Case Report

Application of LC–MS Analysis to a Colchicine Fatality

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Abstract

A 73-year-old man developed nausea, vomiting, and diarrhea 20–30 min after receiving a 1.0 mg intravenous dose of colchicine for the treatment of severe pain due to gouty arthritis in his physician's office. He was hospitalized 8 h later, and his condition deteriorated as he developed renal and respiratory failure. He subsequently died 10 h later, or a total of 18 h after he received the original 1 mg colchicine injection. The patient received a prescription for oral 0.6 mg colchicine tablets 8 days previously and consumed eight tablets during that period, an average of 0.6 mg/day (42 of 50 tablets remained at the time of death). Colchicine concentrations were measured by liquid chromatography–mass spectrometry in selected ion monitoring mode using positive ionization. Chromatography was performed using an Eclipse XDB C_8 analytical column (30 mm × 2.1-mm i.d., 3-µm particle size) and a programmed mobile phase consisting of 50mM pH 4 ammonium acetate buffer and acetonitrile. Colchicine concentrations were as follows: 50 µg/L in cardiac blood, 10 µg/L in vitreous humor, 575 µg/kg in liver, 12,000 µg/L in bile, and 4.4 µg in 60 g received gastric contents (estimated total gastric contents 100 g). The cause of death was ruled to be "acute colchicine toxicity" and the manner of death "accidental."

Introduction

Colchicine is a potent alkaloid that occurs in flowers of the autumn crocus (meadow saffron, *Colchicum autumnale*). It has reputedly been used since at least 600 A.D. for the treatment of gout, its most common use to this day (1). Colchicine has been described as anti-inflammatory, although this action is relatively specific for acute attacks of gouty arthritis and prophylaxis of the condition. Colchicine is a relatively potent inhibitor of mitosis, both in vitro and in vivo, and it has been investigated as an antineoplastic agent (2,3), for the treatment of amyloidosis in Familial Mediterranean Fever (4), and even for the treatment of severe constipation where other, less toxic agents are ineffective (5). For the treatment of gouty arthritis, colchicine is generally administered in doses of 0.3 to 1.2 mg, usually orally, although it may be administered intravenously. Although colchicine has been regarded as relatively safe for the prophylaxis of gouty arthritis if the recommended doses are not exceeded, it is not unusual for it to cause nausea, vomiting, and diarrhea. The treatment of acute gout attacks with intravenous colchicine, although highly effective in most patients, is increasingly being viewed with caution because of the potential for severe side effects (6). There are several reports of colchicine toxicity in the literature, most attributed to suicidal overdose, although blood or tissue concentrations have only been reported in a relatively small number of cases.

Detection and accurate measurement of colchicine in blood or other specimens has not been easy. The drug has nearly neutral properties and a molecular weight that causes it to elute very late on most gas chromatography (GC)-based drug-screening systems. Coupled with the very low concentrations expected, even after overdosage, colchicine is extremely difficult to detect in blood without a targeted analysis. Spectrofluorimetry (7), radioimmunoassay (8), and gas chromatography–mass spectrometry (GC–MS) (9) have been used for the determination of colchicine, but high-performance liquid chromatography (HPLC) has been the most commonly used technique (10–14). However, non-MS methods lack the desired specificity for forensic applications, and liquid chromatography in combination with mass spectrometry (LC–MS) is the preferred method. One method has been published for the determination of colchicine by ion spray LC–MS (15).

We present a case in which colchicine has been measured by LC–MS with API-electrospray ionization in postmortem blood and other tissues and fluids. In our case, the concentrations of colchicine are consistent with previously reported overdoses, although the case history strongly indicates that colchicine may have accumulated to toxic levels over a period of only a few days.

Case History

This 73-year-old, 56-kg man with a history of atrial fibrillation, severe arthritis, and pseudo-gout, died 18 h after receiving a 1-mg intravenous dose of colchicine in his doctor's office. According to his wife, about 20–30 min after receiving the dose, he complained of feeling unwell and of numbness of the lips, followed by the commencement of vomiting and diarrhea. He was taken to hospital 8 h later. His condition continued to deterio-

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rate in hospital, with development of renal and respiratory failure, followed by death about 10 h after admission.

