Alanine Aminopeptidase and β₂-Microglobulin Excretion in Patients Receiving Vancomycin and Gentamicin

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The effects of vancomycin, gentamicin, and combination vancomycin-gentamicin treatments on alanine aminopeptidase (AAP) and β₂-microglobulin (β₂M) elimination in 30 hospitalized patients were assessed and compared with elimination in a control group. Twenty-four-hour urine excretion values for AAP and β₂M were determined on treatment day 1 and day 5 for patients receiving the three treatment regimens and for the control group. AAP excretion values for the vancomycin-treated group were not found to be statistically different from those of the control group. Both the gentamicin and the vancomycin-gentamicin groups had statistically higher AAP excretion values on treatment day 1 as well as on treatment day 5 when compared with the vancomycin and control groups. AAP excretion on day 5 of treatment was highest for the vancomycin-gentamicin group. Overall, β₂M elimination was variable in all treatment groups. Although the β₂M values were elevated as early as day 1 in all treatment groups, they were significantly elevated only in the vancomycin-gentamicin group on day 1 and only in the gentamicin group on day 5 compared with the vancomycin and the control groups. AAP appears to be a sensitive indicator of renal tubular damage. The combination of vancomycin and gentamicin results in greater AAP excretion than does either agent alone.

Renewed interest in vancomycin, because of the increase in methicillin-resistant Staphylococcus aureus, has coincided with a concern over its potential for nephrotoxicity (13, 14, 22, 23, 25). In addition, the combination of vancomycin and an aminoglycoside has remained an important modality for the treatment of a number of infections (7, 9, 23). Although there appear to be a number of treatment benefits from this combination, it may potentiate nephrotoxicity (3, 7, 22, 23, 27).

The traditional clinical parameter used to detect drug-induced nephrotoxicity has been serum creatinine; however, various indicators have been examined to identify renal toxicity in its earliest stages (4, 5, 8, 18). The plasma protein β₂-microglobulin (β₂M) and the proximal-tubule brush border enzyme alanine aminopeptidase (AAP) have been identified as sensitive indicators of renal injury (4, 18–20, 26). While the mechanism of aminoglycoside-induced tubular toxicity has been described and shown to correlate with increased β₂M and AAP urinary elimination, the vancomycin mechanism of action for nephrotoxicity has yet to be determined (1, 16, 17). In animal studies, kidney damage secondary to treatment with both aminoglycosides and vancomycin has been reported and the mechanisms of the drugs have been postulated as similar (1, 15, 16, 28, 29). To determine the significance of this potentiation of nephrotoxicity, we examined the elimination of AAP and β₂M from patients receiving vancomycin alone, gentamicin alone, or concomitant vancomycin and gentamicin.

MATERIALS AND METHODS

A total of 40 subjects participated in this study. Ages ranged from 18 to 81 years, with a mean of 45.0 ± 7.7 years.

Ten patients (nine male and one female) not receiving an aminoglycoside or vancomycin served as the in-patient control group. Of the remaining 30 subjects, 10 received gentamicin (8 male and 2 female), 10 received vancomycin (5 male and 5 female), and 10 received both gentamicin and vancomycin (8 male and 2 female). Mean demographic data for control and treatment groups are listed in Table 1.

Informed consent was obtained from all subjects prior to participation in the study. Exclusion criteria consisted of any known hypersensitivity to vancomycin or gentamicin, prior exposure to either study agent, prior exposure to nephrotoxic or cytotoxic agents, significant liver or renal disease as determined by comparison to standard laboratory reference values, and recent surgery or infection of the upper urinary tract.

Twenty-four-hour urine collections were obtained from all subjects on day 1 of therapy and were repeated on day 5. From each urine collection, two 10-ml postagulation aliquots were removed for storage. One specimen was stored at 2°C and used for AAP determination while the second specimen was stored at −20°C for urinary creatinine and β₂M quantification. All specimens were assayed within 4 weeks of collection.

