The Significance of β-2 Microglobulinuria Associated with Gentamicin Therapy*

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ABSTRACT

Gentamicin is a nephrotoxic agent known to damage the proximal tubule,—a site of low molecular weight (LMW) protein reabsorption and catabolism. The effect of gentamicin was investigated on three LMW proteins—amylase, light chains, and β2 microglobulin—and the effects were correlated on the latter to renal function as determined by creatine clearance (GFR).

The renal excretion of β2 microglobulin (β2M) was studied in 18 patients receiving gentamicin and eight control patients. Both gentamicin and control patients had similar mean ages and serum β2M. Twelve of the 18 gentamicin treated patients had marked increases in β2M excretion. The mean daily β2 microglobulin excretion for the gentamicin treated group was 10,511 μg while that of the control group was 102 μg.

Serial determinations in 10 of the gentamicin treated patients revealed an increase in β2M excretion within 48 hours of starting therapy. No deterioration of GFR was seen in any patient. In four patients, β2M excretion decreased while still receiving gentamicin. The renal handling of amylase was found to be normal in four patients and mildly abnormal in three patients receiving gentamicin who also had increased β2M excretion. Urinary light chains were determined in four of these seven patients and found to be normal. It is concluded that gentamicin induces an early and often transient tubular proteinuria. This tubular proteinuria is not associated with clinical nephrotoxicity.

Introduction

Gentamicin is an effective and frequently used aminoglycoside antibacterial agent. A major complication of therapy, however, is nephrotoxicity which is characterized clinically by a rising serum urea nitrogen and creatinine associated with a falling glomerular filtration rate and a defect in urine concentrating ability.1,24,36 Gentamicin is known to be concentrated in the proximal tubular

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β-2 microglobulinuria associated with gentamicin therapy

Proximal tubular cells have several functions, one of which is the reabsorption and catabolism of low molecular weight proteins such as insulin, growth hormone, light chains, amylase, and β-2 microglobulin. Human β-2 microglobulin is a low molecular weight protein associated with the human leukocyte antigen (HLA) system (MW = 11,800) and is present in both serum and urine. This protein freely passes through the glomerular capillary membrane and is reabsorbed by and catabolized in the proximal tubules. In subjects with normal creatinine clearance and tubular function, the concentrations found in the urine are quite low.

There are several disorders characterized by proximal tubular dysfunction in which an increase in urinary β-2 microglobulin excretion is found. Cystinosis, cadmium poisoning, Wilson's disease, pancreatitis, and Minimata's disease are examples. More recently, the phenomenon has been described in association with aminoglycoside therapy. The urinary excretion of β-2 microglobulin has been studied by us to examine what relationship such a finding might have to clinical nephrotoxicity.

**Materials and Methods**

Serum and 24-hour urine collections were obtained from 26 patients who were hospitalized at the Roger Williams General Hospital. Eighteen of these patients received a course of gentamicin, while the other eight who were not treated with gentamicin acted as control subjects. The mean age of the gentamicin group was 68 while that of the untreated group was 71. (In table I are listed the diagnoses and drug therapy for both groups.) Twenty-four urine collections were obtained as early in the course of gentamicin treatment as possible, usually within 48 hours of the initial dose, and serial collections were made on 14 patients, usually at two to four day intervals during the duration of treatment. Serum was analyzed for creatinine, amylase, and β-2 microglobulin. Urine samples were assayed for creatinine, β-2 microglobulin, and total protein. Selected urine samples were analyzed for amylase and subjected to immunoelectrophoresis. Creatinine clearance and amylase/creatinine clearance ratios were calculated by standard techniques.

