A fatal case of simultaneous ingestion of mirtazapine, escitalopram and valproic acid

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Abstract

Mirtazapine, escitalopram, and valproic acid are newer antidepressant drugs than traditional tricyclic antidepressants and are supposed to be less toxic. Nevertheless, intoxication cases due to their overdosage have been repeatedly reported. In the case presently reported, a 64-year-old woman with a previous history of chronic depression was found dead in her apartment. Several packages of pharmaceutical drugs were found, including mirtazapine, escitalopram, and valproic acid. During the autopsy, no evidence of natural disease or trauma was found to account for this death. In order to determine whether massive drug assumption might have determined a lethal intoxication, heart blood, urine, and gastric content were collected and submitted to toxicological analysis. Specific liquid chromatography–tandem mass spectrometry protocols were purposely developed and validated. Blood concentrations of mirtazapine, escitalopram, and valproic acid were 20.3, 65.5, and 417 mg/L, respectively, whereas urine concentrations were 17.0, 94.5, and 423 mg/L, respectively. High concentrations of these drugs were also detected in the gastric content, confirming their ingestion shortly before death. The agreement between authoptic examination by forensic pathologists and toxicological findings are consistent with the suicidal hypothesis, where the death arose by drug intoxication due to simultaneous high-dosage ingestion of mirtazapine, escitalopram, and valproic acid.

Introduction

Depression is a common psychiatric disease, affecting about 20% of people during their lifetimes. Severe depression is also a major risk factor for suicide, and up to 15% of patients hospitalized for depression eventually commit suicide (1). In order to treat this common mental illness, several antidepressants drugs are daily prescribed by physicians. The use of antidepressants has substantially increased worldwide during the past two decades and several new antidepressant drugs have been introduced, gradually replacing tricyclic antidepressants (TCAs), such as amitriptyline, clomipramine, doxepine, and imipramine, which have been available since the 1960s. The new generation of antidepressants is designed on the basis of molecule targeting, with focus on serotonine level control. Drugs such as citalopram, fluoxetine, paroxetine, and sertraline, acting as selective serotonin reuptake inhibitors (SSRIs), have become increasingly popular for the treatment of depression (2). Other antidepressant drugs include the monoamino-oxidase inhibitor moclobemide and other mixeduptake inhibitors (mirtazapine, nefazodone, and venlafaxine). Ingestion of TCAs is still an important cause of suicidal death, as serious poisoning from TCAs lead to cardiac disturbances, respiratory depression, metabolic acidosis, convulsion, and coma. Even if SSRIs are considered to be less toxic than the TCAs (3), numerous case reports have shown that the newer antidepressants may also cause severe intoxication (4).

Among the antidepressants, mirtazapine is a tetracyclic piperazinoazepine compound, structurally related to mianserin, which has been used as an antidepressant since 1994. Mirtazapine is a strong α2 antagonist, enhancing noradrenergic and serotonergic neurotransmission without inhibiting the serotonin re-uptake. Daily doses for adults are normally in the range of 15–45 mg. The oral bioavailability is approximately 50% (5). Adverse effects associated with mirtazapine therapy include somnolence, dizziness, agitation, hypertension, and loss of appetite. Overdosage may cause disorientation, drowsiness, and tachycardia.

Escitalopram is the S-enantiomer of the racemic derivative citalopram that was approved by the Food and Drug Administration (FDA) for use as an antidepressant in 2002. It selectively inhibits the re-uptake of serotonin with little effect on norepinephrine or dopamine re-uptake. Adverse effects associated with its overdosage include fatigue, somnolence, hyponatremia, dizziness, and nausea. Adult doses of 10–20 mg are normally administered once daily. The oral bioavailability of escitalopram averages 80% (5). Valproic acid has been used since 1967 as an anticonvulsant drug. More recently, it has also been employed in the treatment of the manic depressive phase of bipolar disorders. Daily oral doses range from 250 to 2500 mg. The oral bioavailability of valproic acid approaches 100% (5). Adverse reactions to valproic acid therapy include vomiting, sedation, weakness, pancreatitis, and hepatic damage.

