system (CNS) including dopamine, serotonin, adrenergic, and muscarininc cholinergic receptors (2). Similar to clozapine, it is considered an atypical neuroleptic agent and with fewer extrapyrimidal side effects than the classical neuroleptic agents such as haloperidol (1). Reported side effects following the use of olanzapine include drowsiness, dry mouth, hypotension, *paresthesias,* elongated Q-T intervals, and weight gain (1,3,4).

Until recently, very few studies described the expected therapeutic concentrations of olanzapine during therapy. Perry et al. (5) suggest that the minimum effective therapeutic concentration of olanzapine is 9 ng/mL, and following a single that dose of 12.2 mg Levine (4) states that average peak serum concentrations were 11 ng/mL. Further, Ereshefsky (6) proposed a therapeutic range of 9 to 23 ng/mL.

Similarly, very little has been reported about olanzapine concentrations contributing to, or causing, death. In one reported case of suicide involving olanzapine, the olanzapine concentration in whole blood was 4100 ng/mL (7). Anderson and Kuwahara (8) reported one case of acute olanzapine toxicity leading to death. In this case the concentration of olanzapine in femoral blood was 1200 ng/mL and in heart blood was 1300 ng/mL (8). Levine et al. (4) also reported one death due to olanzapine toxicity. In this case the blood olanzapine concentration was 980 ng/mL. Stevens et al. (9) described the suicidal ingestion of as much as 600 mg of olanzapine. Postmortem blood concentrations were determined to be 1238 ng/mL.

The absence of reference values in the literature made the interpretation of olanzapine concentration in clinical and postmortem specimens very difficult. Therefore, the aim of this study, using a large population of clinical and postmortem specimens, was to determine the expected concentration of olanzapine following therapeutic administration and, further, to determine at what concentrations olanzapine may be associated with toxicity and/or death.

Methods

Reagents and Glassware

Drug-free serum was obtained from Quality Assurance Science Corp., (GA). Olanzapine (free base) and LY-170222 (internal standard) were obtained from Lilly Research Labs. Sodium phosphate, monobasic (Sigma, USA), sodium phosphate, dibasic (Aldrich, USA), acetic acid and ammonium hydroxide (Fisher, USA) were of analytical grade. Methanol, toluene and acetonitrile (Fisher, USA) were of HPLC grade. Solid phase extraction cartridges were International Sorbent Technology Isolute™ HCX cartridges.

Standards and Controls

Stock drug solutions were prepared in methanol at a concentration of 100 ng/mL. Calibration samples were prepared by adding methanolic olanzapine solutions to serum followed by serial dilution of the serum specimens with blank serum to give a final concentration ranging from 3 to 250 ng/mL. Parallel whole blood calibration curves were prepared using fresh, drug-free postmortem blood.

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Serum specimens with known concentrations of olanzapine (12, 40 and 75 ng/mL) were prepared in-house using blank serum and assayed with each analysis batch to provide a measure of quality assurace. Negative controls were also analyzed with each analysis batch.

Specimens

Clinical serum and postmortem blood specimens were received by National Medical Services Inc., during 1997 and 1998. Prior to analysis the specimens were stored refrigerated until analysis. Postmortem blood specimens contained 1% sodium fluoride/potassium oxalate as the preservative. The clinical samples typically did not contain preservatives.

Identification and Quantification of Olanzapine

Olanzapine was identified and quantified in serum and blood by capillary gas chromatography using nitrogen-phosphorus detection (GC/NPD), as described previously (10).

Results

The procedure for olanzapine determination was linear over the range of 3 to 250 ng/mL, with an average recovery of 68%. Olanzapine and internal standard eluted at 7.4 and 7.6 min, respectively. Percent coefficients of variation (% C.V.) for within-run and between-run variability were 8.3%, 3.6%, and 3.8% and 13%, 10%, and 8.5% at olanzapine concentrations of 12, 40 and 75 ng/mL, respectively. Specimens with olanzapine concentrations exceeding the highest calibrator were diluted appropriately with blank serum and re-extracted.

