Effect of Tobramycin on Urinary γ-Glutamyltransferase Activity: Studies in a Case of Renal Carcinoma

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γ-Glutamyltransferase activity was studied in a man presenting with recurrent septicemia owing to pyonephrosis and renal carcinoma. Increased activity in the urine was ascribable to administration of the aminoglycoside antibiotic, tobramycin. That the renal carcinoma did not contribute to the increased values was confirmed by homogenization and enzyme histochemistry of the tumor. Although the activity of this enzyme in serum was greater than normal, this persisted postoperatively, and thus was not related to the renal carcinoma.

Studies of the activity of urinary γ-glutamyltransferase [γ-GT; (γ-glutamyl)-peptide:amino-acid γ-glutamyltransferase, EC 2.3.2.2] in renal disease are scanty. Recent German papers (1–3) have shown that urinary γ-GT activity may increase in inflammatory renal disease and decrease in chronic noninflammatory disease. These papers have also shown the important relationship between urinary γ-GT and creatinine clearance.

Here, we describe results of our various γ-GT investigations in a patient with renal carcinoma, and emphasize the importance of the aminoglycoside antibiotic, tobramycin, in altering urinary γ-GT activity.

Case Summary

The patient, a 75-year-old man, was admitted on December 15, 1974, with Escherichia coli septicemia, which was treated with tobramycin. He was anemic (80 g of hemoglobin per liter of blood) and had a persistently elevated erythrocyte sedimentation rate of about 130 mm/h. A double-dose intravenous pyelogram at this time demonstrated splayed midcalyces in the left kidney, caused by either a cyst or a tumor. He was readmitted on February 20, 1975, with recurrence of E. coli septicemia, again treated with tobramycin. He improved and his tobramycin was stopped on March 11. E. coli persisted in his urine, and this infection was treated with nalidixic acid.

On April 15, 1975, a left nephrectomy was performed. The specimen consisted of a shell of normal renal tissues that surrounded a pyonephrosis, enclosing a renal carcinoma, about 1.5 cm in diameter. Histological examination showed a well-differentiated clear-cell carcinoma with large areas of hemorrhage. His postoperative course was uneventful and he was discharged home well at the end of April 1975.

Methods

γ-GT assay. γ-GT was assayed at 37 °C, pH 8.1, and 410 nm, with use of an LKB 8600 reaction-rate analyzer. Buffered substrate was freshly prepared by heating to 70 °C L-γ-glutamyl-p-nitroanilide (Boehringer), 4.6 mmol/liter in 230 mmol/liter tris(hydroxymethyl)aminomethane buffer, pH 8.1.

To 1.4 ml of buffered substrate, 0.1 ml of serum or urine was added, and the reaction was initiated with 0.1 ml glycyglycine (640 mmol/liter; 40 mmol/liter in the final reaction mixture). The results were expressed as U/liter by using the following equation:

\[
γ-GT \text{ activity, } U/\text{liter} = [(\text{absorbance change/min}) \times 1000 \times V)/(E \times v) = \text{absorbance change/min} \times 1882
\]

where \(V\) = total volume in tube, 1.6 ml; \(v\) = serum (urine) volume, 0.1 ml; and \(E\) = millimolar absorptivity of \(p\)-nitroaniline at 410 nm and pH 8.1, 8.502 (4).

Urine specimens were complete 24-h collections, without preservative, stored at 4 °C, and were well-mixed before assay. The enzyme is stable under such
conditions (5). Creatinine was measured with the Jaffé reaction in a Technicon AutoAnalyzer I system. A highly significant positive correlation, \( r = 0.855, P < 0.001 \), exists between urinary \( \gamma \)-GT and creatinine clearance in normal individuals (6), and the regression equation for this, \( y = 20.4x + 8.13 \), has been used to calculate a \( \gamma \)-GT/creatinine clearance ratio (\( \gamma \)-GT/ml):

\[
\gamma \text{-GT/ml} = \frac{\gamma \text{-GT(U/24 h)} - 8.13}{\text{creatinine clearance (ml/s)}}
\]

**Homogenization.** About 1 g of tissue was homogenized in 20 ml of cold 230 mmol/liter tria(hydroxy-methyl)aminomethane buffer, pH 8.1, with a mechanically driven Teflon-and-glass homogenizer. The homogenates were centrifuged for 1 h at 4 °C and 2000 \( \times \) \( g \), and the supernates used for \( \gamma \)-GT assays. Protein contents were estimated by the technique of Lowry et al. (7).

The histochemical technique, that of Albert et al. (8), was used on acetone-fixed tissues. \( \gamma \)-GT in tissue sections reacts with \( \gamma \)-glutamyl-\( \alpha \)-naphthylamide to release \( \alpha \)-naphthylamine. This latter combines with Fast Garnet GBC Salt to give an insoluble red-brown diazo complex, which indicates the site of enzyme activity.

**Results**

**Blood and Urine**

The first \( \gamma \)-GT assays were done on March 5, during the patient’s second admission, and when he had been receiving tobramycin for 13 days. Relevant results were:

Serum creatinine, 140 \( \mu \)mol/liter (normal, 35–115 \( \mu \)mol/liter). Creatinine clearance, 0.17 ml/s (normal, 1.42–2.33 ml/s). Serum \( \gamma \)-GT, 78 U/liter (normal, 1–35 U/liter). Urinary \( \gamma \)-GT, 67 U/24-h specimen (normal, 22–55. U/24-h specimen). \( \gamma \)-GT/CC, 325.8 (normal, 10.2–31.2) (CC = creatinine clearance).

We thought the abnormally high \( \gamma \)-GT values were attributable to the renal tumor, but serial measurements showed clearly that the urinary \( \gamma \)-GT values decreased almost to normal within one week after tobramycin injections were stopped (Figure 1). The urinary \( \gamma \)-GT activities of 25–30 U/24 h that were found pre-operatively fell slightly to 18–23 U/24 h in the early postoperative period.