Case History

A 64-year-old woman (weight 85 kg, height 167 cm) with a previous history of chronic depression was found dead on the kitchen floor of her apartment. She had attempted to commit suicide in 2000. Since then, she had been under psychiatric treatment. On the table next to her, many packages of pharmaceutical drugs were found, suggesting the occurrence of drug-related suicidal death. Specifically, the police seized four boxes of Mirtazapina EG® 30 mg (mirtazapine) containing seven empty blister packs, originally corresponding to 105 tablets; three boxes of Cipralex® 10 mg (escitalopram) containing five empty blister packs, originally corresponding to 70 tablets; and one box of Depakin Chrono® 300 mg (valproic acid) containing three empty blister packs, originally corresponding to 30 tablets. It was not known whether the woman had started consuming these packages at a previous time, so it was not possible to estimate the amount of drugs possibly ingested by the victim the day of her death. No letters or explanation of her act were found in the apartment. The death was reported to the Public Prosecutor's office, which took jurisdiction of the case. At the autopsy, the decedent did not show any specific pathology and appeared wellnourished. Internal examination presented no evidence of any trauma to account for her death. Therefore, our laboratory received the responsibility to determine whether massive drug assumption could be taken into account as the cause of the death. The specimens sampled during the autopsy included heart blood, urine, and gastric content. Femoral blood was not collected.

Experimental

Materials

Mirtazapine, valproic acid (as sodium salt), 17α-methyltestosterone, sodium hydrogen carbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), sodium phosphate dibasic dehydrate ($Na_2HPO_4 \cdot 2 H_2O$) and potassium phosphate monobasic (KH_2PO_4), methanol, formic acid, acetonitrile, t-butyl methyl ether (TBME), and β-glucuronidase (from Escherichia coli) were purchased from Sigma-Aldrich (Milan, Italy), and escitalopram (as oxalate salt) was from Toronto Research Chemicals (North York, ON, Canada). Sodium hydroxide was obtained from Carlo Erba Reagents (Milan, Italy). All solutions and buffers were prepared using deionized water obtained from Milli-Q System (Millipore, Billerica, MA). Phosphate buffer was prepared by dissolving 4.63 g of KH_2PO_4 and 11.75 g of $Na_2HPO_4 \cdot H_2O$ in 1 L of water, and carbonate buffer was prepared by dissolving 2.12 g of Na_2CO_3 and 6.72 g of $NaHCO_3$ in 1 L of water.

Sample preparation

For screening and confirmation analysis, samples and calibrators were extracted with the following procedure. Blood (1 mL), urine (1 mL), and gastric content (1 g) samples were fortified with 5 μg of

the internal standard 17α-methyltestosterone (IS). Only urine samples were preliminarily buffered at pH 7.4 with 2 mL of a 0.1 M phosphate buffer, and 30 µL of β-glucuronidase was added prior to incubate the mixture at 55°C for 1 h. Then, blood, urine, and gastric content samples were buffered to pH 9.6 with 2 mL of a 0.1 M carbonate buffer. Liquid–liquid extraction was performed by adding 10 mL of tert-butylmethylether (TBME) and shaking the mixture in a multimixer for 10 min. The organic layer was separated and then dried under a gentle flow of nitrogen. The resulting residue was reconstituted with 50 µL of methanol. Lastly, a 1-µL aliquot was injected (split ratio of 10:1) into the gas chromatography–mass spectrometry (GC–MS) system for screening analysis. For confirmation analysis, the organic extract was dried and subsequently dissolved into 50 µL of liquid chromatography (LC) mobile phase (component A/component B, 9:1). The elution solvents were water/formic acid 5 mM (component A) and acetonitrile (component B). Ten microliters of the final extract was injected into the LC–MS–MS instrument.

