Comparative Effects of Gentamicin and Tobramycin on Excretion of N-Acetyl-$\beta$-$\delta$-Glucosaminidase

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Received 4 March 1985/Accepted 9 July 1985

Patients receiving gentamicin or tobramycin were studied to determine whether there were differences in the urinary excretion of N-acetyl-$\beta$-$\delta$-glucosaminidase (NAG) between the two groups. The average daily increases in NAG excretion were significantly higher in the group receiving gentamicin. In individual patients, the best correlation with nephrotoxicity was with high initial rates of NAG excretion. If abnormally high initial rates of increase can serve as a prognosticator of nephrotoxicity, it may be possible to alter aminoglycoside therapy and to avoid renal damage.

Aminoglycoside antibiotics remain an important component of the therapy for infections caused by gram-negative bacilli. The potential for nephrotoxicity has, however, limited the clinical usefulness of these agents. Various parameters have been examined in an attempt to detect nephrotoxicity during its earliest stages, including measurements of urinary casts, $\beta$-2-microglobulin, and lysosomal enzymes (1, 6, 15, 17, 19). Of the lysosomal enzymes excreted into the urine, N-acetyl-$\beta$-$\delta$-glucosaminidase (NAG) appears to be the most sensitive indicator of renal injury (1, 8, 13). Furthermore, assays for measurement of NAG levels are readily adaptable to most clinical microbiology laboratories.

Gentamicin and tobramycin are the two most commonly used aminoglycosides. The results of several animal and clinical comparative studies suggest that tobramycin may be the less nephrotoxic agent (7, 18, 21) although considerable controversy still exists regarding the clinical significance of these observations (3, 4, 22). Various risk factors may accentuate the potential for nephrotoxicity, thus confounding the ability to predict impending nephrotoxicity in any given patient (2, 5, 11, 14, 20). In an attempt to improve the early detection and predictability of nephrotoxicity, this study was designed to examine the kinetics of urinary NAG excretion during and after the clinical use of gentamicin and tobramycin.

MATERIALS AND METHODS

The activity of NAG was assayed fluorometrically by modifications of the methods of Leaback and Walker (9), Price et al. (13), and Wellwood et al. (23). The substrate, 4-methylumbelliferyl-$n$-acetyl-$\beta$-$\delta$-glucosaminide (Sigma Chemical Co., St. Louis, Mo.), was dissolved by gentle heating at a concentration of 0.5 mg/ml in C-P buffer (0.05 M citrate, 0.1 M sodium phosphate [pH 4.5]). The substrate mixture was stable at 4°C for up to 1 week. The reaction mixture contained 1.8 ml of substrate and 0.2 ml of urine, diluted at least eightfold with C-P buffer. This final concentration of substrate ensured that the reaction rate was dependent on the amount of enzyme present in the urine. Urinary enzyme activity was stable at 4 or $-20^\circ$C for up to 1 week after collection. As noted by Wellwood et al. (23), centrifugation or dialysis of urine before assay was not necessary. Urine samples were obtained from single excretions, usually as first-morning specimens.

After incubation at 37°C for 30 min, the reaction was stopped by the addition of 3.0 ml of 0.5 M glycine (pH 10.4). The fluorescence of the released methylumbellifereone was measured in a Beckman Acta II spectrophotometer equipped with a fluorescence accessory (Beckman Instruments, Inc., Fullerton, Calif.), using an excitation wavelength of 373 nm and a standard curve based on 1.0 $\mu$g of methylumbellifereone $= 100\%$ transmission. Urine samples were assayed in triplicate, and internal methylumbellifereone standards were included to assess both recovery and quenching. (The presence of gentamicin, tobramycin, or cephalosporin, in concentrations up to 1 mg/ml, had no effect on fluorescence or the assay.) The final results were expressed as nanomoles of methylumbellifereone released per hour of assay per milligram of urine creatinine.

Assays of urine creatinine, serum creatinine, and blood nitrogen were all performed by automated methods by the clinical laboratories. Aminoglycoside levels were assayed by a commercially available radioimmunoassay.

A total of 22 patients, ranging in age from 36 to 84 years, were studied. There were no significant differences in age between the group of 10 patients receiving gentamicin and the group of 12 patients receiving tobramycin. The underlying diagnoses and indications for antibiotic treatment were comparable in both groups. Four patients in each group received additional cephalosporin therapy. Both groups received comparable amounts of daily (means of 220 versus 250 mg) and total (means of 2,050 versus 2,600 mg) aminoglycosides and were treated for similar durations (means of 8.9 versus 9.1 days). Some of these data are displayed in Tables 1 and 2.

RESULTS

Soon after the administration of either aminoglycoside antibiotic, the excretion of NAG into the urine increased over that of base-line and control values (Fig. 1). Excretion rates appeared to be generally higher with gentamicin, and the differences between the average daily increases in urinary NAG values (5.691 ± 1.477 for gentamicin versus 3.296 ± 0.628 for tobramycin) were statistically significant ($P < 0.05$). Peak enzyme activities were observed at similar times during therapy (6.5 ± 1.0 days for tobramycin versus 7.0 ± 0.6 days for gentamicin).
1.4 days for gentamicin). After cessation of antibiotic therapy, urinary enzyme values rapidly declined, with no apparent differences in these rates between antibiotics.

