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Colchicine poisoning: report of a fatal case with body fluid and post-mortem tissue analysis by high-performance liquid chromatography

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ABSTRACT: A case involving a suicide by the ingestion of colchicine tablets is presented. Liquid chromatography has been used to measure the drug level in blood and in post-mortem tissues of the patient (a 42-year-old man). Plasma concentration 24 h after ingestion was 4.5 ng/mL. On autopsy, the kidney showed the highest concentration (396 ng/g). High concentrations were also found in the liver (347 ng/g) and heart (334 ng/g). Low concentrations were detected in the lung (58 ng/g), muscle (10 ng/g) and brain (5 ng/g). Copyright © 1999 John Wiley & Sons, Ltd.

INTRODUCTION

Colchicine has been used in the therapy of acute attacks of gout arthritis for centuries (Nadius *et al.*, 1977). More recently, colchicine was shown to be effective in the prophylaxis of acute attacks of familial Mediterranean fever (Levy and Eliakim, 1990). Its use was extended to the treatment of immune or inflammatory diseases such as Beçhet's syndrome (Kershennobich *et al.*, 1973), recurrent pericardis (Millaire and Ducloux, 1991), primary biliary cirrhosis (Kaplan *et al.*, 1986) and systemic scleroderma (Alarcon-Segovia *et al.*, 1979).

Toxicity associated with colchicine therapy has been recognized since the first use of the plant extracts. Manifestations of toxicity may range from minor signs to fatality.

Although the early and reversible stage of colchicine toxicity (nausea and diarrhea) is well known to clinicians (Stapczynski *et al.*, 1981; Murray *et al.*, 1983), few reports exist in the toxicology literature describing the detection or monitoring of colchicine levels (Walaszek *et al.*, 1960; Jarvie *et al.*, 1979; Lhermitte *et al.*, 1985; Rochdi *et al.*, 1992). Before the last decade, no sensitive analytic technique was available for determination of plasma colchicine concentrations. Recent studies using sensitive radioimmunoassays have reported low plasma concentration (Thomas *et al.*, 1989). High-performance liquid chromatography (HPLC) has been used to measure colchicine in the body fluids of poisoned patients (Jarvie *et al.*, 1979; Caplan *et al.*, 1980; Lhermitte *et al.*, 1985; Kintz *et al.*, 1997), as have gas chromatography/mass spectrometry (GC/MS) methods (Clevenger *et al.*, 1991). This paper presents here a case of suicidal colchicine overdose and a method of extraction of colchicine from the organs, obtained post-mortem.

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MATERIALS AND METHODS

Case report

A 42-year-old male ingested in a suicide attempt an unknown amount of colchicine, allopurinol, steroid and benzodiazepine. Ten hours later, he was admitted in a local hospital in a comatose state (Glasgow coma scale, (GSC: 7)), vomiting and with a severe diarrhea. Eight hours later, he deteriorated, requiring tracheal intubation and mechanical ventilation. Hypotension and oliguria appeared. The patient was referred to our Intensive Care Unit.

On admission, 24 h after ingestion, he was deeply comatose (GCS: 4). His haemodynamic state was unstable (mean arterial pressure 55 mmHg) and he required vasoactive and inotropic drugs. He was anuric. Serum creatinine was 610 μmol/L (118 μmol/L on initial admission), ASAT was 277 UI/L, ALAT was 97 UI/L (normal $<$100 UI/L), prothrombin time was 40 s (control 12), white blood count was 15.5 10^9/L, erythrocyte count was 4.34 10^{12}/L, and platelet count was 660 10^9/L.

A right-heart catheterization was performed showing mean arterial pressure 76 mmHg, mean pulmonary artery pressure 35 mmHg, pulmonary artery occlusion pressure 14 mmHg, and

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cardiac index 4.8 L/min/m^2. Two hours later, his haemodynamics further deteriorated and the patient died in acute heart failure.

Plasma sample procedure

Blood was collected into heparinized tubes. After centrifugation, the plasma was separated, frozen and stored in the dark at −20°C, until analysed. Colchicine concentration was determined by using a modification of the method described by Lhermitte *et al.* (1985). After addition of borate buffer to 2 ml of plasma to obtain pH 9.0, colchicine was extracted by ethyl acetate using methyl clonazepam as an internal standard. The determination of colchicine was performed by HPLC using a Lichrospher 100 RP-18 5 μm (250 × 4 mm) (Darmstadt, Germany). Colchicine and internal standard were detected at 340 nm.