Apparatus and methods

Preliminary screening analyses for amphetamines, triciclic antidepressant, barbiturates, benzodiazepines, cannabinoids, methadone, cocaine, and opiates were performed by fluorescence polarization immunoassay (FPIA, Abbott Laboratories, Abbott Park, IL). Screening analysis for unknown substances was performed using a 6890N GC (Agilent Technologies, Milan, Italy) equipped with a 17-m fused-silica capillary column (J&W Scientific HP-5), of 0.2-mm inner diameter and 0.33-µm film thickness, for GC separation. Helium was employed as the carrier gas at a constant pressure of 23.24 psi. The GC oven temperature was set at 90°C for 1 min and then raised to 180°C with a 30°C/min heating rate. The oven temperature was maintained at 180°C for 7 min and then raised to 315°C at a 15°C/min heating rate. The gas chromatograph injector and transfer line were maintained at 280°C. Full scan spectra in the interval 50–500 amu were acquired using a 5975 inert mass selective detector (Agilent Technologies) operating in the EI mode at 70 eV. For confirmation analyses, two specific LC–MS–MS procedures were developed and validated: one for the determination of mirtazapine and escitalopram and one for valproic acid. In both methods, LC was performed using an Agilent 1100 series LC (Agilent Technologies) equipped with a Phenomenex Synergi Fusion-RP column (150 × 2.00-mm i.d. × 4 µm), protected by a guard column. The mobile phase eluted under the following linear gradient conditions: (A/B, v/v) from 90:10 to 0:100 in 10 min, with 5 min for re-equilibration. Each chromatographic separation was run at flow rate of 300 µL/min. Detection was carried out by an Applied Biosystems API 3200 triple-quadrupole MS (Applied Biosystems, Foster City, CA) equipped with turbo ion spray source, operating either in the positive ionization mode for detecting mirtazapine, escitalopram and the IS, or in the negative ionization mode for valproic acid. Best results were obtained using a source block temperature of 350°C. Data were recorded in the selected reaction monitoring (SRM) mode. In order to establish appropriate SRM conditions, each analyte was individually infused into the

electrospray ionization (ESI) capillary and the cone voltage (CV) was adjusted to maximize the intensity of the protonated or deprotonated molecular species. Collision offset voltage (CE) was selected to preserve approximately 10% of precursor ion. Nitrogen was employed as collision gas (5 × 10–3 Pa). Precursor ions and the corresponding product ions for analytes and IS are presented in Table I. Only the parent-parent transition was obtained for valproic acid, as already reported in previous literature (6).

Validation of the LC–MS–MS confirmation method

Blood was chosen as the target matrix for the method validation. Two standard calibration curves at low and high concentration levels were prepared by fortifying blank blood samples in the ranges 0.002–1.0 mg/L (0.3–1.0 mg/L for valproic acid) and 1.0–50.0 mg/L. Whenever the effective drug concentration resulted higher than the calibration range, the sample was diluted in order to fit the quantitation interval considered in the curves. The limits of detection (LOD) were estimated as the analyte concentration whose response provided a S/N value equal to 3, as determined from the least abundant among qualifier ions. The estimated LOD was extrapolated from the S/N value of the lowest concentration level (LCL) using the corresponding calibration curve. The limit of quantitation (LOQ) was consequently calculated as three times the LOD value. Relative extraction recovery were determined by comparing the responses of the extracted blood samples containing the analytes at a final concentration of 5 mg/L with the responses of a blank blood sample in which the drugs were added at the same concentration after the extraction step. Within-batch precision (expressed as percent variation coefficient, CV%) and accuracy (expressed as bias %) were assessed by extracting and analyzing a series of five blood samples fortified at 5.0 mg/L. Matrix effects were evaluated by comparing the signal obtained when the analytes were fortified into the matrix extract with the responses obtained from a methanolic elution containing the analytes at the same concentration.

Results and Discussion

The LODs determined in blood were 0.0005, 0.0006, and 0.1 mg/L for mirtazapine, escitalopram, and valproic acid, respectively. Consequently, the corresponding LOQ values were 0.002, 0.002, and 0.3 mg/L. Calibration curves were generated by linear regression and exhibited correlation coefficients (R2) between 0.9961 (valproic acid) and 0.9990 (escitalopram). The extraction recoveries were 94.6%, 88.2%, and 76.9% for mirtazapine, escitalopram, and valproic acid, respectively. From the analysis of five replicates of blood samples fortified at the final concentration of 5.0 mg/L, the within-batch precision ranged from 2.0% to 4.6%, and the accuracy (expressed as percent bias) was +22.6%, +18.9%, and +9.6% for mirtazapine, escitalopram, and valproic acid, respectively. No significant matrix effects were found for all the tested analytes. All the validation