During 1997 and 1998, 1653 clinical specimens were received by National Medical Services Inc., and analyzed for olanzapine, usually within 48 h of receipt. The mean concentration of olanzapine was 36 ± 40 ng/mL, with a median concentration of 26 ng/mL. The range of positive samples was 3 to 390 ng/mL. Eighty-six percent of specimens had olanzapine concentrations between 5 and 75 ng/mL. These data are shown in Figs. 1 and 2.

No data were available with respect to the patients' therapeutic regime or the time of specimen collection relative to the time of ingestion.

Fifty-eight postmortem specimens were analyzed. The mean concentration of olanzapine was 358 ± 758 ng/mL, with a median concentration of 130 ng/mL. The range of positive samples was 10 to 5200 ng/mL. These data are shown in Fig. 2.

For the majority of the postmortem cases, no information was available with respect to cause and manner of death, or with respect to the extent of toxicological analysis that may have been performed at other laboratories.

Discussion

This GC/NPD method provided a sensitive and reliable analysis for the detection and quantitation of olanzapine in biological specimens. The analysis of 1653 patient samples showed that the vast majority of specimens contained concentrations of olanzapine below 75 ng/mL, and in fact closer to 26 ng/mL. Previously reported therapeutic concentrations for olanzapine suggest a range in concentrations up to 23 ng/mL during therapy (6). Our findings, although consistent with previous findings, suggest that the concentration of olanzapine expected during therapy may be as high as 75 ng/mL. Unfortunately, little information could be obtained regarding