Beta-2 microglobulin was assayed in the serum in urine by a radioimmunoassay technique. The suppliers' normal values are 1.1 to 2.4 mg per L for serum and 30 to 370 μg per 24 hours for urine. The manufacturer's control sample and a frozen urine pool were used to establish day-to-day reproducibility. The manufacturer's supplied β-2 microglobulin reference serum assayed to a mean of 0.92 mg per L with a day-to-day coefficient variation of 13 percent (N = 20). A urine pool assayed to a mean of 133 μg per L with a CV of 23 percent (N = 16). Aliquots of a single urine sample were frozen, stored at 12°C, and assayed over a four month period to establish the stability of frozen samples. The urine pool was stable for the entire period. The pH was determined on all patient samples when received in the laboratory. In all but one case, the pH was greater than 6.5. One sample was treated with sodium bicarbonate prior to storage. Creatinine assays, both serum and urine, were performed by a standard procedure using the Jaffe reaction. Serum and urine amylase assays were performed according to the method of Caraway. Urine total protein was assayed by trichloroacetic acid turbidimetry. Immunelectrophoresis was performed on serum and urine (50-fold concentration) in agar gel against anti-human serum, anti-IgA, anti-IgG,

* Pharmacia Diagnostics, Uppsala-Sweden.
## TABLE I

**Gentamicin**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Drugs</th>
<th>GFR ml/min</th>
<th>Urine B2 µg/day</th>
<th>Plasma B2 mg/l</th>
<th>Urine B2 C %</th>
<th>C. Creat.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 79 F</td>
<td>Urinary tract infection, sepsis Cholecystitis</td>
<td>Ampicillin, carbenicillin, cephaloxin</td>
<td>27</td>
<td>46,200</td>
<td>2.3</td>
<td>55</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 79 F</td>
<td>Urinary tract infection, sepsis</td>
<td>Furosemide, carbenicillin, ampicillin</td>
<td>9</td>
<td>899</td>
<td>6.6</td>
<td>4.73</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D 43 M</td>
<td>Pneumonia</td>
<td>Furosemide, carbenicillin, ampicillin</td>
<td>100</td>
<td>248</td>
<td>1.4</td>
<td>0.17</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E 70 M</td>
<td>Pneumonia</td>
<td>Furosemide, carbenicillin, ampicillin</td>
<td>42</td>
<td>6,336</td>
<td>2.1</td>
<td>9.26</td>
<td>5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 83 M</td>
<td>Urinary tract infection</td>
<td>Ampicillin, carbenicillin, cephaloxin</td>
<td>100</td>
<td>13</td>
<td>2.7</td>
<td>0.01</td>
<td>0.003</td>
<td></td>
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<tr>
<td>G 84 M</td>
<td>Sepsis, cholangitis</td>
<td>Ampicillin, carbenicillin, cephaloxin</td>
<td>74</td>
<td>7,280</td>
<td>1.6</td>
<td>7.57</td>
<td>4.2</td>
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<tr>
<td>I 73 F</td>
<td>Chronic obst., lung disease, pneumonia</td>
<td>Penicillin, oxacillin, simethide, furosemide</td>
<td>34</td>
<td>43,200</td>
<td>5.3</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>L 78 F</td>
<td>Subacute bacterial endocarditis</td>
<td>Penicillin, oxacillin, furosemide</td>
<td>55</td>
<td>840</td>
<td>-</td>
<td>1.87</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>O 73 F</td>
<td>Pneumonia</td>
<td>Penicillin, oxacillin</td>
<td>54</td>
<td>6,375</td>
<td>1.4</td>
<td>10.2</td>
<td>5.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 89 F</td>
<td>Infected decubiti</td>
<td>Ampicillin, oxacillin</td>
<td>32</td>
<td>9,936</td>
<td>5.0</td>
<td>15.33</td>
<td>4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q 74 M</td>
<td>Pneumonia, decubiti, hypertension</td>
<td>Ampicillin, oxacillin, cephaloxin</td>
<td>40</td>
<td>50,000</td>
<td>5.8</td>
<td>71.4</td>
<td>17.2</td>
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<tr>
<td>T 56 M</td>
<td>Cholecystitis, pulmonary edema, wound infection</td>
<td>Lasix, oxacillin, erythromycin</td>
<td>38</td>
<td>7,248</td>
<td>3.6</td>
<td>13.</td>
<td>8</td>
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<tr>
<td>U 80 F</td>
<td>Pneumonia, hypercalc., urinary tract infection</td>
<td>Ampicillin, furosemide, carbenicillin</td>
<td>18</td>
<td>48</td>
<td>8.0</td>
<td>0.01</td>
<td>0.2</td>
<td></td>
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<tr>
<td>V 32 M</td>
<td>Abdominal wound</td>
<td>Cefazolin, furosemide, clindamycin</td>
<td>136</td>
<td>164</td>
<td>-</td>
<td>0.14</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>W 52 F</td>
<td>Pyelonephritis</td>
<td>Cefazolin, clindamycin</td>
<td>174</td>
<td>86</td>
<td>2.8</td>
<td>0.04</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z 38 F</td>
<td>Sepsis, cirrhosis</td>
<td>Dialysate, clindamycin, clindamycin</td>
<td>69</td>
<td>745</td>
<td>1.5</td>
<td>0.04</td>
<td>0.5</td>
<td></td>
<td></td>
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<tr>
<td>CC 80 F</td>
<td>Fever unknown, etiology</td>
<td>Ampicillin, oxacillin, carbenicillin</td>
<td>15</td>
<td>415</td>
<td>5.8</td>
<td>0.65</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD 61 M</td>
<td>Diabetes, abdominal abscess</td>
<td>Cefazolin, clindamycin</td>
<td>72</td>
<td>9,170</td>
<td>-</td>
<td>11.0</td>
<td>-</td>
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</tbody>
</table>

