Urinary Clusterin in Chronic Nephrotoxicity in the Rat (43564)

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Abstract. **The excretion of clusterin was compared with that of *N*-acetyl-β-glucosaminidase (NAG) in rats given gentamicin daily for 2 months to determine whether clusterin excretion stays elevated after NAG excretion falls during chronic gentamicin administration. Clusterin was measured by radioimmunoassay and NAG by the hydrolysis of 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide. Gentamicin at 110 mg/kg was given daily for 44 days and thereafter, at 90 mg/kg daily. The excretion rate of both proteins rose rapidly, peaked, and then declined; however, the clusterin values stayed significantly above control values for the entire study, whereas NAG values were close to normal during the last 10 days, even though tubulointerstitial disease was active at that time. For this reason, the further evaluation of clusterin as a marker of renal tubular cell injury or death is warranted.** [P.S.E.B.M. 1993, Vol 202]

Long-term, low-dose administration of gentamicin to rats is associated with pathologic evidence of mild chronic tubulointerstitial nephritis with progressive renal failure (1). *N*-Acetyl-β-glucosaminidase (NAG) excretion, a marker of renal cell injury, may not stay elevated during continued gentamicin therapy, despite the fact that renal injury is continuing to occur (2, 3). Because of this lack of persistent urine NAG elevation during continuing renal injury, we initiated a study comparing it to clusterin excretion.

Clusterin is an 80-kDa glycoprotein that is involved in a number of physiologic functions, including regulation of complement, reproduction, cell aggregation, and programmed cell death (apoptosis) (4–6). Clusterin protein or mRNA has been given a number of different names, including sulfated glycoprotein-2 (7), testosterone-repressed prostate message-2 (8) or protein-2 (9), and human serum protein (SP-40,40) (10). Its expression is induced in a variety of regressing and dying tissues. In some tissues, clusterin has been associated with programmed cell death and tissue remodeling (7). In the kidney, clusterin is a component of immune deposits and its expression is increased after ischemia or obstruction (11). Acute gentamicin nephrotoxicity increases the urinary excretion of clusterin protein which can be detected prior to increases in serum creatinine levels (12). The present study was designed to test the hypothesis that tubulointerstitial nephritis in gentamicin-treated rats will cause elevated levels of clusterin in the urine at a time when the elevated NAG excretion has subsided during chronic gentamicin administration.

Materials and Methods

Male Sprague-Dawley rats weighing 150–200 g were housed in individual metabolism cages, allowed free access to water, and fed a standard Purina diet *ad libitum*. The rats were divided into two groups of eight rats each, with one being gentamicin treated and the other saline treated. The rats were acclimatized for 7 days before the study began.

Rats were injected subcutaneously with either saline or gentamicin (110 mg/kg/day) daily at 8:30 AM. One rat in the gentamicin-treated group expired on the 44th day of treatment. After that, the dose of gentamicin was lowered to 90 mg/kg/day. Rats received gentamicin or saline for 58 days. The animals were weighed every 5 days and appropriate adjustments were made in the gentamicin dose per rat as they gained weight to keep the gentamicin dose per kilogram correct.

Urine samples were collected into tubes on ice three

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times a week between 8:00 AM and 5:00 PM. Samples were assayed for *N*-acetyl-glucosaminidase and clusterin. Urinary NAG was assayed by a modification (13) of the method of Leaback and Walker (14). The urinary NAG activity is expressed as nanomoles of 4-methylumbelliferone liberated from its substrate, 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide, per hour of incubation at 37°C per milligram of urine creatinine. The creatinine in urine and serum was measured by the alkaline picrate (Jaffe) reaction. The concentration of clusterin in urine was measured by radioimmunoassay using previously described procedures (12). Results were expressed as ng protein/mg of urine creatinine.

Blood samples were taken from the orbital venous sinus under methoxyflurane anesthesia every 2 weeks. Gentamicin-treated rats and control rats were sacrificed approximately 24 hr after their last dose of study drug on the 59th day of the study. At the time of sacrifice, blood was obtained by cardiac puncture and the kidneys were removed for renal histopathology, fixed in 10% buffered formalin, and stained with hematoxylin and eosin by standard techniques.