Organ sample procedure

To determine the concentration of colchicine in the various human organs, the procedure was at first performed on animal tissue (rat, Wistar male, 250 g).

Calibration curve. Various tissue standards were prepared at different concentrations of colchicine (0–400 ng/g) by injecting an aqueous solution of colchicine. Spiked tissue samples were stocked at −20°C until used.

Sample procedure. Each sample (1 g), after defrosting and weighing, was introduced into a glass tube containing 500 ng of internal standard (methyl clonazepam) and was pounded with ultraturrax for 30 seconds, after addition of 2 mL phosphate buffer saline (PBS). The homogenate was then centrifuged at 4000×***g*** for 5 min and the supernatant was transferred to another tube. HCl (2M) was added to obtain pH values from 3.5 to 9.1, and 4 mL ethylacetate. Extraction of colchicine and internal standard was performed manually for 2 min. The tube was then centrifuged at 4000×***g*** for 5 min. The aqueous phase was re-extracted in a similar manner and the corresponding organic phases were pooled and evaporated to a dry residue under a nitrogen stream at 30°C. The residue was solubilized in 500 μL of mobile phase (acetonitrile–0.03 M ammonium sulphate–triethylamine) and 100 μL were injected into the column.

Apparatus

A Varian model 5000 liquid chromatograph equipped with a UV variable wavelenght detector SPD6A (Shimadzu, Touzart and Matignon, France) was used. Chromatography was performed on a 125 × 4.6 mm i.d. stainless-steel C18 Hypersil ODS 5 μm particule size column (Touzart and Matignon, France) with a precolumn (10 × 4.6 mm i.d.) that contained the same stationnary phase. The mobile phase was a mixture of acetonitrile (A) and 0.03 M ammonium sulphate containing 1‰ of triethylamine (v/v) solution adjusted to pH 3.0 with phosphoric acid (B). The column was conditioned with the mixture A–B (25–75 v/v) and eluted with the same mobile phase during the first 5 min, then a gradient was applied to the column to get a mixture of A–B (40–60 v/v) at 10 min. This elution was maintained for 10 min and initial conditions were restablished in the remaining 5 min. The flow rate was 1.0 mL/min and the pressure 14 MPa. Colchicine and internal standard were detected at 340 nm.

Quantitation

Calibration standards covering the anticiped concentration range (0–400 ng/g) in methanol and tissue were processed. Peak-area ratios of colchicine to the internal standard were measured, and the calibration was obtained from linear regression of the peak-area ratios against concentrations. This line was then used to calculate the concentration of colchicine in unknown samples.

Recovery

Extracts from tissue, prepared as described above, were done at different pH: 3.5, 4.8, 6.8, 8.6 and 9.1, and were compared with a direct assay of standards in methanolic solution. The relative recoveries were determined at a concentration of 400 ng/g.

RESULTS AND DISCUSSION

In our case, the findings clearly demonstrate that colchicine was responsible for the death. In addition, the patient's clinical presentation was typical of colchicine intoxication. As is well described in a number of clinical reviews (Stapczynski *et al.*, 1981; Murray *et al.*, 1983; Levy *et al.*, 1991), early clinical complications include gastrointestinal symptoms, volume depletion and a peripheral leukocytosis, all demonstrated in this patient. Subsequently, multisystem organ failure involving the cardiorespiratory, renal, gastrointestinal, nervous and haematologic systems ensues. This patient demonstrated prior to death severe alterations in all of these organ systems. On admission into our hospital 24 h after ingestion, the blood level of colchicine was 4.5 ng/mL.

This value, found 24 h after ingestion, confirmed the importance of ingestion, since Rochdi *et al.* (1992) showed in two groups of patients plasma terminal half-lives ranging from 10.6 to 31.7 h. Caplan *et al.* (1980) in a single case report detected colchicine in the blood after 2, and not after 40 h, after ingestion. Clevenger *et al.* (1991) in a single case detected colchicine in the urine, not in the blood of the intoxicated patient. A large volume of distribution and a low body clearance have been showed for colchicine (Rochdi *et al.*, 1992).

