Interference of the Chemotherapeutic Agent Etoposide with the Direct Phosphotungstic Acid Method for Uric Acid

Mary Lou Metha, Gerald Kessler, and Kwok-Ming Chan

A 62-year-old woman receiving chemotherapy with etoposide showed discrepant uric acid values as measured by a direct phosphotungstic acid (PTA) method (150 mg/L) compared with a uricase technique (40 mg/L). After ultrafiltration, the positive interference for the direct PTA method was retained in the protein fraction, but not in the filtrate. Adding exogenous etoposide to drug-free serum confirmed this interference for the direct PTA method, but not for the uricase procedure or a PTA technique preceded by dialysis. Decisions for aggressive patient management are often based on the magnitude of hyperuricemia. We do not recommend that the direct phosphotungstic acid method be used to measure uric acid in patients receiving etoposide.

Additional Keyphrases: acute myelogenous leukemia • analytical error • monitoring therapy

Uric acid, being the major product of purine catabolism, is frequently used as an indicator of tumor lysis after chemotherapy (1–3). Therefore, interference by a chemotherapeutic agent in its measurement is important, because falsely increased uric acid values could affect case management.

Etoposide, a semisynthetic podophyllotoxin derivative used in conjunction with other chemotherapeutic agents to treat a number of solid tumors as well as childhood and adult leukemias (4), binds strongly to human serum protein (5). Here we document the interference with uric acid measurements in serum of a patient undergoing chemotherapy with etoposide.

Case Report

The patient, a 62-year-old white woman with acute myelogenous leukemia, presented two months prior to this admission with fatigue, malaise, vaginal bleeding, and epistaxis. She was admitted then with a leukocyte and platelet counts of 325 000 and 30 000 per microliter. After a chemotherapeutic course of cytosine arabinoside and daunorubicin, she was discharged with a leukocyte count of 4400 per microliter. Her post-chemotherapy course was complicated by pancytopenia and *Staphylococcus* bacteremia.

At the present admission, she reported having noticed an increase in lymphadenopathy over the preceding two to three weeks, and she developed a non-pruritic rash about a month before. At this admission her leukocyte and platelet counts were 44 200 and 79 000 per microliter. She was admitted for consolidation chemotherapy, which included etoposide, 4.32 g in 10.8 L of isotonic saline, given over 34 h, starting on the third day of hospitalization. She was also given cyclophosphamide, 2.7 g in 500 mL of a 50 g/L solution of dextrose in water, every morning starting on day 5, and allopurinol, 300 mg orally every day, starting on day 3. On day 4 a discrepancy was noted between the value for uric acid measured with a uricase method (in the Du Pont "a za"), 40 mg/L, and that measured by a direct phosphotungstic acid method (in the American Monitor "Parallel"), 150 mg/L. Etoposide was discontinued on day 5. By day 8, the uric acid measurements were no longer discrepant.

Materials and Methods

Direct phosphotungstic acid (PTA) measurements of uric acid were made with the Parallel (American Monitor, Indianapolis, IN), which monitors at 760 nm the reduction of PTA to tungsten blue in basic solution. The indirect PTA method of the SMA II (Technicon Instruments Corp., Tarrytown, NY) includes a dialysis stage for separation from macromolecules before this reaction and measures the reduced PTA at 660 nm. The uricase (EC 1.7.3.3) method used in the aza (Du Pont Co., Wilmington, DE) measures the decrease in absorbance at 293 nm as uric acid is enzymatically converted to allantoin (6).

Ultrafiltration consisted of centrifuging 1 mL of the patient's serum in CentriFuge Micropartition System (Amicon Corp., Danvers, MA 01923) for 2.25 h at 2000 × g, in a Sorvall RC2B Refrigerated Centrifuge (Du Pont Instruments, Wilmington, DE 19898) until approximately 0.5 mL of serum remained above the filter.

Vepesid (etoposide [VP-16-213]; Bristol Laboratories, Syracuse, NY) was added to either pooled serum samples or an aqueous uric acid solution (23 mg/L) in phosphate buffer, pH 7.4 to give concentrations of 0–40 mg/L. Uric acid was obtained from the National Bureau of Standards, Office of Standard Reference Materials, Gaithersburg, MD 20899.

Results and Discussion

Concentrations of uric acid are often increased in serum of patients who are undergoing chemotherapy, because of cell destruction and increased catabolism of nucleoproteins (1–3). Detection of increased concentrations of uric acid dictates more-aggressive treatment. Thus, it is essential for the laboratory to provide accurate uric acid measurements.
Our study clearly indicates that etoposide interferes with the direct PTA method for uric acid measurement. To investigate the mechanism of etoposide interference, we subjected to ultrafiltration samples of the patient’s serum. Before ultrafiltration, the uric acid concentration by the direct PTA method was 2.5 times greater than that by the uricase technique (127, SD 7 vs 49, SD 2 mg/L). After ultrafiltration, the uric acid values of the ultrafiltrate were similar for both the direct PTA (50, SD 2 mg/L) and uricase (41, SD 2 mg/L) methods. On rediluting the retentate to its original volume, we observed interference only with the direct PTA method for uric acid.

The direct PTA uric acid measurement (71, SD 1 mg/L) exceeded the uricase result (17, SD 1 mg/L) by approximately 50 mg/L. These findings suggested that the interfering substance either could have a high molecular mass or could be a low-molecular-mass substance bound to serum proteins and (or) the Amicon membrane.

Table 1 summarizes the effect of etoposide on uric acid measurements when added to drug-free serum or phosphate buffer containing uric acid. The direct PTA method demonstrated interference proportional to the concentration of etoposide in serum. Both the indirect PTA and uricase methods were unaffected. Etoposide added to an aqueous buffer solution containing uric acid behaved similarly to the serum-based samples. The direct PTA method showed interference of similar magnitude, while the indirect PTA and uricase techniques were unaffected. The results suggest that the nondialyzable nature of this interference was not attributable solely to protein binding but could also be related to the hydrophobic nature of the molecule and its inability to pass through the dialysis membrane.

Patients receiving chemotherapy for a responsive tumor can develop tumor lysis syndrome (1), which is characterized by hyperuricemia, hyperphosphatemia, hypocalcemia, and hyperkalemia. However, the presence of hyperuricemia, even in the absence of other signs, could still prompt aggressive intervention (e.g., alkalinization of urine) is often used to enhance uric acid excretion by ensuring its solubility (1), but this practice could lead to calcium deposition in the kidneys. Therefore a falsely increased uric acid value of the magnitude observed for this patient would lead to inappropriate further actions. We therefore recommend that direct PTA methods not be used to measure uric acid in patients receiving etoposide chemotherapy.

Table 1: Interference on Uric Acid Measurement by Etoposide Added to Drug-Free Serum

<table>
<thead>
<tr>
<th>Etoposide concn, mg/L</th>
<th>Direct PTA*</th>
<th>Indirect PTA*</th>
<th>Uricase*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum-based samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>34 ± 1</td>
<td>39</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>41 ± 2</td>
<td>38</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>10</td>
<td>53 ± 5</td>
<td>38</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>20</td>
<td>72 ± 6</td>
<td>38</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>40</td>
<td>107 ± 6</td>
<td>38</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>Aqueous sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20 ± 1</td>
<td>26</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>40</td>
<td>98 ± 1</td>
<td>27</td>
<td>37 ± 4</td>
</tr>
</tbody>
</table>

* Mean ± SD of triplicate measurements.

References