The high \( \gamma \)-GT/CC ratios found during tobramycin therapy (Table 1) had returned almost to normal pre-operatively, and became so postoperatively.

Serum \( \gamma \)-GT activity was persistently increased to between 55 and 90 U/liter at all times pre-operatively, but did not decrease postoperatively, a sample taken on April 29 giving a result of 79 U/liter.

\( \gamma \)-GT activities of homogenized tissues taken from the nephrectomy specimen are given in Table 2. The protein concentration of the tumor tissue could not be estimated satisfactorily because of the large amount of blood present. It can be seen that the activity of \( \gamma \)-GT (expressed as U/g fresh tissue) in the carcinomatous tissue is a quarter to a fifth that of normal renal cortical tissue, and is also less than that of renal medulla.

**Histology.** Figures 2–4 show respectively the \( \gamma \)-GT activity in renal cortex, renal medulla, and renal carcinoma. In Figure 2 the very high \( \gamma \)-GT activity is seen as dark staining in proximal tubules, particularly the “brush borders.” In medulla (Figure 3) the \( \gamma \)-GT activity is entirely confined to “casts” within the tubules. Many bacteria are visible. The carcinoma itself (Figure 4) shows small amounts of activity, mainly in the periphery of the cells.

<table>
<thead>
<tr>
<th>Date and situation</th>
<th>Urinary ( \gamma )-GT, 24-h spec. (normal 22–55)</th>
<th>( \gamma )-GT/CC ratio (normal 10.2–31.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>03-05-75 (admission; on tobramycin)</td>
<td>67</td>
<td>325.8</td>
</tr>
<tr>
<td>03-11-75 (end of tobramycin therapy)</td>
<td>117</td>
<td>427.2</td>
</tr>
<tr>
<td>03-25-75 (2 weeks post-tobramycin)</td>
<td>48</td>
<td>71.4</td>
</tr>
<tr>
<td>04-09-75 (6 days pre-op.)</td>
<td>30</td>
<td>43.2</td>
</tr>
<tr>
<td>04-27-75 (12 days postop.)</td>
<td>23</td>
<td>29.4</td>
</tr>
</tbody>
</table>

**Table 2. \( \gamma \)-GT Activities of Homogenates of Normal Renal Tissue and of Renal Carcinoma**

<table>
<thead>
<tr>
<th>Tissue examined</th>
<th>( \gamma )-GT activity, U/g fresh tissue</th>
<th>( \gamma )-GT activity, U/g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial renal cortex</td>
<td>17.8</td>
<td>310</td>
</tr>
<tr>
<td>Deep renal cortex</td>
<td>20.4</td>
<td>562</td>
</tr>
<tr>
<td>Renal medulla</td>
<td>6.0</td>
<td>196</td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td>4.0</td>
<td>—</td>
</tr>
</tbody>
</table>
Discussion

Serum. Only three studies (9-11) have specifically mentioned an increased \( \gamma \)-GT activity in the serum in cases of renal tumor. None of them gives a detailed analysis of their findings. The present case, although showing an increased serum \( \gamma \)-GT, lends no support to the idea that renal carcinoma is accompanied by an increase, because \( \gamma \)-GT activity remained constant for a considerable period postoperatively. Although the cause of the increase is unknown, it may be related to repair processes, as has been suggested (12) for heart disease.

Urine. Kley et al. (2) are the only authors to mention urinary \( \gamma \)-GT in renal tumor, but they give no details, and their results are difficult to interpret. We at first attributed the increased values for urinary \( \gamma \)-GT in the present case to the renal carcinoma. The presence of \( E. \) coli does not affect urinary \( \gamma \)-GT activity (6). The \( \gamma \)-GT activity that we found in the homogenized carcinoma might be low because of contamination by a considerable quantity of blood. However, the histochemistry confirmed that the \( \gamma \)-GT activity of the carcinoma was low, and was considerably less than that of normal renal cortex. The carcinoma, therefore, would contribute little to the urinary \( \gamma \)-GT activity.

The urinary \( \gamma \)-GT values returned to normal after treatment with tobramycin was stopped. The slight decrease in urinary \( \gamma \)-GT activity that occurred postoperatively would be expected with removal of a partly functioning kidney. We have shown that tobramycin does not affect the \( \gamma \)-GT assay in vitro.

The increase in urinary \( \gamma \)-GT values that results from administration of tobramycin is both interesting and important, in view of the doubt concerning nephrotoxicity of aminoglycoside antibiotics (13). The change in \( \gamma \)-GT (two- to three-fold the upper limit of normal) was even greater (10- to 15-fold normal) when the \( \gamma \)-GT/CC ratio was calculated, illustrating that the calculation of such a ratio provides a more useful index for the interpretation of the urinary \( \gamma \)-GT values than does excretion rate alone.

The change in urinary \( \gamma \)-GT was not associated with other evidence of renal dysfunction; serum creatinine and creatinine clearance remained constant, and proteinuria was insignificant. We do not know whether the change in urinary \( \gamma \)-GT was the result of renal damage or of enzyme induction. In view of the propensity of the liver to respond to drug administration by inducing \( \gamma \)-GT (14), such induction is a distinct possibility.

Kanamycin similarly increases urinary \( \gamma \)-GT (6), and a more extensive study of the effect of the aminoglycoside antibiotics on urinary \( \gamma \)-GT is in progress.

We thank Mr. R. Scott for allowing access to the patient while under his care during surgery. Thanks are also due to the Department of Histopathology, Royal Infirmary, for preparing the tissues before histochemistry and for taking the photomicrographs.

References