Figure 1 shows the separation and quantitation of colchicine in tissue, using methylclonazepam as internal standard. As compared with the chromatogram obtained after extraction of 0.5 g of drug-free tissue, no additional peaks that might interfere with the determination of colchicine and internal standard were present [Fig. 1(a)]. Retention times for colchicine and methylclonazepam were 6.8 and 18.2 min, respectively. Because the percentage of colchicine varied according to the pH, a study of extraction of colchicine at various pH was performed. Results reported in Table 1 showed that

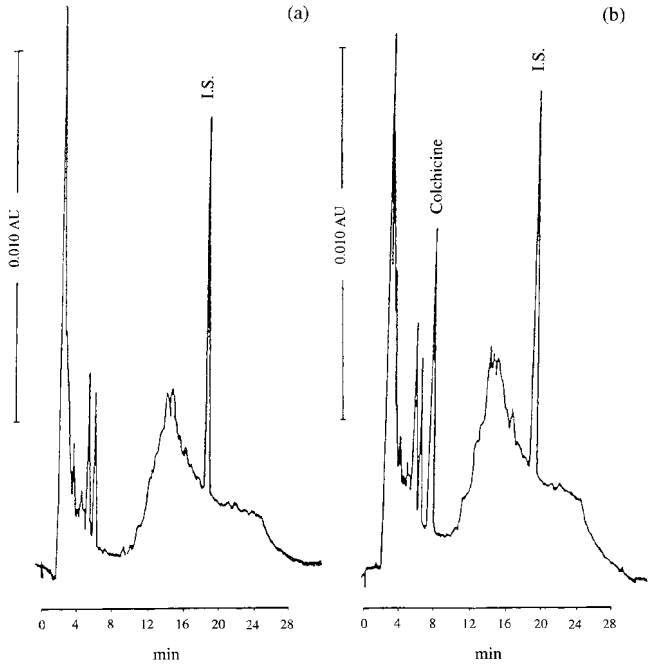

Figure 1. Representative chromatograms of extracted drug-free tissue (a) and human tissue (1 g) containing 400 ng/g colchicine and 500 ng/g internal standard (b).

colchicine is extracted with greatest efficacy at pH 3.5. The other experiments were done at this value of pH. The calibration curves were obtained using a methanolic solution of standards or tissue spiked with 0–400 ng/g colchicine and 500 ng/g internal standard. There was a good correlation between the amount of colchicine added to tissue and the amount detected in the sample of 0.5 g of tissue. The linear regression equations were: $y = 0.002645\ x + 0.001$ for methanolic solutions and $y = 0.002075\ x - 0.002$ ($r = 0.999$) for standards in tissue (y = peak area ratio of colchicine to internal standard; x = colchicine concentration). Calibration curves showed good linearity between peak-area ratios and concentrations from 0 to 400 ng/g. The limit of quantitation in tissue was defined as the lowest concentration of colchicine resulting in a signal to noise ratio of 2. The present method was able to detect 5 ng/g colchicine in tissue.

The HPLC assays of post-mortem tissues are reported in Table 2. Tissue concentrations ranged from 5 ng/g in brain to 396 ng/g of wet weight in the kidney.

Post-mortem tissue analysis shows an ubiquitus colchicine distribution with tissue/plasma ratio ranging from a value near 0 for brain to 88 for kidney. Only traces of colchicine were found in the brain. Bennet *et al.* (1981) found that colchicine did not reach the brain of mice and rats. The results are not in agreement with those of Rochdi *et al.* (1992), who found concentrations of 125 ng/g in the brain. However, high concentrations of colchicine were found in the liver, kidney and heart, as described also by Terrien *et al.* (1989) in mice, and Rodchi *et al.* (1992) in humans. Kintz *et al.* (1997) reported colchicine level of 12 and 29 ng/mL in liver and heart, respectively, of a fatal case of colchicine poisoning. In this last experiment, colchicine determination was done without internal standardization and colchicine was extracted from post-mortem tissues at pH 9.0.

Our HPLC method measuring colchicine in plasma can also be easily applied to the measurement of colchicine in post-mortem organs.

Table 1. Yield of extraction for colchicine (400 ng/g) at various pH values

pH	Yield (%)
3.50	78
4.80	56
6.80	53
8.60	43
9.10	25

Table 2. Colchicine concentration in post-mortem tissues

Tissue	Concentration (ng/g) ($n = 3$)
Lung	58 ± 1.2
Heart	334 ± 7.5
Liver	347 ± 8.5
Kidney	396 ± 7.8
Muscle	10
Brain	5

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