that the most obvious explanation for our findings is that the concentration of HC is influenced by the vitamin B\textsubscript{12} status of the patient. The issue has important implications for the interpretation of low cobalamin in relation to low HC concentrations in patients undergoing examination for vitamin B\textsubscript{12} deficiency. If genetic factors were the only regulators of the HC concentration, the physician could decide that the low concentration of cobalamins reflected an inherited low HC. If HC is regulated by the vitamin B\textsubscript{12} status in addition to the genetic factors, as suggested by us, such a patient should always undergo further analysis to rule out a deficiency state.

References


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Perchloric Acid Treatment To Stabilize Uric Acid Concentrations in Blood Samples of Patients Receiving Uric Acid Oxidase (Rasburicase) Therapy

To the Editor:

Sample handling requirements for uric acid analysis during recombinant uric acid oxidase (rasburicase, Sanofi-Synthelabo) therapy are a matter of concern. Rasburicase catalyzes the oxidation of uric acid to allantoin, which is easily excreted by the kidney. It is indicated for the treatment and prophylaxis of malignancy- or chemotherapy-associated hyperuricemia (1).

For monitoring uricemia in patients receiving rasburicase therapy, the manufacturer recommends keeping blood samples in ice water immediately after collection and during specimen transport until analysis. When samples are maintained at 4 °C, uric acid concentrations are reported to be adequately preserved (2). However, cold inactivation of the enzyme requires a cooling period to bring the collection tube to 4 °C, during which further degradation of uric acid is possible (3). Furthermore, rack-based modern random access analyzers do not allow maintaining the sample temperature at 4 °C. Moreover, in practice, laboratories are often unaware that the patient has received rasburicase because the blood samples were sent at room temperature to the laboratory as part of routine biochemistry testing (2). In view of the rapid uricolytic activity by rasburicase, the accuracy of uric acid determinations in patients under rasburicase treatment can be questioned under routine conditions.

We studied inactivation of the therapeutic enzyme to preserve uric acid. A blood sample (0.5 mL) was supplemented with 1 mL 8% perchloric acid (PCA) (4). After centrifugation, (900g, 10 min, room temperature) 1 volume of supernatant was neutralized with 1/2 volume tri-potassium phosphate 0.7 mol/L (pH = 13). After centrifugation, the supernatant was analyzed. Uric acid analysis was performed by an enzymatic colorimetric assay (Modular P, Roche Diagnostics) with a detection limit of 12 µmol/L, and between-run imprecision (CVs) of 1.2% and 1.3%, at mean concentrations of 297 and 714 µmol/L, respectively. The influence of hematocrit was evaluated by reconstituting plasma and blood cells of a donor in different proportions, resulting in hematocrit values in the range 0.2–0.5. Based on measurements with 3 different donors, the plasma uric acid concentration (y, µmol/L) correlated to the measured uric acid concentration (x, µmol/L) as follows:

\[ y = x \cdot 4.5/(0.905 - 0.63 \cdot hct), \]

in which hct represents the hematocrit value.

To investigate its in vitro uricolytic activity, rasburicase (concentration: 1.5 mg/L) was added to heparinized blood samples from healthy volunteers (n = 4). An aliquot was treated with PCA, a 2nd aliquot was promptly placed on ice water, and a 3rd aliquot was kept at room temperature. Uric acid analysis was performed at 30-min intervals for 4 h (Table 1). In untreated samples stored at room temperature (~20 °C), plasma uric acid concentration decreased to <12 µmol/L within 3 h. When untreated samples were stored at 4 °C and, as under routine conditions, centrifuged at room temperature, uricolyis was much less pronounced. However, uric acid values still showed a marked decrease of 30%, thereby exceeding the maximum error budget of 14.8% (5). In PCA treated samples, calculated uric acid values remained stable at room temperature.

To mimic sampling conditions, heparinized blood samples from 2 volunteers (uric acid concentrations: 321 and 506 µmol/L) were placed in a heating bath at 37 °C before addition of 1.5 mg/L rasburicase. After addition, an aliquot of 0.5 mL was treated with PCA, while the remaining tube was placed in ice water. Residual uric acid concentrations were highest with PCA treatment (at least 95%), while storage in ice water and centrifugation at 4 °C resulted in a higher but stable loss (up to 30%), probably due to degradation during the equilibration time needed for complete enzymatic inactivation. Overall, our findings are in agreement with Lim et al. (1, 2) who demonstrated that the in vitro uricolytic activity of rasburicase is minimized in samples maintained at 4 °C.

We also evaluated a sample of a patient with a hematologic malignancy who was given rasburicase for

clinical chemotherapy-associated hyperuricemia, receiving rasburicase therapy, the manufacturer recommends keeping blood samples in ice water immediately after collection and during specimen transport until analysis. When samples are maintained at 4 °C, uric acid concentrations are reported to be adequately preserved (2). However, cold inactivation of the enzyme requires a cooling period to bring the collection tube to 4 °C, during which further degradation of uric acid is possible (3). Furthermore, rack-based modern random access analyzers do not allow maintaining the sample temperature at 4 °C. Moreover, in practice, laboratories are often unaware that the patient has received rasburicase because the blood samples were sent at room temperature to the laboratory as part of routine biochemistry testing (2). In view of the rapid uricolytic activity by rasburicase, the accuracy of uric acid determinations in patients under rasburicase treatment can be questioned under routine conditions.