Twenty days before his death, the patient had been prescribed oral colchicine 0.3 mg daily, with instructions to increase the dose to 0.6 mg daily if he could tolerate the side effects. However, he did not fill the prescription until 8 days prior to death, when he obtained 50 0.6-mg tablets; 42 remained unused at the time of his death. He apparently did not obtain adequate relief from the prescribed oral dosage and therefore received the intravenous 1-mg dose the day prior to his death. The patient was also prescribed digoxin and warfarin. His serum digoxin determined in hospital was 0.9 µg/L, well within the therapeutic range of 0.5–2.2 µg/L. Hematology results showed his clotting time was within the normal range, indicating his warfarin dosage was properly adjusted.

The autopsy revealed an enlarged (500 g) heart with atherosclerotic coronary artery disease with 75–80% stenosis of the left anterior descending coronary artery and 75–80% stenosis of the right coronary artery, transmural myocardial scarring of the posterior wall of the left ventricle, pulmonary edema with congestion, and aortic atherosclerosis. The left lung had been previously surgically excised; the right lung weighed 727 g. Postmortem cardiac blood, vitreous humor, liver, bile, and gastric contents were collected for toxicology testing. Antemortem specimens were not available.

Liver function tests were not performed during hospitalization. A concentration of urea that was only marginally elevated (9.3 mmol/L; normal 2.5–8.0) and a normal concentration of creatinine (102 µmol/L; normal 45–125) indicated grossly normal renal function at the time of admission to hospital.

Experimental

Reagents and standards

Colchicine and demecolcine were purchased from Sigma Chemical Co. (Oakville, ON, Canada). All chemicals were reagent grade or better and used without further purification. ELISA kits were purchased from Diagnostix Ltd. (Mississauga, ON, Canada). Fluorescence polarization immunoassay (FPIA) kits were purchased from Abbott Diagnostics (Chicago, IL). Solvents were purchased as distilled-in-glass grade and used without further purification.

Drug screening

Extracts of blood for bases and neutrals and for strong bases were screened by GC with nitrogen-phosphorus detection (NPD), electron capture detection, and GC–MS in total ion mode. Blood was also diluted 1:10 with deionized water and subjected to a panel of ELISA screening tests for barbiturates, benzodiazepines, and opiates using a Hyperion (Miami, FL) Hyprep analyzer and 4-Plus MicroReader. Abbott TDx kits were used to screen for acetaminophen and salicylate.

Morphine quantitation

Morphine analysis was performed with and without hydrolysis of the glucuronides with β-glucuronidase. Liquid–liquid extraction was performed with ethyl acetate after buffering with pH 8.7 sodium carbonate/bicarbonate solution. Morphine-d_3 was used as the internal standard. Extracts were derivatized by *N*-methyl-bis-trifluoroacetamide (MBTFA, to form trifluoracetyl derivatives). A six-point blood based calibration was prepared. Urine and blood-based morphine-3-glucuronide controls were used to verify the efficiency of the hydrolysis step and accuracy of the calibration. Extracts were analyzed by GC–MS in selected ion monitoring (SIM) mode using electron impact positive ionization.

Colchicine method

Instrumentation. An Agilent (Palo Alto, CA) model G1946B LC–MS system was used, consisting of a degasser, a binary pump, a thermostated column compartment, autosampler, and an MS operated with an API-electrospray source set to positive ionization mode. The following MS parameters were set: drying gas temperature 350°C, fragmentor voltage 90V, nitrogen drying gas 12 L/min, nebulizer pressure 35 psi, and capillary voltage 3000V.