results are summarized in Table I. The presence of mirtazapine, escitalopram, and valproic acid was confirmed in all specimens. The toxicology results for this case are shown in Table II. The overlay of two LC–MS–MS profiles resulting from the detection of mirtazapine, escitalopram and valproic acid in blood is shown in Figure 1. The heart blood concentration of mirtazapine was 20.3 mg/L. In order to interpret this finding, some considerations are needed. The influence of postmortem redistribution is well known to modify the drug levels in tissues and in blood specifically. Regarding mirtazapine, this kind of phenomenon is likely not to be predominant. In eight postmortem cases in which mirtazapine was identified but did not contribute significantly to the cause of death, the mirtazapine concentration in heart blood was found to be approximately equal to the one present in peripheral blood, indicating that postmortem redistribution was not a factor to be taken into account in evaluating the postmortem blood concentrations (7). Moreover, several mirtazapine metabolites are known to have some pharmacological activity, but they are not believed to significantly contribute to the overall effects of the drug because of their low plasma concentrations (8). Thus, the pharmacological level of mirtazapine is generally expressed by its parent drug concentration. The serum therapeutic concentration of mirtazapine ranges from 0.02 to 0.1 mg/L (9), but no published data are available about its toxic concentration. In a single fatal case in which mirtazapine was the only ingested drug, the postmortem blood concentration was found at 2.7 mg/L (10). In the case reported here, the mirtazapine blood concentration is much higher than the expected therapeutic concentration, so there is good reason to believe that a toxic concentration may have been reached. The heart blood concentration of escitalopram was 65.5 mg/L. To our knowledge, evaluation of postmortem redistribution of escitalopram has never been reported. Plasma levels of N-desmethylescitalopram, likely being the main escitalopram metabolite, are about one-third those of the parent drug during chronic administration, whereas the concentrations of another important metabolite, N-N-didesmethylescitalopram, are generally so low as to be practically undetectable (5). In the cases of suspected escitalopram intoxication, the primary determination of the parent drug, not its metabolites, appears to be reasonable. Although some lethal intoxications involving citalopram are reported (11–14), the same does not emerge for escitalopram. A series of 14 occurrences of intentional overdosage with 100–600 mg of the drug by persons aged 16–44 years resulted in no significant adverse effects in half the patients and relatively minor effects in the remainder (15). Therefore, escitalopram ingestion is not likely to account for the main cause of death in this case, but its high level detected in blood may have contributed to the progression leading to the woman's decease. The heart blood concentration of valproic acid was 417 mg/L. A heart/femoral blood concentration ratio of 1.0 was reported in a single case (16). Several metabolites of valproic acid were found in the plasma of patients receiving the drug, but their low pharmacological activity together with their relatively low concentration make it consider that valproic acid itself accounts for at least 90% of the overall anticonvulsant drug activity (17). The suggested valproic acid therapeutic level in plasma is about

50–100 mg/L (18). A multicenter case series study of valproic acid intoxication revealed that 186 out of 335 reports exhibited serum levels higher than 100 mg/L, and peak concentrations exceeding 850 mg/L were more likely to be associated with coma, respiratory depression, aspiration, or metabolic acidosis (19). In the few reported cases of lethal ingestion, blood concentrations of valproic acid ranged from 720 to 2204 mg/L (20–24). In the case presented here, the blood concentration of valproic acid appears to be at least four times higher than the therapeutic level and within the reasonable range of acute intoxication. However, it remains questionable whether such a concentration of valproic acid could have caused death when taken alone or the combined ingestion with mirtazapine and escitalopram represents the key explanation for the fatality.

Conclusions

The fatal case reported in the present study, involving the concomitant intake of mirtazapine, escitalopram, and valproic acid, suggests that the synergic pharmacological effects of the three drugs are likely to account for the decease. Among the three drugs, only the blood concentration found for mirtazapine appears to be fully compatible with acute intoxication leading to death, whereas escitalopram and valproic acid are more likely to have played a subsidiary role in the fatality. Nonetheless, their high concentrations found in blood have almost certainly accelerated the progression of the lethal intoxication. The agreement between the authoptic examination executed by forensic pathologists and the toxicological findings presented here are consistent with the suicidal hypothesis beyond any reasonable doubt.