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We also evaluated a sample of a patient with a hematologic malignancy who was given rasburicase for
prophylaxis of hyperuricemia. Following the manufacturer’s instructions, uric acid concentration was 83 μmol/L, compared to 95 μmol/L after immediate PCA treatment, and <12 μmol/L after 1 h storage at room temperature.

In conclusion, sample pretreatment with PCA appears to be a useful tool for monitoring of plasma uric acid concentrations during rasburicase treatment. We recommend supplying plastic tubes containing 2 mL PCA. A 1 mL syringe can be used to add 1 mL whole blood to this tube. Immediately after addition, the tube should be shaken vigorously for 30 seconds before being sent to the laboratory.

We thank Sanofi-Synthelabo for providing rasburicase (Fasturtec®).

References

Table 1. Residual uric acid concentrations in whole blood after addition of rasburicase.

<table>
<thead>
<tr>
<th>Time</th>
<th>4 °C</th>
<th>RT</th>
<th>+ PCA, RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>70 (3)</td>
<td>57 (6)</td>
<td>101 (&lt;0.1)</td>
</tr>
<tr>
<td>60 min</td>
<td>76 (2)</td>
<td>45 (5)</td>
<td>103 (5)</td>
</tr>
<tr>
<td>120 min</td>
<td>70 (6)</td>
<td>18 (6)</td>
<td>102 (1)</td>
</tr>
<tr>
<td>180 min</td>
<td>68 (5)</td>
<td>6 (4)</td>
<td>105 (7)</td>
</tr>
<tr>
<td>240 min</td>
<td>68 (5)</td>
<td>0</td>
<td>94 (2)</td>
</tr>
<tr>
<td>300 min</td>
<td>70 (5)</td>
<td>ND</td>
<td>97 (4)</td>
</tr>
</tbody>
</table>

The original uric acid concentrations before addition of rasburicase in the 4 donors were 482, 358, 336, and 290 μmol/L, respectively. At defined intervals after addition of rasburicase (time 0), samples were centrifuged, supernatants were collected and prepared for analysis. For the PCA pretreatment, all aliquots were treated with PCA at time 0, with further sample preparation at the indicated time points. PCA, perchloric acid 8%; RT, room temperature; ND, not determined.

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Does Bilirubin Cause Interference in Roche Creatinine Methods?

To the Editor:

Bilirubin interference in kinetic alkaline picrate (Jaffe) creatinine assays has been well documented (1, 2). The exact mechanism of interference is still unclear, however, and both conjugated and unconjugated bilirubin have been implicated. Enzymatic assays, which reportedly suffer less interference, are an alternative, but the extra reagent cost has restricted their use. Historical data continue to be used to estimate the extent of bilirubin interference, although modifications to these assays or instruments may have taken place since the original interference studies were performed. We used liquid chromatography tandem mass spectrometry (LC-MS/MS) as the reference method to investigate the extent of bilirubin interference in 2 automated creatinine assays. We have previously shown that this LC-MS/MS assay compares well to the 2 automated methods at creatinine concentrations of <150 μmol/L (3) by analyzing >100 samples with bilirubin concentrations within reference intervals.

We added varying amounts of creatinine (Sigma) to phosphate buffered saline (PBS) pH 7.4, containing 40 g/L bovine serum albumin, to give concentrations of 37.5–1000 μmol/L. One liter of PBS contained 8 g sodium chloride, 0.2 g potassium chloride, 1.44 g disodium hydrogen phosphate, and 0.24 g potassium dihydrogen phosphate. Unconjugated bilirubin (Sigma) was then added to give bilirubin concentrations up to 511 μmol/L. Anonymized icteric sera (n = 73) with creatinine concentrations <150 μmol/L were stored at −20 °C for ≥2 weeks, and total and conjugated bilirubin were determined by the Roche liquid diazonium ion and Jendrassik-Grof based assays, respectively. All samples were analyzed by the following 3 different creatinine methods.

The automated creatinine assays were the rate-blanked, compensated Jaffe method and the creatinine plus enzymatic assay performed on the Roche Modular according to the manufacturer’s instructions (Roche Diagnostics). The reagent set insert states that there is no significant interference by bilirubin for concentrations <150 μmol/L were stored at −20 °C for ≥2 weeks, and total and conjugated bilirubin were determined by the Roche liquid diazonium ion and Jendrassik-Grof based assays, respectively. The LC-MS/MS assay employed was used as described by Owen et al. (3).

The comparative LC-MS/MS method shows no significant interference by bilirubin (see Fig. 1 in the Data Supplement that accompanies the online version of this Letter at http://www.clinchem.org/content/vol53/issue2). Surprisingly, the Jaffe method (Fig. 1A) did not show significant interference in most PBS samples; however, the samples with the lowest creatinine concentrations