

Case Report

Two Cases of Fatal Zopiclone Overdose

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Abstract

Two cases of death due to the ingestion of zopiclone are presented. Quantitative determinations of zopiclone yielded 1.4–3.9 mg/L in the blood, 0.81 and 8.7 mg/kg in the liver, and 13.5 and 133 mg in the stomach contents. Drug concentrations were interpreted relative to the case findings, published data, and a limited evaluation of therapeutic concentrations found in two cases of therapeutic zopiclone use. Zopiclone was extracted from buffered blood or digested liver or both with *n*-butyl chloride and analyzed by high-performance liquid chromatography with ultraviolet detection. Liver samples were digested at pH 7 to avoid zopiclone decomposition.

Introduction

Zopiclone (Imovane®) (Rhône-Poulenc, Essex, England) is a pyrrolopyrazine drug with hypnotic effects that has been shown in insomniac patients to possess rapid onset of action and few associated side effects (1–3). It is chemically unrelated to any existing drug and acts on a site distinct from that of benzodiazepines. Its human tissue distribution and post-mortem toxicokinetics are unknown, and we are aware of only one published fatality from an overdose (4).

We report here two cases involving zopiclone overdose and two cases involving therapeutic zopiclone use. Blood, liver, and stomach content samples were analyzed in each overdose case, whereas blood and liver were analyzed in the therapeutic cases.

Case Histories

Case I. The deceased was an 81-year-old Caucasian who had been suffering from depression for the last 5 years because of physical disability and the likelihood of going into a rest home. He had threatened to commit suicide on several occasions. He was found dead approximately 16 h after last being seen. A

cup bearing tablet residues was found beside him. The cause of death was zopiclone overdose.

Case II. The deceased was a 24-year-old Caucasian who had attended a party on the night of her death. She phoned a male friend and fell asleep while talking to him. The friend ended the conversation at 0200 h. She was found dead at 1940 h in her bedroom by her parents. She had been bleeding from the mouth and nose and was discovered next to a plastic drink bottle containing liquid and tablet residues. The cause of death was zopiclone overdose complicated by ethanol use.

Case III. The deceased was an 82-year-old hospitalized male suffering from depression and suicidal thoughts. He was being treated with buspirone, paroxetine, pericyazine, and 7.5 mg zopiclone nightly. The last 2 days of his life he was eating very little, losing weight, and saying he wanted to die. He was found dead in the morning. Autopsy revealed many paroxetine tablets in his stomach and coronary atheroma. The cause of death was paroxetine overdose complicated by coronary atheroma.

Case IV. The deceased was a 38-year-old Indian who arrived much earlier than usual for work. He was found dead 4 h later in his car. He had a serious alcohol abuse problem and was prescribed 7.5 mg zopiclone nightly. The cause of death was chronic fatty liver disease complicated by mild head injury and bronchopneumonia.

Experimental

Materials

Zopiclone and its internal standard, 6-(6-chloro-2-quinolyl)-7-[(4-methyl-1-piperazinyl)carbonyloxy]-6,7-dihydro-[5H]pyrrolo[3,4-*b*]pyrazine-5-one, were gifts of Rhône-Poulenc. *n*-Butyl chloride (synthesis grade) was obtained from Merck (Munich, Germany) and redistilled before use. All other chemicals and reagents were analytical-reagent grade and were used as purchased. Both zopiclone and the internal standard are unstable in nucleophilic solvents such as methanol or ethanol. Stock standard solutions of zopiclone and the internal

standard for quantitation were therefore prepared in the non-nucleophilic solvent, acetonitrile, and stored at -15°C . The high-performance liquid chromatographic (HPLC) system consisted of a 1050 quaternary pump, a 1050 autosampler with a high-pressure sampling valve (1–100 μL), a 1050 vacuum degassing unit, a 1040M diode-array detector supplied by Hewlett-Packard (Waldbronn, Germany), and a silica reversed-phase column (220 \times 4.6-mm i.d.; 5- μm particle size) (Brownlee Spheri 5, Foster City, CA) operated at room temperature. The retention times and areas were measured using Hewlett-Packard HPLC Chemstation (DOS series) software. The flow rate was 1.0 mL/min, and the wavelength used for quantitation was 305 nm.

The mobile phase consisted of 40% (v/v) phosphate buffer in acetonitrile. The phosphate buffer was prepared by dissolution of 1.15 g ammonium dihydrogen phosphate in 1.0 L distilled water. Triethylamine (1.0 mL) was added, giving a final unadjusted pH of approximately 6.8.

Tris buffer for liver digestion was prepared by dissolution of 121 g tris(hydroxymethyl)methylamine in 1.0 L of distilled water, and the pH was adjusted to 7.0 with concentrated sulfuric acid.

Quantitation was performed by the addition of known amounts of zopiclone standard along with the appropriate amount of internal standard into blank transfusion blood or blank digested liver and by the preparation of a calibration curve. Unknown concentrations were calculated using linear regression.