Statistical Analysis

The serum creatinine values were normally distributed and the *t* test was used for the comparison. The NAG and clusterin values were not normally distributed. For this reason, median values and 99% confidence interval of the median values are presented (15). When the median value of the experimental group exceeds the 99% confidence interval of the control group, it is very unlikely that the difference between the groups is due to chance.

Results

During the 2-month period, the gentamicin-treated animals gained weight more slowly than the control group and the weights (mean ± SD) were 566 ± 34 g and 428 ± 26 g for control and gentamicin groups, respectively, at the end of the study.

The serum levels of creatinine increased in the gentamicin-treated group so that they were significantly above control levels on Days 15, 30, and 45, but were the same as control by Day 60 (Fig. 1). This confirms the work of others (2, 3) indicating that serum creatinine falls to normal in rats during chronic gentamicin administration that initially caused azotemia.

Histologic examination of the control group given saline was normal. The gentamicin-treated rats showed histologic abnormalities. These changes were predominantly in the proximal tubules. The epithelium of most of the proximal tubules appeared with some segments showing cellular vacuolization which varied in size and number. There were some dilated tubules that were lined with flattened cells. In some instances, the

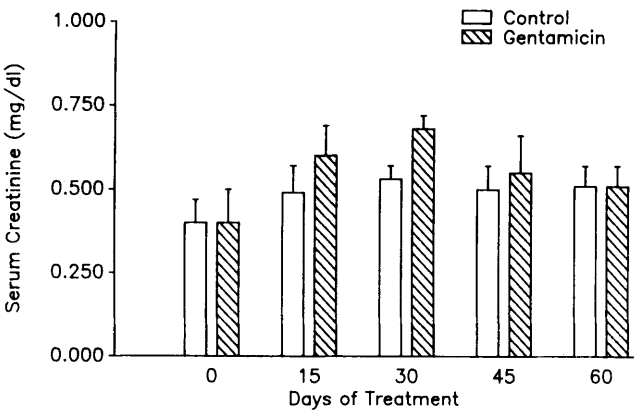

Figure 1. Mean ± SD of serum creatinine values in rats during study.

epithelial cells were layered. Patchy mononuclear inflammatory infiltrates were scattered throughout the interstitium. These histologic changes in the gentamicin-treated group provided evidence of chronic tubulointerstitial disease.

Gentamicin induced an increase in urinary excretion of NAG that was first detectable on Day 2. The NAG increased progressively during the first 13 days of gentamicin administration (Fig. 2). Since one animal was very sick and died, the gentamicin dose was reduced to 90 mg/kg/day on the 44th day of the study. The NAG levels eventually returned toward predrug levels, despite continued drug administration.

Urinary clusterin levels reached a maximum on the twelfth day of the study and fell as NAG excretion fell (Fig. 3), but they remained elevated throughout the study.

If one looks at the urine protein values after Day 45, the apparent end of the azotemic period, the median clusterin values appear definitely higher than the upper 99% confidence interval of the control clusterin values, while the median values of NAG only slightly exceed the upper 99% confidence interval of the control NAG.

Discussion

This study was done to see whether urinary clusterin excretion stayed elevated during chronic gentamicin administration after urine NAG excretion fell despite ongoing active tubulointerstitital nephritis. We found that it did. The excretion of both proteins was high during the initial period of treatment and stayed high during the period of azotemia. But the test of our hypothesis of persistence of clusterin elevation after NAG excretion fell was just the last 2 weeks of gentamicin administration, when the renal impairment as measured by serum creatinine had subsided but active renal disease as assessed histologically was present. During this period, the ratio of median clusterin excretion of gentamicin-treated to control rats was higher than the ratio of median NAG excretion of treated to control