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Compound	RT (min)	MS conditions			Low levels		High levels		LOD (mg/L)	LOQ (mg/L)	Within-batch precision (CV%)	Accuracy (bias%)	Recovery (%)
		SRM transitions	CV (V)	CE (V)	Linearity Range (mg/L)	Correlation coefficient (R^2)	Linearity Range (mg/L)	Correlation coefficient (R^2)					
Mirtazapine	6.30	266.1→195.1 266.1→72.1 266.1→209.3	+38 +38 +38	+33 +30 +28	0.002-1	0.9972	1-50	0.9983	0.0005	0.002	4.7	+22.6	94.6
Escitalopram	7.88	325.1→109.1 325.1→262.2 325.1→234.2	+68 +68 +68	+35 +26 +35	0.002-1	0.9987	1-50	0.9990	0.0006	0.002	2.0	+18.9	88.2
Valproic acid	9.47	143.0→143.0	-34	-13	0.3-1	0.9984	1-50	0.9961	0.1	0.3	3.0	+9.6	76.9
17α-methyltestosterone	10.25	303.0→109.0 303.0→97.0	+36 +36	+34 +39			-	-	-	-	-	-	-

CV = Cone Voltage (Volt); CE = Collision Energy (Volt)

Table 1. Mass-chromatographic conditions and validation data

Table 2. Drugs concentrations detected in blood, urine and gastric content of the victim.

	Specimen (Concentration)		
Drug	**Heart Blood (mg/L)**	**Urine (mg/L)**	**Gastric content (g/kg)**
Mirtazapine	20.3	17.0	0.5
Escitalopram	65.5	94.5	0.6
Valproic Acid	417	423	4.8

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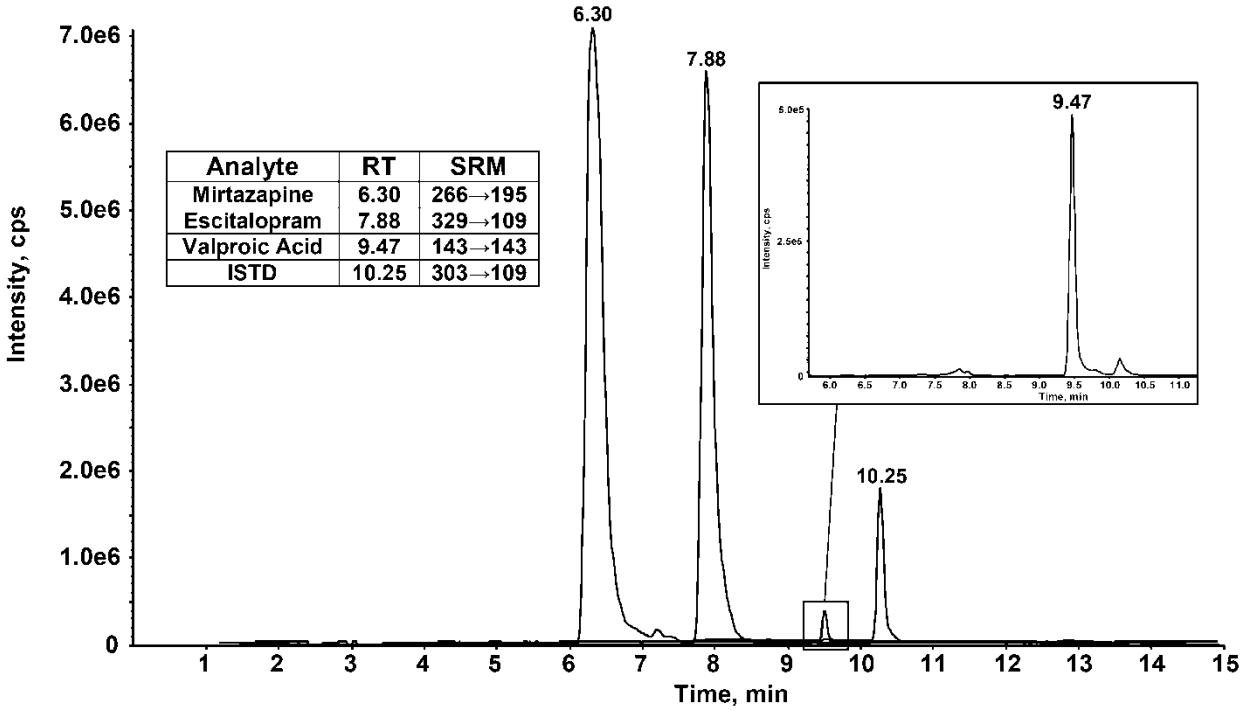

Figure 1. Overlay of the two SRM chromatograms resulting from the detection of mirtazapine, escitalopram and valproic acid in blood; the inset shows in detail the mass-chromatographic peak corresponding to valproic acid.