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Case Report

Mitragynine 'Kratom' Related Fatality: A Case Report with Postmortem Concentrations

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A 24-year-old man whose medical history was significant for alcohol abuse and depression was found unresponsive in bed. He had several prior suicide attempts with 'pills' and had also been hospitalized for an accidental overdose on a previous occasion. Autopsy findings were unremarkable apart from pulmonary edema and congestion, and urinary retention. Postmortem peripheral blood initially screened positive for mitragynine 'Kratom' (by routine alkaline drug screen by gas chromatography–mass spectrometry, GC–MS), which was subsequently confirmed by a specific GC–MS selective ion mode analysis following solid-phase extraction. Concentrations were determined in the peripheral blood (0.23 mg/L), central blood (0.19 mg/L), liver (0.43 mg/kg), vitreous (<0.05 mg/L), urine (0.37 mg/L) and was not detected in the gastric. Therapeutic concentrations of venlafaxine, diphenhydramine and mirtazapine were also detected together with a negligible ethanol of 0.02% (w/v). The results are discussed in relation to previous cases of toxicity, and the lack of potential for mitragynine postmortem redistribution.

Introduction

Mitragynine (Figure 1) is the primary indole alkaloid extracted from the leaves of the rubiaceous plant *Mitragyna speciosa*. Kratom, which may contain over 25 different alkaloids, is the name given to the leaves and preparations from the species. It is commonly chewed, smoked or brewed as tea in Southeast Asia where it has been used for hundreds of years. It was reported in 2005 to have significant abuse potential in the USA. (1). Marketed as a euphoriant dietary supplement, 'incense' or 'legal opioid', at low doses it has been described to produce stimulant effects, while at higher doses it produces sedative and euphoric effects. It is sold as tablets, capsules, concentrated extracts or chopped leaves as 'Kratom' or 'Krypton' (2). The effects are generally noted within 5–10 min of ingestion and may last up to 6 h (3).

The pharmacology and toxicity of mitragynine are not fully understood. However, the coca-like stimulant and opium-like sedative effects are most likely due to its dual binding to α-adrenergic and opioid-μ receptors (4). Case studies have reported individuals self-medicating with Kratom for pain management being admitted to the hospital following seizures (5, 6). Other adverse side effects reported by users include nausea, vomiting, diarrhea and tolerance development. Withdrawal symptoms were also reported upon terminating use (2). Users who combine the drug with central nervous system depressants can experience respiratory depression (3).

Despite descriptions of toxicity and even drug-related deaths, there are limited reports of biological mitragynine concentrations. A urine concentration of 0.167 mg/L was found in a man suffering a seizure following Kratom use (5). Kronstrand and colleagues (7) presented postmortem blood concentrations ranging from 0.02 to 0.18 μg/g in nine cases of accidental intoxication where both mitragynine and *O*-desmethyltramadol were detected. A fatality, attributed to propylhexedrine toxicity, found mitragynine together with low concentrations of morphine, promethazine and acetaminophen (8). Their investigation of tissue distribution revealed the highest mitragynine concentration in urine (1.2 mg/L) with other tissues being heart blood (0.39 mg/L), liver (0.12 mg/kg), vitreous (0.15 mg/L), kidney (0.16 mg/kg), spleen (0.18 mg/kg), lung (0.01 mg/kg) and bile (0.48 mg/mL). Another mixed drug-related fatality was reported where mitragynine (postmortem blood concentration of 0.60 mg/L) was considered a contributing factor together with dextromethorphan, diphenhydramine, temazepam and 7-aminoclonazepam (9).

We describe a death attributed to mixed drug toxicity—primarily mitragynine. The current report revealed the detection of mitragynine by routine toxicology screening, describes a specific gas chromatography–mass spectrometry selective ion mode (GC–MS SIM) analysis and presents the postmortem concentrations in peripheral blood, central blood, liver, vitreous, urine and gastric contents.

Methods

Case report

The decedent was a 24-year-old man whose medical history was significant for alcohol abuse and depression. He had been drinking alcohol since age 15, had several suicide attempts with 'pills' and had been hospitalized for an accidental overdose. His mother spoke with him by phone the night before his death and he sounded fine to her and he had no complaints. Less than 1 h later a friend picked him up from his residence and described him as appearing 'out of it', tired and depressed. They drove to the friend's residence and watched television for about an hour, and during that time the decedent reportedly consumed a glass of wine and a beer. He then took a 'sleeping pill' and they retired to bed at approximately midnight. At 03:00 h, the friend awoke because the decedent was encroaching on his sleeping space, but could not move him and found that he was cold and unresponsive. The friend called rescuers at 03:03 h, moved the decedent to the floor and started chest compressions. Medics arrived at 03:07 h and initiated advanced resuscitative efforts. Resuscitation was unsuccessful and he was declared dead at 03:30 h. Vomitus was noted on the bedding and around the decedent's head on the floor. The decedent's belongings contained prescription bottles for venlafaxine (75 mg), mirtazapine (15 mg) and omeprazole (20 mg). Pill counts of the remaining medications, from the bottles collected at the scene, suggested that he had taken the amounts prescribed—or even less than prescribed. A loose loperamide caplet (2 mg) was also among his possessions.

