Colchicine Poisoning: Report of a Fatal Case and Presentation of an HPLC Procedure for Body Fluid and Tissue Analyses

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Abstract

A case involving a suicidal overdose resulting from the ingestion of colchicine tablets is presented. The drug was quantitated using liquid chromatography. The femoral blood level was 62 ng/mL, and the maximum concentration found in bile was 2921 ng/mL. Therefore, bile appears to be the sample of choice for toxicological analysis when a poisoning case involving colchicine is suspected.

Introduction

Colchicine is a naturally occurring alkaloid that can be found in the flowers of autumn crocus (Colchicum autumnale), a member of the liliaceae family. Concentrations of colchicine in the plant are approximately 0.1–0.8% by weight. The drug is used for the treatment of acute gouty arthritis, for which it was first used in 1763. Colchicine is a potent inhibitor of cellular mitosis by binding to tubulin, which prevents its polymerization into microtubules.

In France, the drug is only available as 1-mg tablets (Colchicine Houdé®, Laboratoires Houdé, Puteaux, France). Plasma concentrations in 10 subjects after a 1-mg oral dose of colchicine reached an average peak of 2.2 ng/mL at 2 h; by 24 h, the level had declined to an average of 0.4 ng/mL (1). Colchicine is primarily deacetylated by the liver, although as much as 30% may be excreted unchanged by the kidney. Cancer and gout patients eliminate about 4% as unchanged drug, whereas normal or asthmatic subjects eliminate about 28% in this manner (2). Large amounts of both parent drug and metabolites are excreted in the bile and intestinal secretions, resulting in an enterohepatic circulation (3).

Most serious poisonings result from the suicidal ingestion of colchicine tablets. Colchicine is a potent gastrointestinal toxin and causes intractable multiorgan failure. Overdosage is manifest by nausea, vomiting, confusion, fever, shock, respiratory distress, hematuria, renal failure, cardiovascular collapse, thrombocytopenia, granulocytopenia, and hypervolemia (4–6).

Colchicine can be quantitatively measured in biological specimens by fluorimetry (7), radioimmunoassay (8), liquid chromatography (9), liquid chromatography–ionspray mass spectrometry (10), and gas chromatography–mass spectrometry (11).

Although several papers report colchicine fatal overdose (12,13), drug measurements in fatalities were seldom performed. However, when measurements were taken, blood or plasma levels in the range of 10 to 250 ng/mL were found (3,10,14–17). In some cases, although colchicine was detected at admission to the intensive care unit, none was found in postmortem blood (11,15). For this reason, data concerning colchicine distribution in body fluids and tissues are useful for presenting information on forensic cases involving the drug.

We present here a fatality in which colchicine was identified and quantitated in postmortem samples by an accurate high-performance liquid chromatographic (HPLC) procedure.

Case History

A 42-year-old caucasian male, 1.80 m in height, was found dead in his car with several vomit stains on his clothing. Numerous tablets were found near the body, and several empty blister packs of Colchicine Houdé (colchicine, 1 mg) were found as well. The autopsy revealed massive edema and congestion of the lungs. The respiratory passages contained mucopurulent secretion. Examination of the gastrointestinal tract revealed esophageal hemorrhage. No samples were taken for histology during the autopsy. The following postmortem samples were taken for toxicological analysis: femoral blood, urine, gastric content, bile, vitreous humor, liver, and heart.

Experimental

Materials

Methanol, acetonitrile, and dichloromethane were HPLC grade (Merck, Darmstadt, Germany). All other chemicals and reagents were analytical grade (Merck and Prolabo, Paris, France). Colchicine as pure standard was obtained from Sigma (St. Louis, MO). Phosphate buffer for extraction was a 1 mol/L solution of di-ammonium hydrogen phosphate that was adjusted to pH 8.0.
Instrumentation
The HPLC system consisted of a pump (Waters 6000 A, Milford, MA) and an automatic sample-injection module (Waters Wisp 710B), which were coupled to a programmable multiwavelength detector (Waters) simultaneously set at 254 and 350 nm. The column was a 5-µm Lichrosorb (Alltech, Deerfield, IL) RP 18 column (250 x 3.9-mm i.d.), which was thermostated at 26°C during all experiments. Elution was performed isocratically, with a flow rate of 0.8 mL/min. The mobile phase consisted of methanol, acetonitrile, and 0.1M KH₂PO₄ buffer (pH 7.6, 4:15:44, v/v/v).