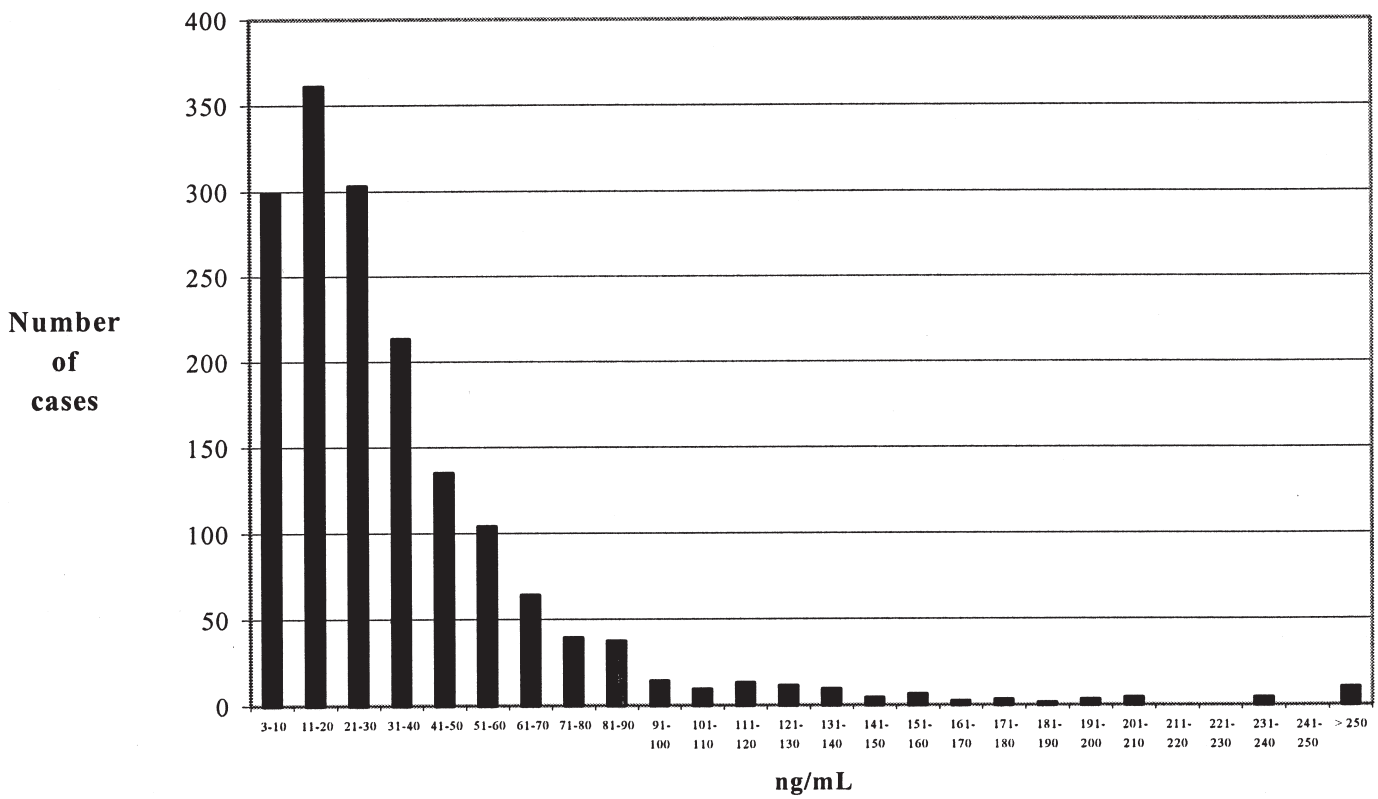

FIG. 1—*Graph showing concentration distribution of olanzapine in clinical serum specimens received during 1997 and 1998 (*n *= 1653).*

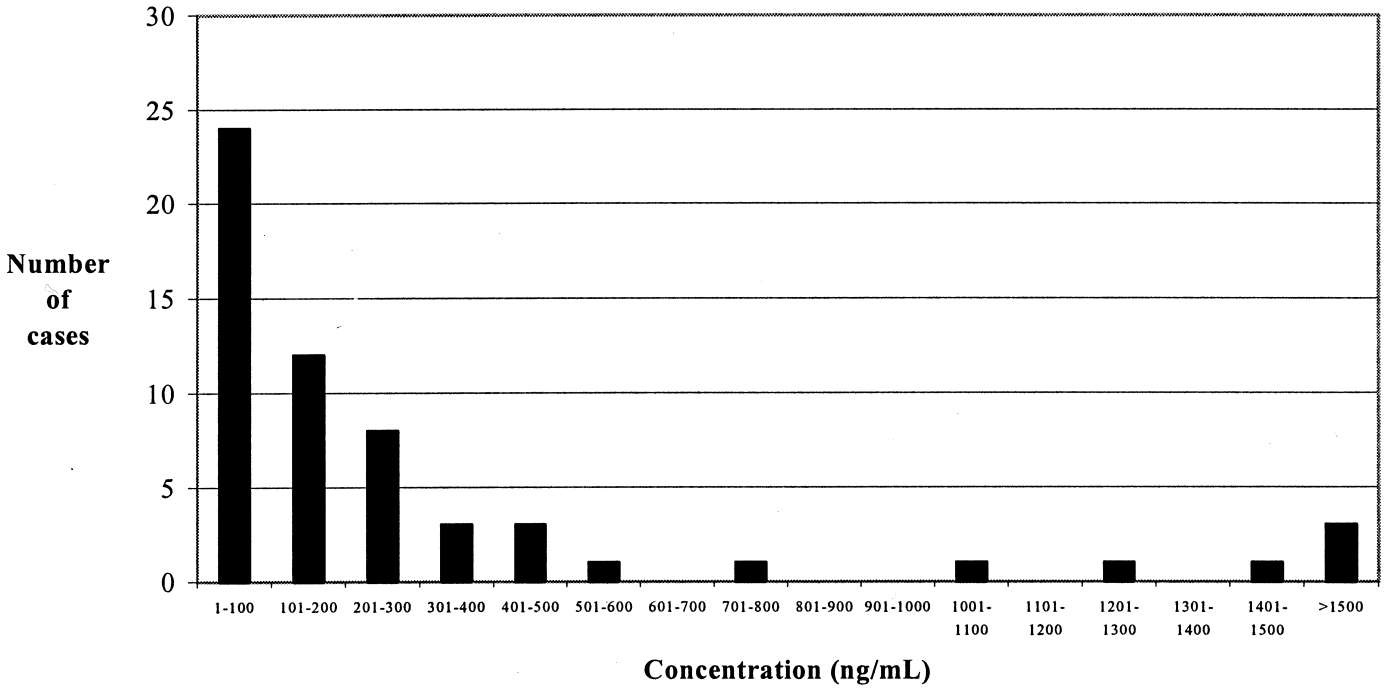

FIG. 2—*Graph showing concentration distribution of olanzapine in postmortem blood specimens received during 1997 and 1998 (*n = *58).*