Patients receiving gentamicin developed slightly greater changes in serum creatinine values (0.34 ± 0.25 mg/dl) during therapy than did the tobramycin-treated group (0.20 ± 0.09 mg/dl). Of the patients receiving gentamicin, two increased their serum creatinine by more than 0.6 mg/dl to values greater than 1.5 mg/dl, and one eventually required renal dialysis. In contrast, none of the tobramycin-treated group developed increases in serum creatinine values greater than 0.4 mg/dl.

When the data from individual patients were replotted as the slopes of the initial rates of increase to peak levels, differences in patient responses were more readily observable (Fig. 2). Two groups of responders appeared in the tobramycin-treated group, and another group with distinctly higher slopes (≥4.0) was apparent in the gentamicin-treated group. Of the three patients in the gentamicin-treated group with the steepest slopes, two developed the greatest increases in urinary NAG and serum creatinine values. The other patient with the steepest slope (i.e., 8.0) had acute pancreatitis, and the enzyme elevations may have been due to pancreatic, rather than renal, damage. Patients in either treatment group who developed only minimal increases in urinary NAG had little or no changes in their serum creatinine. Positive correlations between the rates of increased NAG and increases in serum creatinine were also apparent in patients composing the intermediate group of responders.

**DISCUSSION**

The greatest limitation in the clinical use of aminoglycosides is their potential for nephrotoxicity. A variety of early indicators of toxicity have been sought, but no consensus has been reached on the best prognostic test (1, 3, 6, 8, 13, 15, 17). Logically, if the release of cellular enzymes is an indication of toxicity, as it seems to be, then a quantitative analysis of these enzymes should correlate with toxicity. Previous studies, as well as our current one, have tended to support this relationship. The novel observation made in this study is that the initial rate of increase may be the key indicator of toxicity, perhaps even more so than the absolute amount of enzyme excretion at any given point in time. From the data collected, slopes of <1.5 were not associated

**TABLE 1. Patients receiving gentamicin**

| Patient no. | Age | Diagnosis* | Length of treatment (days) | Amt of gentamicin (mg) | Other antibiotics* | Serum creatinine (mg/dl) | Increase during therapy | Increase after therapy | Peak | Slope of increase in NAG%
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<tr>
<td>1</td>
<td>43</td>
<td>UTI</td>
<td>10</td>
<td>1,800</td>
<td>Ce</td>
<td>0.1</td>
<td>0.3</td>
<td>0.7</td>
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<td>2</td>
<td>48</td>
<td>Penile implant; prophylaxis</td>
<td>2</td>
<td>400</td>
<td>Ce</td>
<td></td>
<td>1.4</td>
<td>0.2</td>
<td></td>
<td></td>
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<td>3</td>
<td>72</td>
<td>UTI</td>
<td>15</td>
<td>3,420</td>
<td>Cl</td>
<td>0.3</td>
<td>1.0</td>
<td>0.8</td>
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<tr>
<td>4</td>
<td>65</td>
<td>Myonecrosis</td>
<td>17</td>
<td>3,240</td>
<td>Cl</td>
<td>0.6</td>
<td>1.5</td>
<td>0.6</td>
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<td>5</td>
<td>40</td>
<td>Acute pancreatitis</td>
<td>8</td>
<td>2,180</td>
<td>Cl</td>
<td>0.2</td>
<td>0.7</td>
<td>8.0</td>
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<td>Radiation cystitis</td>
<td>6</td>
<td>1,290</td>
<td>Ce</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
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<td>7</td>
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<td>Osteomyelitis</td>
<td>7</td>
<td>1,920</td>
<td>Ce</td>
<td>0.2</td>
<td>1.0</td>
<td>0.1</td>
<td></td>
<td></td>
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<tr>
<td>8</td>
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<td>Septicemia</td>
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<td>1,560</td>
<td>Ce</td>
<td>1.4</td>
<td>2.8</td>
<td>4.0</td>
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<td>9</td>
<td>47</td>
<td>Aspiration pneumonia</td>
<td>10</td>
<td>2,970</td>
<td>Ce</td>
<td>0.1</td>
<td>2.6</td>
<td>1.0</td>
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<tr>
<td>10</td>
<td>70</td>
<td>Infected skin ulcer</td>
<td>5</td>
<td>1,800</td>
<td>Ce</td>
<td>0.8</td>
<td>9.1</td>
<td>11.0</td>
<td>4.2</td>
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*UTI, Urinary tract infection.

* Ce, Cephalosporin; Cl, clindamycin.

† Calculated slopes of initial rates of increase in urinary NAG excretion (Fig. 2).

‡ ND, Not determined.