Procedure
Phosphate buffer (1.0 mL, 1 mol/L, pH 8.0) and 10 mL of dichloromethane were added to 3.0 mL postmortem fluids or tissue homogenates (1 part tissue for 3 parts deionized water, v/w). After agitation and centrifugation, the organic phase was removed and evaporated to dryness. The dry extract was then dissolved in 100 µL of mobile phase, and 75 µL was injected into the column.

Results and Discussion
Analytical performance
Under the described conditions, colchicine was eluted in 7.6 min. Chromatograms were cleaner when recorded at 350 nm rather than 254 nm. Colchicine was quantitated by external standard calibration because of the unavailability of suitable internal standard. The method was linear for whole blood (n = 3) over the range of 5 to 500 ng/mL (r = 0.996). Recovery was about 65% in whole blood. The limit of detection was estimated to be 1 ng/mL using a 3.0-ml blood sample. Day-to-day precision in blood, studied at 50 ng/mL, was 14.6%. For each other medium (fluid or tissue), the accuracy of the procedure was tested at 100 ng/mL or 100 ng/g. Coefficients of variation ranged from 6 to 17% in the assay.

Postmortem findings
Colchicine was detected in all the autopsy samples. Concentrations are presented in Table I. Femoral blood concentration was 62 ng/mL, which was in the range of previous papers. Confirmation of colchicine in blood was performed by liquid chromatography–mass spectrometry (10).

It was not possible to evaluate the amount of drug ingested as it was demonstrated that postmortem colchicine blood concentrations definitely do not correlate with the severity of ingestion (18). In several reports, even after high-dose acute poisoning, it was not possible to detect colchicine in postmortem blood because of the extremely short plasma half-life of the drug (approximately 20 min), although poisoned persons frequently undergo a prolonged agony. For example, the 2-h post-ingestion blood colchicine concentration was 250 ng/mL in a 39-year-old woman who consumed 30–35 colchicine tablets (0.6-mg), but no drug was detected at death 40 h post-ingestion (15). Toxicological evaluation of colchicine ingestion appears to be better when bile is considered as the sample of choice. Because of enterohepatic circulation, colchicine is concentrated in bile, and the bile to blood ratio is always greater than 1. Bile concentrations are generally in the micrograms-per-milliliter range, compared with the nanograms-per-milliliter range for blood (3,17). However, the concentration of colchicine in bile in this case may be excessively elevated if the decedent was on colchicine therapy before committing suicide. The drug may already have been accumulated in the bile during his prior use of colchicine. The medical report of the subject was missing, and this hypothesis could not be documented. It is of interest to note that a low amount of colchicine was detected in the liver, particularly considering the large concentration detected in the bile. These findings are in concordance with the report of McIntyne et al. (17), who did not evidence the drug in the liver, although the bile concentration was 30 ng/mL. The heart was not a major site of colchicine deposition. The unique finding of colchicine in vitreous humor could be of interest in forensic science, particularly when a blood sample is not available.

No other drugs were detected by general screening, which was performed with liquid chromatography coupled to diode array and gas chromatography coupled to mass spectrometry. In view of the circumstances, the manner of death was listed as suicide.

Table I. Colchicine Concentrations in Postmortem Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentrations (ng/mL or ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral blood</td>
<td>62</td>
</tr>
<tr>
<td>Bile</td>
<td>2921</td>
</tr>
<tr>
<td>Urine</td>
<td>1024</td>
</tr>
<tr>
<td>Gastric content</td>
<td>39</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>20</td>
</tr>
<tr>
<td>Liver</td>
<td>12</td>
</tr>
<tr>
<td>Heart</td>
<td>29</td>
</tr>
</tbody>
</table>

References

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