The activity of AAP was assayed spectrophotometrically by using modifications of the methods of Mondorf (19) and Jung and Scholz (10). The substrate mixture was uniformly prepared immediately prior to laboratory analysis and protected from light. The reaction mixture contained 0.15 ml of L-alanine-p-nitranilide as the substrate, 1.0 ml of phosphate buffer, and 0.2 ml of patient urine. AAP activity was determined at 37°C at a wavelength of 405 nm. The amount of AAP activity was determined by measuring the change in absorbance due to the release of 4-nitranilide. The spectrophotometer used was a Beckman 34 (Beckman Instruments, Inc., Irvine, Calif.). Stability of AAP at 2°C was determined...
by using stored standard samples over a 4-week period. No significant loss of activity was noted (<0.5%). The coefficient of variation between day runs ranged from 1.1% (50 U/liter, standard) to 8.4% (6.25 U/liter, standard). Since enzyme elimination and 24-h creatinine production are known to be greater per unit time in men than in women, volume variations encountered during the investigation were corrected for by dividing the 24-h AAP elimination by 24-h urinary creatinine elimination, thus removing any possible sex bias (18). The final results were expressed as units of AAP per 24 h per gram of urinary creatinine. Urinary \( \beta_2 \) M quantification was performed by a radioimmunosorbent assay (Beta-2 Micro RIA 100; Pharmacia, Uppsala AB, Sweden). The sensitivity detection limit for this assay is less than or equal to 0.4 mg/liter. No loss of activity has been reported in \( \beta_2 \) M samples stored at \(-20^\circ\mathrm{C}\) for up to 4 weeks (20). Data for \( \beta_2 \) M excretion were also recorded for volume by using 24-h urinary creatinine elimination rates as a standard (24).

Assays of urine creatinine, serum creatinine, and blood urea nitrogen were all performed by using standard automated methods in the clinical laboratory with an ASTRA-8 (Beckman Instruments). Actual creatinine clearances for all subjects were calculated on treatment day 1 as well as on day 5. Creatinine clearance rates were determined by the method of Wagner (21). All doses of gentamicin and vancomycin were diluted in 50 to 100 ml of 5% glucose in water or 0.9% sodium chloride and infused for 3 to 5 min to 1 h. Gentamicin and vancomycin peak and trough concentrations in serum were determined on days 3 to 5 as part of routine monitoring. Trough concentrations in serum were obtained within 2 min prior to the dose, and peak concentrations in serum were obtained for gentamicin and vancomycin at 30 min and 1 h postinfusion, respectively. Aminoglycoside and vancomycin concentrations in serum were determined by a commercially available fluorescence polarization immunoassay (TDX; Abbott Laboratories, Irving, Tex.).

A multifactorial, repeated-measures model of analysis of variance was used to assess the differences in response variables (SAS Statistical Software, Cary, N.C.). Intergroup differences were assessed by the Tukey test for multiple comparisons. The level of significance was set at \( P < 0.05 \).

## RESULTS

Antibiotic dosages were not statistically different between the groups when like agents were compared. Mean dosages and peak and trough concentrations in serum for each treatment group are listed in Table 1. All patients included had serum creatinine values on treatment day 1 not greater than 2.0 mg/dl. Actual creatinine clearances, as calculated from 24-h urine collections, ranged from 35 to greater than 120 ml/min. There were no significant differences in base-line creatinine clearance values for the control or treatment groups. Although no changes were noted in creatinine clearance values for any of the treatment groups, two patients (one in the gentamicin group and one in the vancomycin-gentamicin group) had an increase in serum creatinine during the study of greater than 0.5 mg/dl. Mean creatinine clearance values for control and treatment groups are listed in Table 1.