The 1100 series LC used an Eclipse XDB C_8 analytical column

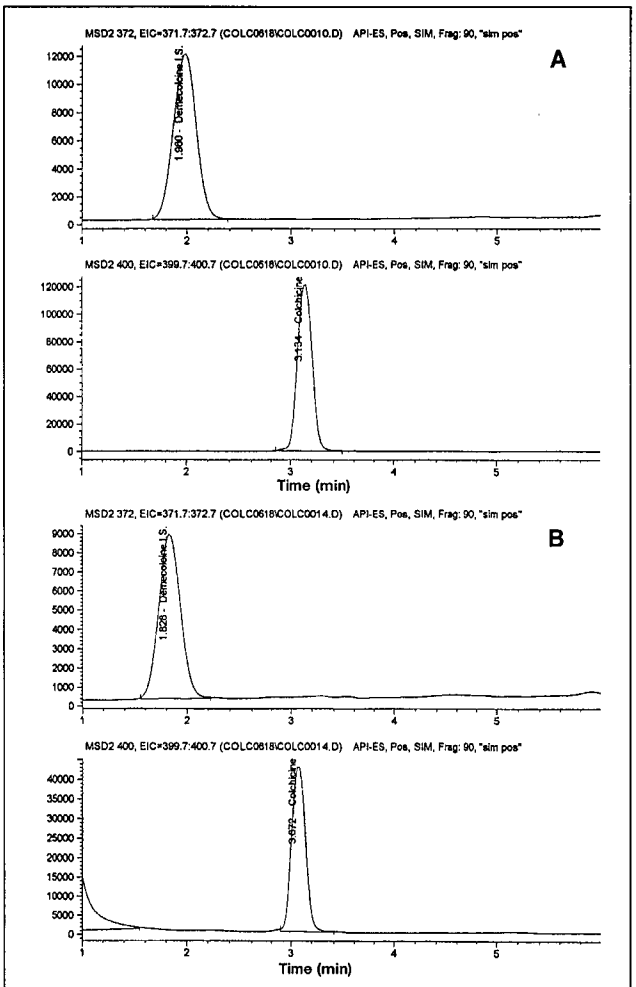

Figure 1. LC–MS SIM chromatograms for A, colchicine 100 µg/L calibrator and B, case blood (*m/z* 400 colchicine quantitating ion; *m/z* 372 demecolcine internal standard).

(30 mm × 2.1-mm i.d., 3-µm particle size, Agilent) using a C_8 guard column (4 mm × 2 mm cartridge, Phenomenex, Torrance, CA). The mobile phase was programmed as follows: solvent A 50mM pH 4 ammonium acetate buffer with 5% acetonitrile, solvent B acetonitrile; 90% A initially, ramping to 70% A until 3.0 min, held until 4.0 min, ramping back to 90% A at 5.0 min with equilibration at 90% A for an additional 3 min. The column flow was 0.5 mL/min initially, ramped to 0.75 mL/min at 3.0 min, and held for an additional min. The column compartment was ramped from 25 to 33°C during the run in order to enhance chromatography. Under these conditions, the colchicine eluted at about 3.1 min and the demecolcine internal standard at 1.9 min.