### Non-Gentamicin Group

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Drugs</th>
<th>GFR ml/min</th>
<th>Urine B2 µg/day</th>
<th>Plasma B2 mg/l</th>
<th>Urine B2 C %</th>
<th>C. Creat.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B 69 M</td>
<td>Rheumatoid arthritis</td>
<td>Gold</td>
<td>18</td>
<td>62</td>
<td>4.6</td>
<td>0.17</td>
<td>0.051</td>
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<td></td>
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<tr>
<td>X 66 F</td>
<td>Rheumatoid arthritis</td>
<td>Gold</td>
<td>76</td>
<td>&lt; 7</td>
<td>2.4</td>
<td>0.01</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K 66 F</td>
<td>Rheumatoid arthritis</td>
<td>Gold</td>
<td>38</td>
<td>24</td>
<td>1.9</td>
<td>0.09</td>
<td>0.024</td>
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</tr>
<tr>
<td>M 65 F</td>
<td>Urinary tract infection</td>
<td>Ampicillin</td>
<td>32</td>
<td>4.5</td>
<td>1.5</td>
<td>0.01</td>
<td>0.00061</td>
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<tr>
<td>N 63 M</td>
<td>Chronic obst., lung disease</td>
<td>Ampicillin, furosemide</td>
<td>82</td>
<td>520</td>
<td>-</td>
<td>0.27</td>
<td>-</td>
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</tr>
<tr>
<td>R 88 M</td>
<td>Gout, urinary tract infection</td>
<td>Cefazolin, furosemide, clindamycin</td>
<td>23</td>
<td>32.5</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
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<td>Urinary tract infection</td>
<td>Sulfisoxazole</td>
<td>34</td>
<td>33</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
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<tr>
<td>X 65 M</td>
<td>Cellulitis</td>
<td>Oxacillin</td>
<td>58</td>
<td>38</td>
<td>-</td>
<td>0.65</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Mean Values

- **Gentamicin group**: 60 10,511 3.7 12.6 6.5
- **Non-gentamicin group**: 45 102 2.6 0.16 0.04

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**GFR** = Glomerular filtration rate.
anti-IgM, anti-IgD, anti-κ light chains, and anti-λ light chains.\textsuperscript{7}

**Results**

The group of non-gentamicin treated patients had a mean GFR of 45 ± 9 ml per min and a mean serum β2 concentration of 2.6 ± 0.6 mg per L. The peak β-2 microglobulin excretion ranged from 5 to 520 μg per 24 hrs, with a mean of 102 ± 71 μg per 24 hrs (mean ± 1 SEM). These results compare favorably with the normal values reported by others.\textsuperscript{34} The mean fractional β-2 microglobulin clearance \( \left( \frac{C_{\beta-2}}{C_{\text{cr}}} \right) \) was 0.043 ± 0.023 percent. In comparison, 12 of the 18 patients who received gentamicin had a peak β-2 microglobulin excretion greater than the highest values found in our control group. This was also true for urinary β-2 microglobulin per mg creatinine and fractional β-2 microglobulin excretion. The mean GFR of the group was 60 ± 11 ml per min, and the mean serum β-2 microglobulin concentration was 3.7 ± 0.7 mg per L. Neither differed significantly from the non-treated group. Peak urine β-2 microglobulin excretions had a mean value of 10,511 ± 3,205 μg per 24 hrs. The mean fractional β-2 microglobulin clearance was 6.52 ± 3.41 percent.