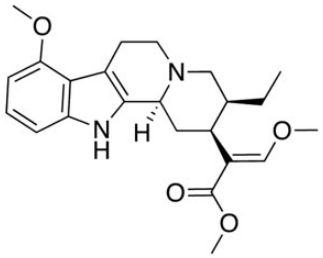

Figure 1. Structure of mitragynine.

An autopsy was performed (beginning 29.5 h after death was declared) and documented pulmonary edema and congestion (950 g, right lung; 890 g, left lung), moderate urinary retention (300 mL) and no natural disease or trauma.

Postmortem specimen collection

All specimens analyzed were collected at autopsy at the San Diego County Medical Examiner's Office. Peripheral blood (~20 mL) was drawn from the left common iliac vein (blood returning from the leg and visually identified in the pelvis at autopsy) and stored in standard glass tubes containing sodium fluoride (100 mg) and potassium oxalate (20 mg). Central blood was collected from the intra-pericardial inferior vena cava and placed in identical tubes. Vitreous humor was withdrawn from the eye with a syringe and stored in a glass tube without preservative. Urine was collected into in a 4-ounce container without preservative. Sections of the right lobe of liver were collected and also stored in a 4-ounce container without preservative. The entire gastric contents were collected and stored in a new 4-ounce container without preservative. All samples were stored at 4°C until analyzed.

Toxicology

A comprehensive toxicological screening regimen was requested and performed. Postmortem blood was screened for alcohol and volatile compounds (GC-FID headspace), drugs of abuse by enzyme linked immunoassay (ELISA) (cocaine metabolite, opiates, methamphetamine, benzodiazepines, cannabinoids, fentanyl, phencyclidine, oxycodone, methadone, zolpidem, carisoprodol and buprenorphine) (Immunalysis Inc., Pomona, CA), an alkaline drug screen by GC-MS following solid-phase extraction (SPE) and an acid/neutral drug screen with HPLC-photodiode array detection following specimen precipitation with acetonitrile. Where necessary, positive results were confirmed and quantified by subsequent and specific techniques.

Alkaline drug screen (GC-MS)

The drug screening procedure (10) has been utilized by this laboratory for over 6 years. It consists of a routine SPE technique utilizing Trace J extraction cartridges (SPEWare, Baldwin Park, CA). Two milliliters of calibrators, controls and casework were extracted after the addition of cyclizine (1.0 μg: internal standard) and ascorbic acid (200 μL, 2% solution). Samples were then precipitated with zinc sulfate (5 mL, 5% methanolic solution) and treated with sodium acetate buffer (4 mL, pH 6.0). SPE cartridges were pre-treated with 3 mL of methanol, 3 mL deionized water and 2 mL sodium acetate buffer before the addition of samples. Following the extraction of samples, the SPE cartridges were washed with 3 mL deionized water, 2 mL acetic acid (0.1 M) and 3 mL methanol. Cartridges were dried for 3 min and the specimens were eluted with dichloromethane/isopropanol/ammonium hydroxide solution (78/20/2). Samples were then evaporated (30°C, under a stream of nitrogen) and reconstituted with 150 μL of ethyl acetate. One microliter (splitless) of each extract was then injected on to the 7890A GC-MS system (Agilent Technologies, Santa Clara, CA) to attain separation and identification of alkaline drugs. A 15 m, 0.25 mm diameter and 0.25 μm film thickness, analytical column (Zebron, ZB-5MS) (Phenomenex, Torrance, CA) was used with helium as the carrier gas (1.1 mL/min). The inlet temperature of the gas chromatograph was 250°C, and the oven temperature was initially 85°C, ramped 40°C/min up to 170°C (held 4 min), then 40°C/min to 190°C (held 5 min) and finally 10°C/min up to 300°C (held 7 min). The MS Aux was 280°C. The mass selective detector (5975C, Agilent Technologies) was set in scan mode with a solvent delay of 2.64 min. Peak identification was determined by relative retention time (relative to the internal standard) and then mass spectral matching from a commercial MS library (at least 70% match). Several commonly detected compounds with established five-point calibration curves (0.10 mg/L to 1.0 mg/L) were extracted with each analytical run. Other compounds were extracted using a single point calibrator to provide concentration estimates for casework. In casework, concentrations of compounds when detected at therapeutic levels may be reported directly from this procedure if the identification and quantitation criteria were attained: relative retention time match; mass spectral library match; the respective positive control concentration within 20% of the target concentration and the calibration curve meets acceptable criteria ($r^2 \geq 0.98$). Otherwise, compounds are subsequently confirmed and quantitated by an appropriately validated second and different technique. The retention time of mitragynine under the listed conditions was 23.3 min.