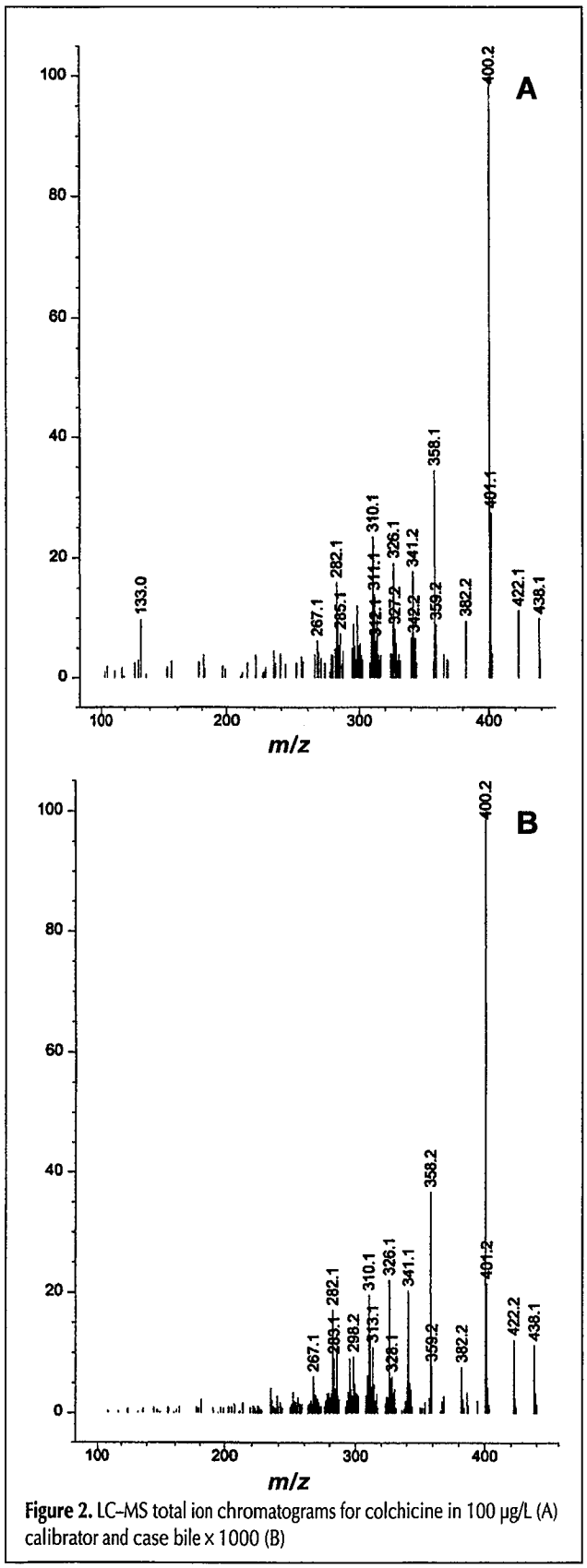

Figure 2. LC–MS total ion chromatograms for colchicine in 100 µg/L (A) calibrator and case bile x 1000 (B)

Extraction. To whole blood or other specimen (1 mL), 0.1 mL internal standard solution (demecolcine 10 ng/100 µL methanol) and 1 mL sodium bicarbonate (1M) were added. The solution was extracted with 5 mL dichloromethane and mixed by rotation for 15 min. The phases were separated by centrifugation for 10 min, transferred to conical vials, and the extract concentrated to dryness under nitrogen at 60°C. The residue was reconstituted in 60 µL acetonitrile/acetate buffer (1:4) and 10 µL analyzed by LC–MS.

Quantitation. Seven calibrators were prepared in sheep blood (range 1–100 µg/L), plus a control that was independently prepared using a separate weighing of the same stock powder at a target concentration of 5 µg/L. Quantitation was based on monitoring the quasi-molecular ions for colchicine (*m/z* 400, M+H) and internal standard (demecolcine; *m/z* 372, M+H). The calibration gave an r^2 value of 0.9992 and all calibrators were within 10% of nominal values when read against the graph. The limit of detection (LOD) for the assay was estimated to be less than 0.5 µg/L. The control read within 20% of the target value. Example chromatograms are shown in Figure 1. Specimens were diluted as necessary with DIW (bile × 1000 and liver × 40 because of high concentrations of colchicine; vitreous was diluted fourfold because of low specimen volume).

Confirmation. Extracts of the 100-ng/mL calibrator and bile diluted 1:100 were reinjected on the LC–MS under conditions similar to those used for the quantitation, except that the MS was set to full scan and the fragmentor voltage increased to 140V to produce diagnostic mass spectra. In the spectrum for colchicine (Figure 2), adduct ions are present at *m/z* 422 (M+Na) and 438 (M+K).

Results

Routine drug screening by GC–NPD and GC–MS revealed the presence of low concentrations of diphenhydramine and metoclopramide, which was consistent with doses charted and administered during hospitalization. The ELISA screen was positive for opiates, and morphine was subsequently confirmed and quantitated by GC–MS. Unconjugated morphine in blood was 69 µg/L and total morphine 115 µg/L, which were also consistent with the prescribed doses. Based on the medical history, an LC–MS assay for colchicine was developed and the following concentrations measured: 50 µg/L in cardiac blood, 10 µg/L in vitreous, 575 µg/kg in liver, 12,000 µg/L in bile, and 4.4 µg in 60 g in gastric contents received (estimated total gastric contents 100 g).

Discussion

The medical history strongly suggested that this man was suffering from colchicine toxicity on his admission to hospital, and that the colchicine played a major role in the decline in his medical condition and ultimate death. The medical progression in our case is consistent with that previously reported for colchicine caused fatalities (16–18).

Plasma concentrations of colchicine after single therapeutic concentrations are relatively low; typically less than 10 ug/L. In one study, single oral 1mg doses given to healthy male volunteers resulted in an average peak plasma concentration of 5.5 µg/L (range 4.0–7.6) after 1 h, which by 24 h had declined to an average of 0.4 µg/L with a half-life of 30 h (19). In a separate study, 1-mg doses of colchicine given orally to 6 healthy males for 15 days resulted in steady state trough concentrations averaging 0.8 µg/L, declining after the last dose with an elimination half-life of 58 h (20). After an intravenous dose of 2 mg given to 16 patients, colchicine plasma levels averaged 11.4 µg/L (range 4.5–33) after 15 min, declining with a distribution half-life of 19 min (21).