Procedure

Working standards of zopiclone and its internal standard were prepared by dilution of the stock standard solutions. The extraction procedure for blood was as described in our previous paper (5). Briefly, to 1.0 mL of blood (sample, blood standard, or blank) were added 85 ng of internal standard (10 μL of a 8.5- $\mu\text{g}/\text{mL}$ solution) followed by 0.3 mL saturated borate buffer (pH 9) and 3 mL *n*-butyl chloride. The resultant mixture was vortex mixed for 2 min and centrifuged, and the upper organic phase was transferred to a clean test tube. The extraction was repeated with a further 3 mL *n*-butyl chloride, and the combined organic extracts were evaporated to dryness under nitrogen at 40°C and reconstituted in 100 μL acetonitrile. A 10–30- μL portion of the reconstituted extract was subjected to HPLC analysis.

Homogenized liver tissue (10 g) was digested by adding 40 mL 1.0M tris buffer and 10 mg of Subtilisin–Carlsberg protease and stirring overnight at room temperature. The resultant homogenate was filtered through glass wool, and a 1-mL aliquot was initially extracted similarly to blood. After evaporation of the *n*-butyl chloride, 0.5 mL hexane was added, and the solution was extracted with two por-

tions of 1 mL acetonitrile. The combined acetonitrile extracts were evaporated to dryness, reconstituted, and analyzed similarly to blood.

Results and Discussion

The concentration of zopiclone and other drugs determined in these cases is listed in Table I. Cases I and II were fatal overdoses of zopiclone, whereas in Cases III and IV, zopiclone was used therapeutically.

Initially, attempts to analyze zopiclone in liver were performed using our standard liver digestion technique, which involved digestion using a protease enzyme at 55°C for 3 h at pH 10.5 (6). However, no zopiclone was detected in Case I and II liver samples. Subsequent experiments with spiked liver samples showed zopiclone was completely degraded under these conditions. This problem was alleviated with digestion at pH 7, which gave good recoveries of zopiclone from spiked liver. The detection limit of zopiclone in digested liver was approximately 0.01 mg/L (0.05 mg/kg).

The pharmacological actions of zopiclone are similar to benzodiazepines—a sedative–hypnotic action together with anti-convulsant, muscle-relaxant, and anti-aggressive properties (7). After a single oral dose of 7.5 mg to five subjects, plasma concentrations ranged from 18.4 to 35.2 $\mu\text{g}/\text{L}$ at 9 h postdose. Zopiclone was not detectable in plasma at 24 h postdose (8). Cases III and IV represented situations where the deceased died of other causes, so the zopiclone concentrations were presumably therapeutic for blood. The whole blood therapeutic concentrations appeared to be similar to therapeutic plasma concentrations.

The blood concentrations in Cases I and II were significantly higher than any previously reported. A possible explanation for the high blood concentration and relatively low liver concentration in Case I is rapid death of the deceased. Analogously, the

Table I. Drug Concentrations

Case	Tissue	Concentration*		
		zopiclone	ethanol	other drugs
I	Femoral blood	3.5	ND [†]	Quinine, 3.2 (Th) [‡]
	Carotid blood	3.9	–	–
	Liver	0.81	–	–
	Stomach contents	13.5	–	–
II	Subclavian blood	1.4	185	Fenfluramine, 0.08 (Th)
	Liver	8.7	–	–
	Stomach contents	133	–	–
III	Aortic blood	0.05	ND	Paroxetine, 0.47 (f) [‡]
	Liver	ND	–	–
IV	Cardiac blood	0.02	ND	ND
	Liver	ND	–	–

* Ethanol in mg/dL, stomach contents in mg, and all other concentrations in mg/L or mg/kg.

[†] ND = Not detected.

[‡] Th = Therapeutic; f = fatal.

lower blood concentration and relatively high liver concentration in Case II may be due to slower death of the deceased, which allowed time for the liver zopiclone concentration to increase. No zopiclone was detected in the liver in the two cases of presumed therapeutic zopiclone use. The blood and liver concentrations of zopiclone were similar to those reported by Pounder and Davies (4) in a case of zopiclone overdose. Also, in agreement with Pounder and Davies' data, there did not appear to be any significant postmortem redistribution of zopiclone in Case I. The zopiclone blood concentrations in Cases I and II were at least 30 times the expected plasma concentration after single oral dosage, and this together with the case histories strongly suggested that death was due to zopiclone poisoning. The blood ethanol concentration of 185 mg/dL may well be a contributing factor in Case II, although there have been claims that zopiclone, in contrast to benzodiazepines, does not have additive central nervous system depressant effects with ethanol (9).

The effects of coadministration of therapeutic amounts of quinine (Case I) and fenfluramine (Case II) are difficult to predict. There do not appear, however, to be any obvious potentiating effects between zopiclone and the other drugs in Cases I and II (10).

The blood and liver in Cases I and II were also screened for the presence of the *N*-oxide and the *N*-desmethyl metabolites of zopiclone, but none were detected. These metabolites have been detected in urine but not in plasma after therapeutic doses (11). It would seem therefore that, even after zopiclone overdose, there are no measurable concentrations of zopiclone metabolites in blood and liver.

The data presented demonstrated that there are no apparent clear fatal concentrations of zopiclone in blood and liver. From our preliminary data, it did appear, however, that shorter survival times resulted in higher blood concentrations and lower liver concentrations, whereas decedents who survived longer periods of time had blood concentrations that were significantly lower. However, additional cases of therapeutic and fatal zopiclone use would need to be evaluated before any firm conclusions could be reached. Liver concentrations, in combination with blood concentrations and survival times, are probably the most reliable data for determining whether zopiclone was the cause of death.

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