Mean AAP and \( \beta_2 \) M excretion values, corrected for urine volume by using urinary creatinine values for control and treatment groups, are listed in Table 2. Overall, AAP excretion values for the vancomycin group were not found to be statistically different from that of the control group for treatment day 1 or day 5. The AAP excretion values in the gentamicin and vancomycin-gentamicin groups, however, were found to be greater \( (P < 0.05) \) than those in either the vancomycin or control groups on day 1 as well as on day 5. The AAP excretion was significantly greater in the vancomycin-gentamicin group than in the gentamicin group on day 5. In addition, the excretion of AAP rose significantly

## TABLE 1. Patient demographic data

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Treatment</th>
<th>CLcr (ml/min per 1.73 m²)</th>
<th>Dose (mg/kg per day)</th>
<th>Conc in serum (µg/ml)</th>
<th>Infection diagnosis (no. of cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38.0 ± 17.5</td>
<td>Control</td>
<td>126.1 ± 42.2</td>
<td></td>
<td></td>
<td>Pneumonia (2), leg abscess (1), hand abscess (1), bronchitis (1), osteomyelitis (1), empiric treatment for open fracture (2), sepsis (2)</td>
</tr>
<tr>
<td>36.1 ± 7.4</td>
<td>Vancomycin</td>
<td>96.9 ± 25.3</td>
<td>29.6 ± 9.9</td>
<td>30.2 ± 6.0</td>
<td>7.3 ± 4.7</td>
</tr>
<tr>
<td>51.8 ± 19.5</td>
<td>Gentamicin</td>
<td>89.4 ± 29.5</td>
<td>3.6 ± 2.6</td>
<td>5.0 ± 1.3</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>51.0 ± 17.1</td>
<td>Vancomycin-gentamicin</td>
<td>108.2 ± 18.3</td>
<td>24.4 ± 12.2</td>
<td>30.5 ± 7.8/5.4 ± 1.6</td>
<td>8.6 ± 0.7/0.8 ± 0.4</td>
</tr>
</tbody>
</table>

* All values are means ± standard deviation.

## TABLE 2. Mean urine excretion data

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Excretion* of:</th>
<th>Day 1</th>
<th>Day 5</th>
<th>β₂M (mg/day)</th>
<th>Day 1</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAP (U/day)</td>
<td>20.5 ± 9.1</td>
<td>24.5 ± 19.5</td>
<td>1.6 ± 2.7</td>
<td>0.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.0 ± 20.2</td>
<td>36.8 ± 40.6</td>
<td>14.4 ± 12.3</td>
<td>13.4 ± 14.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>115.1 ± 86.9</td>
<td>207.5 ± 108.4</td>
<td>29.1 ± 21.0</td>
<td>54.2 ± 40.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>167.7 ± 149.2</td>
<td>285.2 ± 202.0</td>
<td>31.9 ± 38.5</td>
<td>34.2 ± 37.8</td>
<td></td>
</tr>
</tbody>
</table>

* Corrected for urine volume by using urinary creatinine values. All values are means ± standard deviations.
between day 1 and day 5 in the gentamicin and vancomycin-gentamicin groups (Table 2).

There appeared to be wide variability in \( \beta_2 \)M excretion for both day 1 and day 5 as indicated by the mean excretion values compared with that of the control group (Table 2). Although excretion of \( \beta_2 \)M appeared to be higher for all treatment groups than for the control group on both day 1 and day 5, the values were statistically significant only for the vancomycin-gentamicin group on day 1 and the gentamicin group on day 5. In addition, there was no significant elevation in \( \beta_2 \)M excretion between day 1 and day 5 for any of the groups studied.

Finally, we found no correlation between AAP or \( \beta_2 \)M excretion and peak or trough concentrations of either agent in serum during the study period.

**DISCUSSION**

Aminoglycosides have been well described as being nephrotoxic in both animals and humans, yet the potential of vancomycin for inducing renal damage remains controversial (2, 6, 11, 15-19, 26). Wold and Turnipseed demonstrated that the combination of vancomycin and an aminoglycoside resulted in a higher incidence of nephrotoxicity in the rat model than either agent alone (28). In a more recent study, Wood et al. determined histologically that rats treated with the combination of vancomycin and tobramycin showed a greater degree of proximal tubular necrosis than rats treated with either agent alone (29). In addition, information regarding the combination of vancomycin with aminoglycosides in patients thus far indicates a higher risk of nephrotoxicity with the combination than with either agent alone (7, 20, 25, 28, 29).

