Enzymuria in Gentamicin-Induced Kidney Damage

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To assess their potential value as early indicators of gentamicin-induced kidney damage, lysosomal hydrolases were measured in the 24-h urines of rats receiving 30 or 60 mg of gentamicin per kg per day for 15 days. Proteinuria, urine osmolality, blood urea nitrogen, and creatinine clearance were also measured. Kidney tissue was examined by both light and electron microscopy. Beta-galactosidase, beta-n-acetyl-hexosaminidase, and alpha-fucosidase were sensitive indicators and were significantly elevated above control values by day 3 at both doses (P < 0.01). Proteinuria, urine osmolality, and tests reflecting glomerular filtration rate were later indicators of nephron damage. Changes by light microscopy were detected on day 5. Necrosis was most prominent in the proximal convoluted tubules on day 10. Electron microscopy revealed numerous lysosomes with myeloid bodies within the proximal tubular epithelium on day 5. Lysosomal enzymuria appears to be an early manifestation of gentamicin nephrotoxicity and may possibly be related to the lysosomal abnormalities seen on electron microscopy.

Aminoglycosides are extremely useful in the management of severe bacterial infections; however their side effects have continued to be of concern to the clinician. Renal failure with these potent agents, although relatively uncommon, does occur, and is usually detected after kidney function has declined considerably (5). An earlier detection of drug-induced nephrotoxicity would be desirable and potentially helpful in avoiding permanent or prolonged kidney damage.

The kidneys have a large functional reserve capacity. Physiologic function tests, such as measurements of glomerular filtration, or the more common blood urea nitrogen (BUN) determinations, may only be rendered abnormal after considerable injury has been sustained by the kidney (1). Chemical indicators of anatomic integrity might therefore prove more valuable in detecting kidney damage than the tests of physiologic function.

Schonfeld and his colleagues suggested that the anatomic integrity of the kidney might be reflected by the output of urinary enzymes (13). Robinson et al., as well as Ellis and Price, have shown significant urinary excretion of lysosomal hydrolases in nephrotoxicity induced by agents such as mercuric chloride, uranium nitrate, potassium dichromate, 4-nitrophenylarsonic acid, and ethyleneamine (2, 11).

We compared the excretion of urinary lysosomal enzymes and alkaline phosphatase with other parameters of renal function as indicators of nephron damage induced by gentamicin in a rat model. In addition pathological changes were observed with both light and electron microscopy.

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MATERIALS AND METHODS

Adult, male, Sprague-Dawley rats weighing 200 to 250 g were selected, singly housed in metabolic cages, allowed free access to water, and fed a standard Purina rat diet. Baseline values for 24-h urine volume, urine osmolality, enzyme excretion, protein excretion, BUN and creatinine clearance were obtained for 42 sham-injected animals. Urine protein was determined by a Biuret method (7). BUN and creatinine were measured by the methods of March et al. and Martinez and Doolan, respectively (8, 9). Osmolality was measured by freezing-point depression.

Enzymes measured in urine were: the lysosomal glycosidases, alpha-mannosidase, beta-n-acetyl-hexosaminidase, alpha-fucosidase, beta-galactosidase, beta-glucosidase, and beta-glucuronidase, as well as alkaline phosphatase, a nonslyosomal enzyme chosen because of its association with brush border epithelium (2). The glycosidases were chosen over acid phosphatase because of their specificity for lysosomes,
as well as for technical reasons (12). The lysosomal hydrolases were measured according to a modification of the method reported by Patel et al. (10). The reaction conditions for this enzyme are given in Table 1. Alkaline phosphatase was determined by the procedure of Garen and Levinthal (4).

Two groups of 24 rats each received either 30 or 60 mg of subcutaneous gentamicin per kg diluted in 1-ml normal saline daily for 15 days. Twenty-four-hour urines were collected on days 3, 5, 8, 10, 12, and 15 at which time urine volume, urine osmolality, 24-h urine protein, and the urinary excretion of the aforementioned enzymes were measured. Sets of four animals from each group were sacrificed on days 5 and 10, sets of eight animals on days 12 and 15. Kidney tissue was processed from these animals and serum was analyzed for BUN and creatinine clearance determinations. The renal function studies and enzyme concentrations were compared to controls by a statistical analysis of variance.

Tissue was acquired at sacrifice. Portions of the kidneys were fixed in cold 4% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4), postfixed in cold 1% phosphate-buffered osmium tetroxide solution, and embedded in Epon 812 epoxy resin. Ultra-thin sections were stained with uranyl acetate and lead citrate and viewed under a Phillips 300 electron microscope. Additional samples were fixed in 10% buffered formalin, embedded in paraffin, stained with hematoxylin and eosin or by the periodic acid-Schiff reaction, and examined by light microscopy.

RESULTS

Figure 1 shows the standard parameters of renal function graphically displayed. At the 60 mg/kg dose, proteinuria was significantly increased over controls on day 3 and BUN on day 5 (P < 0.05). Urine osmolality and creatinine clearance were significantly decreased from control values on days 5 and 10, respectively (P < 0.05). At the 30 mg/kg dose, proteinuria was