dosage regime and time of previous dose relative to collection; therefore, it can only be stated that these concentrations are those expected from individuals taking olanzapine therapeutically. In addition, it is possible that some of the cases submitted from clinical facilities with elevated concentrations may be associated with acute overdosage; however, from our information these cases are unlikely to significantly affect the concentrations expected during therapy.

In the postmortem specimens the mean and median concentrations were significantly higher than clinical samples. The olanzapine concentrations in postmortem specimens are consistent with the findings of Levine et al. (4), Elian (7), Anderson and Kuwahara (8), Stephens et al. (9), and Wong (11), who showed the olanzapine concentrations in postmortem cases range from 25 to 4800 ng/mL. Typically, in cases identified by the respective authors as death due to olanzapine, the concentration of olanzapine exceeded 1000 ng/mL. An explanation for this difference in olanzapine concentrations between clinical and postmortem populations is that in a large number of the postmortem cases examined, death was the result of ingestion of multiple medications in an attempt to commit suicide.

Although the olanzapine concentrations found in the postmortem population examined in this study were significantly higher than concentrations detected in the clinical population, deaths due to olanzapine toxicity were observed below 500 ng/mL. In one case a 43-year-old male with a history of seizures was found dead in his bedroom face down on the floor. His prescribed medications were lithium, clonazepam, benztropine, fluoxetine, and olanzapine. Following extensive toxicology, only lithium 0.35 mEq/L and olanzapine 160 ng/mL were detected.

In a second case, a 20-year-old male diagnosed with schizophrenia was prescribed olanzapine and was taking ~70 mg/day. After going to sleep, his mother observed him to have breathing difficulties shortly before death. Toxicological examination detected olanzapine at only 330 ng/mL and 7-amino clonazepam at 16 ng/mL.

In both of these cases the presence of elevated olanzapine concentrations in the absence of a similar or more competent cause of death led to the cause of death being ruled as olanzapine toxicity. In neither of these cases was the mechanism of death discussed by the coroner/medical examiner in association with olanzapine's toxicity. It is however possible that olanzapine toxicity is related to reported pharmacological side effects. These include orthostatic hypotension, a result of adrenergic antagonism, possibly causing tachycardia with cardiac manifestations and antihistamine-related sedation and somnilescence. It is also worth noting that olanzapine is extensively metabolized by the liver enzymes CYP1A2 and 2D6; hence any reduction of enzyme-mediated metabolism, a result of pathophysiological processes or drug interaction, may inadvertently raise olanzapine concentrations in the blood, possibly resulting in toxicity.

The investigation of the stability of olanzapine in postmortem blood during storage is beyond the scope of this paper; however, poor stability may be an explanation for the lower concentrations associated with toxicity described herein than previously described by others. It has been observed by Levine et al. (4), and Anderson and Kuwahara (8) that olanzapine is extremely labile in postmortem blood. This may be related to the presence of a heterocyclic sulfur which Stevens (12), when examining the stability of drugs and poisons in putrefying liver, found were prone to decomposition.

Although not examined in this study, the data presented by Anderson and Kuwahara (8) suggest there are site-dependent concentration variations. The authors showed that in 33 of the 35 cases presented, olanzapine concentrations were higher in the heart blood than in the femoral blood.

In summary, the results from this study suggest that olanzapine concentrations between 5 and 75 ng/mL are expected during therapy. Toxicity may occur as low as 100 ng/mL and concentrations in blood above 100 ng/mL should be considered as a possible con-

tribution to the cause of death. When interpreting olanzapine concentrations, consideration should also be given to possible degradation during storage and to the site of blood collection.

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