Various attempts have been made to find diagnostic indicators of early renal damage in humans, yet no consensus has been reached as to which has the most accurate prognostic value (4, 5, 8, 18, 19, 24, 26). AAP excretion in urine has been previously studied in patients receiving aminoglycoside therapy (4, 8, 11, 18, 19, 24). Several investigators have reported significant correlations between AAP excretion and decreased renal function (4, 8, 11, 18, 19). As with other early indicators of renal damage, elevations of AAP in urine precede increases in serum creatinine in patients with renal toxicity (4, 8, 11). We chose to utilize the proximal-tubule enzyme AAP in this study because of its specificity to the proximal-tubule cell, since this is the area in which aminoglycosides and possibly vancomycin inflict their damage. \( \beta_2 \)M was used for comparison purposes since it has traditionally been used to detect renal damage. It is a low-molecular-weight (12,000) plasma protein synthesized by the nucleated cells of the body and is normally filtered and completely reabsorbed by the proximal tubule of the kidney (11, 20). Disorders affecting the epithelium cells of these tubules will cause enhanced excretion into the urine (8). Increased excretion of \( \beta_2 \)M in the urine has been found to correlate with altered renal function secondary to aminoglycoside exposure (8, 18, 24, 26).

It was interesting to note that significantly greater AAP excretion occurred on day 5 in patients who received the combination of vancomycin-gentamicin than in those who received gentamicin alone. In addition, patients treated with vancomycin alone did not have significant changes in AAP excretion compared with control patients. Although we could not confirm a relationship between AAP excretion and development of nephrotoxicity because of the short duration of the study, the AAP assay was sensitive enough to detect differences in enzymuria between the various treatment groups. Previous studies with AAP and other urinary enzymes have supported the relationship between enzymuria and toxicity (4, 8, 12, 18, 19). In addition, the enhanced enzymuria that occurred in the vancomycin-gentamicin patients may indicate that the vancomycin mechanism of nephrotoxicity is similar to that of aminoglycosides. Data obtained from animal studies may support this hypothesis (15, 16). Marre et al. demonstrated a significant reduction in vancomycin renal toxicity when rats received vancomycin in combination with D-glucaro-1,5-lactam, a substance known to protect against aminoglycoside-induced renal damage (16). Further studies in this area may allow us to correlate changes in AAP excretion with renal toxicity in this patient population. Although there appeared to be differences between the groups in mean excretion of \( \beta_2 \)M as early as treatment day 1, these differences reached statistical significance only for the vancomycin-gentamicin treatment group on day 1 and the gentamicin treatment group on day 5, compared with the vancomycin-alone treatment group or the control group. Large interpatient variability in our study may have accounted for this discrepancy in the excretion of \( \beta_2 \)M. It is also possible that the study was too short to allow a significant change in \( \beta_2 \)M excretion to be noted (24). In addition, low urine pH (<5.5) has been shown to affect \( \beta_2 \)M activity (11). Since we did not routinely test pHs of our urine samples, it is possible that this also contributed to our variable \( \beta_2 \)M results. However, review of the medical charts of our patients revealed that urinalyses obtained on days of urine collection indicated urine pHs of equal to or above 5.5 (5.5 to 8.0). Although our patients appeared to be well matched for dose, mean peak and trough concentrations in serum, and renal function, differences in age and severity of illness were unavoidable due to the differences in types of infections for which these drugs are indicated. It is possible that these variables played a part in the outcome of our study. However, we are unaware of any specific reports which suggest that these factors have a significant effect on AAP and \( \beta_2 \)M excretion.

It appears that AAP elimination is a sensitive indicator of tubular damage and is capable of detecting differences in enzymuria among the various treatment groups. The administration of gentamicin appears to cause low enzymuria, which may indicate a lower potential for inflicting renal tubular damage. In addition, the combination of vancomycin and gentamicin appears to result in greater excretion of AAP when either agent administered alone, possibly an indication of additive tubular damage. With the recent resurgence in the use of vancomycin and with literature supporting its combination with an aminoglycoside, further study is needed to determine whether AAP excretion can predict impending nephrotoxicity in this patient population.

**LITERATURE CITED**

4. Davey, P. G., and A. M. Geddes. 1983. Study of alanine aminopeptidase excretion as a test of gentamicin nephrotox-