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate and concn (mmol)</th>
<th>Buffer and concn (mmol)</th>
<th>pH</th>
<th>Dialyzed urine (µl)</th>
<th>Incubation time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-galactosidase</td>
<td>PNP-β-galactoside (4)</td>
<td>Citrate phosphate (60)</td>
<td>3.8</td>
<td>100</td>
<td>0.50</td>
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<tr>
<td>Beta-glucosidase</td>
<td>PNP-β-glucoside (4)</td>
<td>Citrate phosphate (60)</td>
<td>5.2</td>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td>Beta-glucuronidase</td>
<td>PNP-β-glucosamide (4)</td>
<td>Sodium acetate (60)</td>
<td>5.0</td>
<td>50</td>
<td>0.50</td>
</tr>
<tr>
<td>Beta-N-acetyl-hexosaminidase</td>
<td>PNP-N-acetyl-β-glucosamidine (4)</td>
<td>Citrate phosphate (60)</td>
<td>4.2</td>
<td>50</td>
<td>0.25</td>
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<tr>
<td>Alpha-fucosidase</td>
<td>PNP-α-fucoside (4)</td>
<td>Sodium acetate (60)</td>
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<td>1.00</td>
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<tr>
<td>Alpha-mannosidase</td>
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<td>Sodium acetate (60)</td>
<td>5.0</td>
<td>100</td>
<td>1.00</td>
</tr>
</tbody>
</table>

+ PNP, P-nitrophenyl.

![Graph](http://aac.asm.org/Downloadedfrom http://aac.asm.org)
significant by day 8, the osmolality was depressed by day 8, and the BUN was elevated by day 15 ($P < 0.05$). Creatinine clearance was not altered ($P < 0.05$). Polyuria, which is not displayed, occurred in both groups and was significant on day 5 ($P < 0.05$).

The urinary enzyme excretion is outlined in Table 2. Beta-galactosidase, beta-$n$-acetyl-hexosaminidase, and alpha-fucosidase showed a highly significant increase over controls at both the 30 and 60 mg/kg per day doses by day 3 ($P < 0.01$). Beta-glucuronidase was also elevated by day 3 at the 60 mg/kg per day dose; however the excretion of this enzyme varied considerably. The difference was not significant on day 5 significant on days 8, 10, and 12, but not on day 15. The other lysosomal hydrolases, alpha-mannosidase and beta-glucosidase, were increased during the latter part of the treatment. Alkaline phosphatase, a nonlysosomal enzyme, also increased significantly by day 3 and remained elevated throughout the 15-day experimental period ($P < 0.05$). The excretion of beta-$n$-acetyl-hexosaminidase at the 60 mg/kg per day dose significantly exceeded that of the 30 mg/kg per day dose at all days tested ($P < 0.05$). The other enzymes tested were less uniform in showing a significant difference between the two drug doses.

Figure 2 is a bar graph comparing the urinary excretion of beta-$n$-acetyl-hexosaminidase, alpha-fucosidase, urine protein excretion, and BUN, as indicators of nephron damage at both doses. The enzymes show more striking and earlier elevations.

Light microscopy observations at the 60 mg/kg per day dose showed evidence of tubular necrosis involving primarily the pars convoluta of the proximal tubule (Fig. 3). Cloudy swelling was barely evident by day 5, extensive necrosis of the epithelial cells with an intact basement membrane by day 10, and early regeneration by day 15. Interstitial edema and focal fibrosis also occurred. Whereas the light microscopy changes by day 5 were subtle, the electron microscopy changes were striking as compared to sham-injected controls (Fig. 4). Large numbers of cytosomes containing myeloid bodies appeared within the proximal tubular cells. At the 30 mg/kg per day dose, the changes seen with light and electron microscopy differed only in degree from the 60 mg/kg per day dose, but not in character.

**DISCUSSION**

The gentamicin dosage schedule employed in these rats, which was considerably higher than
those used in man compared on a weight basis, resulted in obvious nephrotoxicity. Kosek and his colleagues reported tubular necrosis, oliguria, and uremia at a dose of 40 mg/kg per day in Fischer 344 rats after 20 days of treatment (6). Their findings demonstrated that gentamicin produced dose-related nephrotoxicity. Their finding as well as ours are at variance with the previous report of Flandre and Damon who noted extensive tubular necrosis at the much higher dose of 200 mg/kg per day administered for up to 60 days to 200-g Wistar rats. Azotemia occurred after 20 days at this dose in their model (3).

Proteinuria and decline in urine osmolality, which reflected the polyuria, were relatively, but not uniformly, early indicators of nephron dysfunction due to gentamicin nephrotoxicity. At the 60 mg/kg daily dose only proteinuria was significantly increased by day 3. BUN and creatinine clearance were later indicators of nephron injury, suggesting that considerable changes occurred before the glomerular filtration rate declined.

The lysosomal enzymes, on the other hand, displayed a prompt rise at both the high and...
low gentamicin doses. These enzymes appeared promptly and were reliable indicators of early nephron damage. Of interest were the pathologic findings on electron microscopy that have been aptly described by Kosek et al. (6), and more recently by Harrison et al. (Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 14th, San Francisco, Calif., Abstr. 70, p. 69, 1974). These changes occurred very early in Kosek’s Fischer 344 rats and were seen with doses as low as 1 mg/kg per day after 48 h. Harrison et al. were able to identify the whorls of myeloid material not only in the proximal tubular cells, but also in special preparations of the urinary sediment. Whether or not the appearance of cytosomes with myeloid bodies is direct morphological evidence of drug-induced lysosomal injury, also manifest by the appearance of lysosomal enzymes in the urine, is yet to be shown; however this would appear to be a possibility.

In conclusion, the appearance of significant quantities of lysosomal enzymes in the urine early in the course of gentamicin nephrotoxicity suggests that they may be of some value in predicting nephron damage before a decline in glomerular filtration occurs. The enzymuria is temporally related to the appearance of myeloid body formation seen on electron microscopy, which antedate changes seen with light microscopy. Whether or not enzymuria would occur to a sufficient degree in man to be useful will require clinical trials; however, the relative ease of measurement of lysosomal enzymes and their promising response as possible markers of gentamicin nephrotoxicity warrant further study.

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LITERATURE CITED