Mitragynine confirmation and quantitation analysis (GC-MS SIM)

Materials

Solvents (dichloromethane, methanol, ethyl acetate and isopropanol) were EMD Chemicals OmniSolv grade, purchase through VWR International (Radnor, PA). Ammonium hydroxide (ACS) and glacial acetic acid (ACS) were obtained from VWR International. Zinc sulfate heptahydrate (Certified ACS) was obtained from Fisher Scientific (Pittsburgh, PA), and anhydrous sodium acetate (GR ACS Mallinckrodt) was obtained from VWR Inc. Mitragynine and mitragynine-D3 were obtained from Cerilliant (Round Rock, TX). SPE columns were Trace-J from SPEWare Corp. (Baldwin Park, CA). GC column Zebron-5MS was purchased from Phenomenex (Torrance, CA).

Aqueous working standard containing 1.0 mg/L of mitragynine and internal standard containing 1.0 mg/L of mitragynine-D3 were prepared. A linear calibration curve from 0.05 to 1.0 mg/L using six calibrators (0.05, 0.10, 0.25, 0.50, 0.75 and 1.0 mg/L) was established by diluting the working standard. Linearity was achieved by applying a linear least squares

calibration curve ($r^2 \geq 0.99$). All calibrators were prepared in deionized water. Whole-blood control samples containing 0.20, 0.40 and 0.80 mg/L of mitragynine were independently prepared using porcine blood as a matrix and were extracted with the calibrators and case specimens. Additionally, both blank (extract containing no additives) and negative control (extract containing only internal standard) specimens were extracted to confirm the lack of interference and/or contamination.

Extraction

Mitragynine was extracted using a SPE procedure. A 1.0-mL sample was extracted for all calibrators, controls and casework (blood, vitreous, liver, urine and gastric). Working internal standard (0.5 mL, mitragynine-D3) was added to all tubes. Five milliliters of 5% zinc sulfate/methanol solution were added to each tube. The tubes were then vortexed and centrifuged at ~2,400 g for 10 min. The supernatant was transferred to new tubes and buffered with 4 mL 0.1 M sodium acetate buffer (pH 6.0). The SPE columns were conditioned by adding sequentially 3 mL of methanol, 3 mL of deionized water and 2 mL acetate buffer (pH 6.0). The buffered supernatant was added to the SPE columns and allowed to flow through at 2–5 mL/min. Columns were then washed by adding sequentially 3 mL of deionized water, 2 mL of 0.1 M acetic acid and 3 mL of methanol. Columns were dried at maximum pressure (40 psi nitrogen) for 3 min. Compounds were eluted with 2 mL elution solvent (dichloromethane/isopropanol/ammonium hydroxide, 78/20/2) using gravity flow. The extracts were evaporated at 30°C under a stream of nitrogen until dry, reconstituted with 50 μL ethyl acetate, mixed by vortexing and then transferred to autosampler vials.

Instrumentation

One microliter splitless injections were made onto a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA). The analytical column was a Zebron ZB-5MS with 15 m, 0.25 mm diameter, 0.25 μm film thickness (Phenomenex, Torrance, CA), with helium as the carrier gas (1.1 mL/min). The oven was programmed to an initial temperature of 100°C. The temperature was then immediately ramped 20°C/min to 300°C and held for 3.5 min. The total chromatography time per injection was 13.5 min. A 5975C MSD (Agilent Technologies) operating in SIM was utilized for detection. The GC–MS was controlled by Chemstation software. The mitragynine retention time under these conditions was 11.4 min. Ions monitored for mitragynine included 214*, 397 and 383, while those for the mitragynine-D3 internal standard were 217*, 400 and 386 (*quantitive ions).

Validation

The limit of detection (LOD) was 0.03 mg/L and limit of quantitation (LOQ) (determined from the lowest calibration concentration) was 0.05 mg/L. Control samples were prepared independently at 0.20, 0.40 and 0.80 mg/L in whole-blood and measured 0.20, 0.40 and 0.81 mg/L, respectively. Any potentially significant matrix extraction effects were negated by the use of deuterated internal standard (mitragynine-D3). This was substantiated by preparation and analysis of comparable control specimens (0.40 mg/L) prepared in water and liver—levels of 0.38 mg/L and 0.39 mg/kg were attained for water and liver, respectively.