All urine specimens from patients receiving gentamicin were tested for total protein. Total protein values ranged from “none detected” to 0.5 g per day and did not correlate with β-2 microglobulin excretion. Daily total protein excretion remained constant in all except patient A, who increased from 0.1 g per 24 hrs to 0.3 g per 24 hrs over a five day period. Six of the 18 patients had urinary β-2 excretions within the range of our non-treated group. Four of these patients had GFR values greater than 90 ml per min, and five of the patients were also receiving cephalosporins. Urinary β-2 microglobulin excretion of all patients receiving gentamicin are represented in figures 1 and 1A. Beta-2 microglobulin excretion usually increased within two days of the initiation of gentamicin therapy. Four patients showed a decline of β-2 microglobulin excretion despite the continuation of gentamicin therapy. Serial creatinine clearance measurements were performed on 11 of these patients, and no change in renal function was observed (figure 2). Patient E was followed for a total of 24 days, the last 14 of which he was not receiving gentamicin. His urinary β-2 excretion gradually returned to normal.

To determine if the renal handling of other low molecular weight proteins was also affected by gentamicin, amylase clearance determinations were performed in seven patients with increased β-2 microglobulin excretion (figure 3). Four of these seven patients also had determinations made of urinary light chains. In these seven patients, there did not appear to be any relationship between the quantity of β-2 microglobulin excretion and the presence of an abnormality of amylase excretion. Of the four patients who had light chains determined, two had normal fractional amylase clearances and two had elevated fractional amylase clearances. No light chains could be found in the urines of these patients.

**Discussion**

The association of gentamicin administration and an increased urinary excretion of renal tubular brush border enzymes, lysosomal enzymes, and protein has long been recognized in both rats and humans.\textsuperscript{2,24,32,33} The association of aminoglycoside administration with the entity “tubular proteinuria” as defined by an increased urinary excretion of serum derived low molecular weight proteins has only recently been documented in humans.\textsuperscript{15,29} In 68 percent of our patients
receiving gentamicin, increased excretion of urinary \( \beta-2 \) microglobulin was found in the presence of a normal serum \( \beta-2 \) and a constant creatinine clearance. Gentamicin induced increases in \( \beta-2 \) microglobulin excretion must result not from an increased filtered load but from a decreased tubular reabsorption of \( \beta-2 \) microglobulin, i.e., tubular proteinuria. This phenomenon occurs soon after the initiation of therapy and may well be the first measurable indicator of tubular dysfunction. Six of our patients on gentamicin showed no increase in \( \beta-2 \) microglobulin excretion. It is of interest that four of these patients had the highest GFR's of the gentamicin group, and five were on cephalosporins. Cephalosporins have been shown to block gentamicin induced enzymbria. However, five of our other gentamicin patients who were also on cephalosporins did show an increase in \( \beta-2 \) microglobulin excretion.

The mechanisms of this tubular proteinuria are speculative. Lower molecular weight proteins such as \( \beta-2 \) microglobulin are catabolized by lysosomes after reabsorption by endocytosis in the proximal tubules. Gentamicin administration causes characteristic proximal tubular changes recognizable by electron microscopy. The changes are characterized by
the accumulation of cytosegrasomes with myeloid bodies. These changes correlate poorly with renal function, and the question exists whether myeloid bodies represent gentamicin’s interaction with the lysosomal matrix or phagocytosed organelles damaged by gentamicin. Gentamicin had been demonstrated in vitro to activate specific lysosomal enzymes and to destabilize lysosomal membranes, and this may account for the observed enzymuria. However, its relationship to tubular proteinuria remains speculative.