Somewhat surprisingly, several, mostly suicidal, cases of colchicine overdose in which antemortem or postmortem concentrations were measured have been reported. In one case, a 45-year-old woman deliberately ingested 7.5 mg colchicine and died 45 h later. Her plasma colchicine concentration was 21 ug/L 6 h after ingestion, falling to less than 5 µg/L after 24 h (10). In another case, a 39-year-old woman died 40 h after ingesting 20 mg colchicine; the plasma concentration was 250 µg/L 2 h after ingestion but was not detected in the postmortem blood (22). A more extensive distribution was measured in a 42-year-old man who died after ingestion of an unknown quantity of colchicine (blood 62 µg/L; vitreous 20 µg/L; liver 12 µg/kg; bile 2921 µg/L; urine 1024 µg/L) (13). Another colchicine overdose in a 42-year-old man resulted in his death after a period of hospitalization. The antemortem plasma colchicine concentration was 4.5 µg/L 24 h after admission; postmortem concentrations were as follows: 396 µg/kg in kidney, 347 µg/kg liver, 334 µg/kg in heart, 58 µg/kg in lung, and 5 µg/kg in brain (14). An apparent overdose of colchicine in a 45-year-old man who had been suffering from nausea and vomiting for 2–3 days prior to his eventual admission to hospital, resulted in his death 33 h later. The postmortem plasma colchicine concentration was estimated as 60 µg/L 3.3 h after admission, although it was not detected in later antemortem specimens; 30 µg was detected in postmortem blood and 4200 µg/L in bile, but any colchicine present in the liver or vitreous humor was less than the assay limit of detection (LOD < 5 µg/L) (12). Two unrelated colchicine homicides have been reported where antemortem serum colchicine concentrations were 160 µg/L and 170 µg/L, respectively; the method of analysis was not stated. Both individuals had a period of survival in hospital before succumbing (23).

In our case, the colchicine concentrations in the blood, and even the liver, bile, and vitreous humor are consistent with concentrations reported in overdose cases. Even if it is considered that with a half-life of 23–58 h and a volume of distribution of 5.0–8.5 L/kg, colchicine is a prime candidate for postmortem redistribution, concentrations in specimens other than blood are still considerably higher than would be expected following the prescribed doses. If the prescribed amount of colchicine (50 0.6-mg tablets) and the amount remaining at death (42 tablets) are reconciled, this man would have consumed 8 tablets over the same number of days, a dose of 0.6 mg per day. This is at the low-end of the recommended oral dose of 0.6–1.2 mg/day. Given the medication count, consumption of a suicidal overdose is highly unlikely. The physician did give the patient a 1.0-mg intravenous dose of colchicine in his surgery, apparently because he was not getting adequate relief from his oral dosage. Because of the timing of the onset of symptoms of colchicine toxicity (numbness, nausea, vomiting, and diarrhea), which started only 20–30 min after the intravenous dosage, it is very likely that the two events are related. However, the intravenous dosage of colchicine given was well within that recommended in the medical literature. Furthermore, in an otherwise healthy individual with normal metabolism and clearance, the contribution of the intravenous colchicine should have declined to less than 1 µg/L in blood by the time this man died. Similarly, even given the average 0.6 mg/day oral dose of colchicine, the blood concentrations would be expected to be well under 5 µg/L, before taking postmortem redistribution into account. However, an unusually prolonged half-life of colchicine has been reported in a previous colchicine intoxication (16).

To complicate matters, it was discovered that the intravenous injection of colchicine was prepared 10 months before by a local pharmacy which was equipped to prepare injectable solutions using sterile water and aseptic technique. A review of the pharmacy records, including the manner in which the solution was prepared (15 mg colchicine base added to 30 mL sterile water to produce 1 mg/2 mL injection), indicated the possibility of a pharmacy error was unlikely. The 15 mg colchicine weighing was checked by a second person during preparation of the injection. The physician stated that doses from this multidose vial were removed for use in other patients in the 10-month period before this patient, without any apparent adverse effects. Unfortunately, the remaining colchicine injection solution was discarded after the death and was therefore not available for analysis.

Conclusions

The postmortem findings and case history are consistent with the death of this man due to colchicine toxicity. However, there is no evidence that a deliberate overdose was given or consumed. Slowed metabolism and clearance are well known in the elderly, despite grossly normal renal and hepatic function. It is possible that the age of the patient and cardiac disease also played a role in his death. The medical cause of death was attributed to "acute colchicine toxicity" with "atherosclerotic cardiovascular disease" as a contributing factor and the manner of death "accidental."

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