Table I
Toxicology Data: Peripheral Blood Drug Concentrations

Drug	Concentration (mg/L)
Venlafaxine	1.1 (G < 1 mg)
O-Desmethylvenlafaxine	1.6
Diphenhydramine	0.45
Mirtazapine	0.24
Ethanol	0.02[a]

G, gastric contents.
[a]Concentration in g/dL.

Table II
Mitragynine Postmortem Tissue Distribution

Tissue	Mitragynine (mg/L)
Peripheral blood	0.23
Central blood	0.19
Liver	0.43[a]
Vitreous	<0.05
Urine	0.37
Gastric	ND

[a]Concentration in mg/kg.

Results and discussion

The initial screening tests confirmed and quantified ethanol (alcohol and volatile screen/quantitation), diphenhydramine and mirtazapine (alkaline drug screen). Venlafaxine and *O*-desmethylvenlafaxine, initially detected by the alkaline drug screen, were quantified by GC-NPD following a previously described procedure (11). The ELISA screen was negative. Concentrations are presented in Table I.

Mitragynine was initially identified from the SWGDRUG Mass Spectral Library (http://www.swgdrug.org) with the GC-MS alkaline drug screen following the extraction of peripheral blood—subsequently confirmed with extraction and a full mass spectral scan of a pure stock of the compound. It was detected at a retention time of 23.3 min (internal standard cyclizine at 8.8 min) with ions of 214, 397, 398, 383, 199, 186, 200 and 269. Later specific GC-MS SIM quantitation (method described above) confirmed concentrations in the peripheral blood, central blood, liver, vitreous and urine which are presented in Table II. It was not detected in the gastric contents. The blood concentrations (peripheral blood 0.23 mg/L and central blood 0.19 mg/L) were within the concentration range of prior reported deaths (7–9).

Similar to the previous reports, this current case described a death resulting from the use of mitragynine while associated with the administration of other medications. Both of the antidepressants detected (venlafaxine and mirtazapine) affect the serotonergic and noradrenergic systems, and diphenhydramine (a first-generation antihistamine) is a potent antagonist to acetylcholine in muscarinic receptors. It was previously concluded that the addition of the potent μ-opioid receptor agonist *O*-desmethyltramadol to powdered leaves from Kratom contributed to nine unintentional deaths (7). One report described a case involving the stimulant propylhexedrine—at a concentration reported in fatalities—concurrently with low concentrations of morphine, promethazine and acetaminophen (8), then another implicated mitragynine concomitant with the benzodiazepines 7-aminoclonazepam (metabolite of clonazepam) and temazepam, together with dextromethorphan and diphenhydramine (9). In reviewing all these cases, we concur with, and

reiterate, the statement from Kronstrand and colleagues (7) who recognized that: 'Kratom (*or mitragynine*) is not as harmless as is often described on Internet websites'. It may exert potentially serious additive effects to numerous endogenous receptors with central nervous system depressant activity.

After a comprehensive toxicology screening, the only other compounds detected were therapeutic concentrations of venlafaxine, diphenhydramine, mirtazapine and ethanol. Based on the circumstances, autopsy findings, histology and toxicology results, the cause of death was certified due to mixed drug intoxication—primarily mitragynine. Despite the detection of the other compounds at therapeutic concentrations, they were considered to have additive toxic central nervous system effects in the presence of mitragynine and were therefore felt to have contributed toward the death. The manner of death was certified as accident. Although the decedent had a history of suicide attempts, he also had a history of substance abuse, prior accidental overdose and no evidence of recent suicidality. Furthermore, he had available much more medicine should he have intended to 'overdose' to die; therefore, the manner of death was classified as accident.

In the case reported herein, the central blood to peripheral blood (C/P) ratio was 0.83, and the liver to peripheral blood (L/P) ratio was 1.9 L/kg. These ratios suggest no potential for mitragynine postmortem redistribution (PMR): established on model criteria that C/P ratios <1.0 (12), and L/P ratios <5 L/kg (13, 14) indicate little to no propensity toward PMR. However, as this deduction results from a single observation, it should be viewed with caution.

In conclusion, the present case describes the distribution of postmortem mitragynine concentrations in a case where it was determined to contribute to death—together with therapeutic concentrations of venlafaxine, diphenhydramine, mirtazapine and alcohol. First confirmed by a routine alkaline GC-MS screen, concentrations were then quantified by a specific GC-MS SIM analysis. Mitragynine is not expected to be prone to substantial PMR.

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