Several mechanisms can be suggested for the increased β-2 microglobulin excretion observed in our patient receiving gentamicin. The alterations of brush border enzymes, lysosomal enzymes, and proximal tubular histology support a process of interference of protein uptake at the brush border membrane or reduction in protein catabolism by lysosomes. Furthermore, gentamicin uptake has been shown to take place by a similar process described for other low molecular weight proteins and competitive inhibition may occur. While gentamicin may compete with β-2 microglobulin for tubular uptake, this cannot account for the entire extent of β-2 microglobulinuria since it was observed by us that β-2 microglobulin excretion may fall while gentamicin is being administered. In addition, six patients showed no increase in excretion. Gentamicin has also been shown to reduce Na+K ATPase in concentrations found in the renal cortex. This inhibition of Na+K ATPase may interfere with the mechanism responsible for protein transport. Our observations that increased β-2 microglobulin excretion was not consistently associated with the excretion of other low molecular weight serum proteins suggest that the mechanism of tubular protein reabsorption may be quite complex. A process causing interference with lysosomal function or reduction in Na+K ATPase might be expected to produce a more generalized proteinuria.

The normal urinary light chains and variable amylase excretions are difficult to interpret in light of the marked β-2 microglobulinuria. Both light chains (MW = 22,000) and amylase (MW = 55,000) are handled by the renal tubules, presumably by the same mechanisms as other low molecular weight proteins, although there is evidence to suggest that amylase reabsorption occurs more distally.

Much controversy exists regarding the selectivity of polypeptide handling in the proximal tubules. Supporting the process of nonselectivity is a study done by Hardwick who infused albumin into patients and noted that clearances of the globulin fractions rose in proportion to the rise in albumin clearance. It was concluded that competitive reabsorption exists between serum proteins. Supporting the specificity of polypeptide handling, Dillard has demonstrated the lack of any consistent correlation between the molecular weight of individual low molecular weight proteins and their relative clearance in renal tubular disorders.

* P. Leitman, personal communication.
 Further support for specificity comes from a study by Harrison who showed that the infusion of low molecular weight protein into dogs had no effect on the excretion of other low molecular weight proteins of similar size. Our observations favor the proponents of polypeptide transport selectivity.

A number of clinical entities are known to be associated with increased \(\beta-2\) microglobulin excretion, such as cadmium poisoning, hypokalemic nephropathy, cystinosis, Minimata's disease and pancreatitis. Interestingly, in Minimata's disease, a form of mercury induced renal tubular dysfunction, both \(\beta-2\) microglobulin and light chains are elevated. However, with gentamicin administration, no such elevations of light chains were noted. In acute pancreatitis, an increased clearance of amylase is associated with an increased \(\beta-2\) microglobulin clearance, suggesting the cause of increased amylase excretion results from tubular dysfunction. In chronic pancreatitis, the amylase clearance is also elevated; however, \(\beta-2\) microglobulin excretion remains normal. To explain this discrepancy, Hegarty has suggested that tubular dysfunction in chronic pancreatitis is less severe than that of acute pancreatitis. However, in our gentamicin treated patients, \(\beta-2\) microglobulin excretion is grossly elevated while changes in amylase excretion are mild and variable. This suggests that the differences in the clearances in low molecular weight proteins in various clinical disorders cannot simply be accounted for by differences in the severity of tubular dysfunction.

Four of our 12 gentamicin patients who showed increased urinary \(\beta-2\) microglobulin actually showed a decline of \(\beta-2\) excretion while still receiving the drug. This phenomenon may be a reflection of Gilbert's observation that tubular regeneration occurs during continuous gentamicin administration. Gentamicin induced tubular dysfunction can be added to the list of disorders associated with increased \(\beta-2\) microglobulin excretion. The increased \(\beta-2\) microglobulin excretion is not necessarily a reflection or prediction of toxicity, and the 67 percent development of tubular proteinuria stands in sharp contrast to the known incidence of gentamicin nephrotoxicity which is 5 to 10 percent. The use of urinary \(\beta-2\) microglobulin excretion as a screen for other tubular disorders should be interpreted with caution in patients who have received gentamicin. A similar degree of caution should be exercised in the interpretation of amylase clearances in patients who are receiving gentamicin.

References