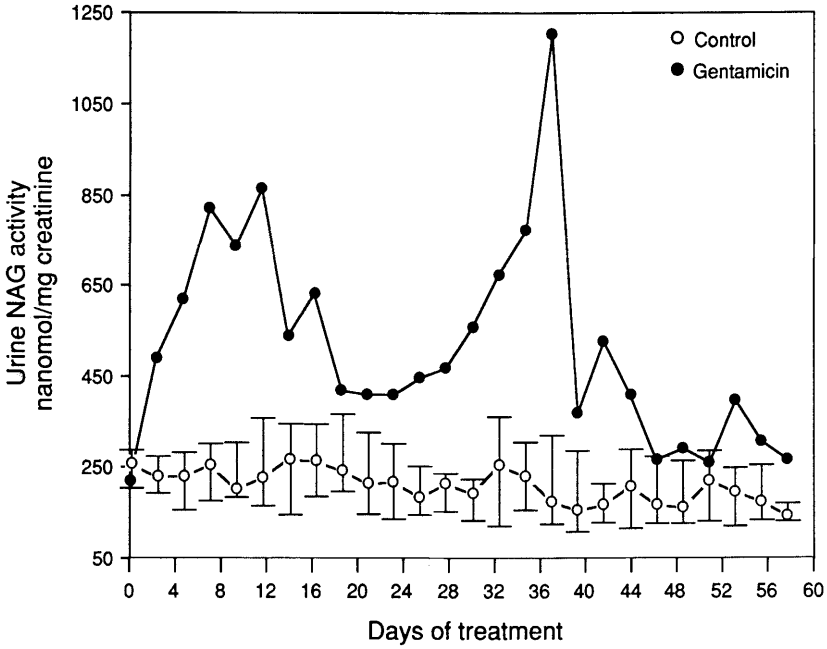

Figure 2. Urinary *N*-acetyl-β-D-glucosaminidase excretion by rats. Median values are presented with the 99% confidence intervals for the median control values.

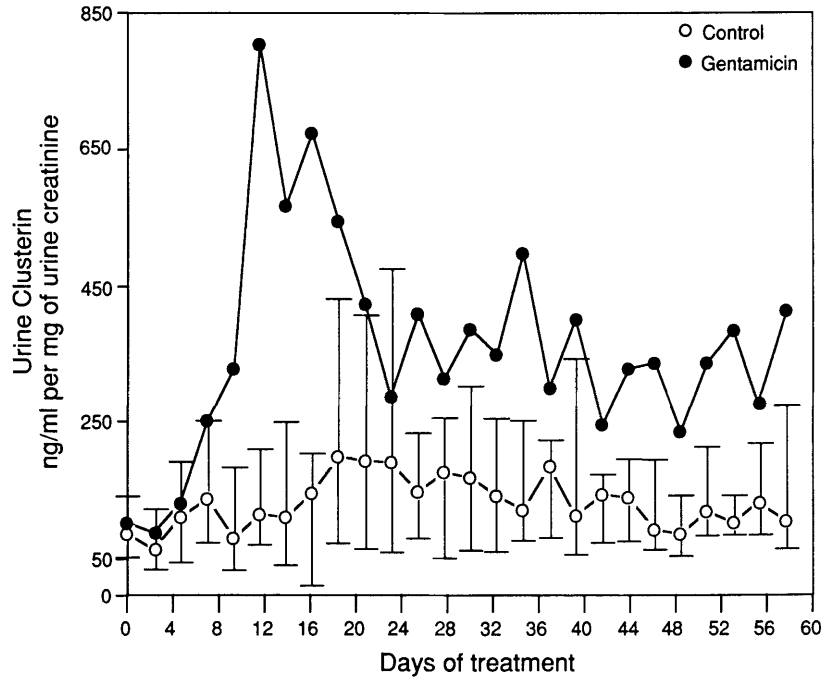

Figure 3. Urinary clusterin excretion by rats. Median values are presented with the 99% confidence intervals for the median control values.

rats ($P < 0.01$, Mann Whitney U test). Since an increase in clusterin protein (9) or mRNA (5) has been demonstrated as a response to tissue-specific injury and tissue remodeling, the further evaluation of the excretion rate of clusterin as a sensitive marker of renal cell injury or death is warranted.

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