**TABLE 2. Patients receiving tobramycin**

| Patient no. | Age | Diagnosis* | Length of treatment (days) | Amt of tobramycin (mg) | Other antibiotics* | Serum creatinine (mg/dl) | Increase during therapy | Increase after therapy | Peak | Slope of increase in NAG%
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<tr>
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<td>UTI</td>
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<td>1,680</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td>2</td>
<td>36</td>
<td>UTI</td>
<td>9</td>
<td>2,400</td>
<td>Ce</td>
<td>ND</td>
<td>ND</td>
<td>1.2</td>
<td>0.3</td>
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<td>3</td>
<td>67</td>
<td>BPH; prophylaxis</td>
<td>4</td>
<td>1,050</td>
<td>Ce</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
<td></td>
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<td>4</td>
<td>48</td>
<td>Epididymitis</td>
<td>4</td>
<td>1,200</td>
<td>Ce</td>
<td>ND</td>
<td>ND</td>
<td>2.4</td>
<td>1.0</td>
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<td>69</td>
<td>UTI</td>
<td>10</td>
<td>2,100</td>
<td>Ce</td>
<td>ND</td>
<td>ND</td>
<td>1.4</td>
<td>1.1</td>
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<tr>
<td>6</td>
<td>51</td>
<td>Aspiration pneumonia</td>
<td>14</td>
<td>5,220</td>
<td>Cl</td>
<td>ND</td>
<td>ND</td>
<td>1.4</td>
<td>0.2</td>
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<td>7</td>
<td>72</td>
<td>Incontinence; prophylaxis</td>
<td>12</td>
<td>3,120</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.4</td>
<td>0.2</td>
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<tr>
<td>8</td>
<td>47</td>
<td>UTI; wound infection</td>
<td>9</td>
<td>2,400</td>
<td>Ce</td>
<td>ND</td>
<td>ND</td>
<td>1.9</td>
<td>1.4</td>
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<td>9</td>
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<td>UTI</td>
<td>5</td>
<td>1,260</td>
<td>Ce</td>
<td>ND</td>
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<td>1.0</td>
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<td>10</td>
<td>65</td>
<td>Cholecystitis</td>
<td>13</td>
<td>4,200</td>
<td>Ce</td>
<td>ND</td>
<td>ND</td>
<td>1.2</td>
<td>1.2</td>
<td></td>
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<tr>
<td>11</td>
<td>71</td>
<td>UTI</td>
<td>17</td>
<td>1,920</td>
<td>Ce</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
<td></td>
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<tr>
<td>12</td>
<td>59</td>
<td>Infected penile prosthesis</td>
<td>16</td>
<td>5,100</td>
<td>Ce</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
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*UTI, Urinary tract infection; BPH, benign prostate hypertrophy.

* Ce, Cephalosporin; Cl, clindamycin; Tc, tetracycline; Md, macrodantin.

† Calculated slopes of initial rates of increase in urinary NAG excretion (Fig. 2).

‡ ND, Not determined.
with nephrotoxicity, and the two patients with nephrotoxicity had slopes of 4.0 and 4.2. This observation seems worthy of further testing and, if confirmed, may prove very useful in monitoring the potential nephrotoxicity of aminoglycoside therapy. Whether nephrotoxicity could actually be averted by earlier modification or cessation of aminoglycoside therapy also needs to be evaluated, even though it seems probable that it would. Current detection parameters (e.g., serum creatinine and aminoglycoside levels) frequently offer little or late correlation with nephrotoxicity, and a better test is needed. The assay of urinary NAG could be readily accomplished by most clinical laboratories and would be less expensive than an assay of aminoglycoside levels.

Normally, the urinary excretion of NAG proceeds at low, but readily detectable, levels. The usual sources of enzymes include the pancreas (10, 12, 16) and the kidney tubule. One of our patients had acute pancreatitis and very large amounts of NAG in his urine without evidence of nephrotoxicity. (That hepatic disease secondary to alcoholic cirrhosis is not associated with high urinary NAG levels was shown by the absence of such elevations in several of our patients who had active liver disease.) Further studies will be needed to evaluate the relationship between pancreatitis and urinary NAG levels and to assess the potential value of this test in the detection of acute pancreatitis.

In this study, the only patients who developed nephrotoxicity were in the gentamicin-treated group. The numbers of patients were too small, however, to confirm previous claims of the greater nephrotoxicogenic potential of gentamicin. Of greater importance is the need to define which patient is at greater risk for nephrotoxicity. Wide interpatient variations in aminoglycoside elimination rates and dosage requirements have been noted (24, 25). Each aminoglycoside derivative has been associated with nephrotoxicity, and a more sensitive prognostic indicator may offer the most important improvement in our current practices. It is possible that further study of the relationship between the rapidity of increase (slope) in urinary NAG and nephrotoxicity will define a subset of the population with an inherent or otherwise greater risk of nephrotoxicity.

ACKNOWLEDGMENT
These studies were supported by a grant from Eli Lilly & Co.

LITERATURE CITED


