Prevention of Acute and Chronic Ascending Pyelonephritis in Rats by Aminoglycoside Antibiotics Accumulated and Persistent in Kidneys

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Gentamicin, because it is stored in renal tissues, can prevent acute retrograde pyelonephritis. Since different aminoglycosides accumulate and persist to various degrees in the kidney parenchyma, the prophylactic activities of gentamicin, tobramycin, amikacin, and netilmicin were compared. The four antibiotics were given intramuscularly to rats 3 days before initiating ascending unilateral pyelonephritis with Escherichia coli. Despite different degrees of renal accumulation at the time of infection (tobramycin and amikacin accumulated significantly less), all four aminoglycosides displayed similar protection against ascending pyelonephritis. This protection was conferred in the absence of active antibiotic detectable in the urine and was therefore attributed to antibiotic stored in the renal parenchyma. Those animals that developed pyelonephritis despite aminoglycoside prophylaxis had less severe acute kidney infection and inflammation. This resulted 3 months later in an almost complete protection against renal scarring (chronic pyelonephritis). These results in rats suggest that renal accumulation and persistence of aminoglycosides may be used to advantage in the prophylaxis or in the treatment of kidney infections.

Renal accumulation and persistence of aminoglycoside antibiotics are thought to be responsible for their nephrotoxicity (5, 6, 12). Recently, the large accumulation of gentamicin has been shown to afford protection against ascending obstructive pyelonephritis (AOPN) with Escherichia coli in rats (9) when the drug is given prophylactically. In comparison to gentamicin, the other aminoglycoside antibiotics accumulate and persist to a lesser degree, and it has not been established whether they display similar prophylactic protection in this disease.

It has been demonstrated that the severity of chronic pyelonephritis that develops after AOPN is related to the intensity of inflammation during the acute phase of the disease (7). Thus, if this inflammation is reduced by prophylactic aminoglycoside treatment in the rats that develop AOPN, protection against developing chronic pyelonephritis might result.

In this study we compared the effect of four different aminoglycoside antibiotics (gentamicin, tobramycin, amikacin, and netilmicin), given prophylactically, on the incidence of E. coli AOPN in rats. In addition, we determined the severity of AOPN in the animals that developed the disease despite prophylaxis and correlated the findings to the degree of subsequent destructive chronic pyelonephritis.

We found that all four antibiotics tested displayed similar protection, despite different degrees of renal accumulation. In those animals that developed infection despite prophylaxis, AOPN was less severe, and this resulted in protection against chronic pyelonephritis 3 months later.

MATERIALS AND METHODS

E. coli O6, the strain used for infecting the rats, has been described previously (3). It is inhibited by 2 μg of gentamicin per ml, 2 μg of tobramycin per ml, 4 μg of amikacin per ml, and 1 μg of netilmicin per ml.

Production of pyelonephritis. Retrograde pyelonephritis was produced in groups of 25 to 36 male Wistar rats (200 to 250 g) as previously described (3). In each experiment, the rats were divided into a control group and two aminoglycoside prophylaxis subgroups. The acute pyelonephritis and the chronic pyelonephritis experiments (sacrifice at 3 and 90 days, respectively) were carried out separately.

Briefly, 10⁷ E. coli organisms are infused into the bladder, and a ligature is tied loosely around the left ureter. After 18 h, the ligature is removed. This procedure produces partial obstruction, and severe unilateral pyelonephritis subsequently develops in about 60% of the rats. By 3 days after the operation, the pyelonephritic kidneys are greatly enlarged and display numerous small abscesses over the cortex. In
animals without macroscopic pyelonephritis, there is no kidney enlargement and the kidney homogenate and urine remain sterile (8, 9). By 3 months after the operation, most animals which developed AOPN have extended scars on the left kidney cortex, with destruction of the kidney parenchyma (7).

Severity of pyelonephritis. Kidney weight provides the best quantitative measurement of the severity of pyelonephritis. During AOPN, the weight increases in proportion to the suppuration, whereas it declines in chronic pyelonephritis, reflecting the amount of kidney destruction (4). To minimize the variation of kidney weights among animals, the ratio of the left kidney weight to the right kidney weight is used (7). The ratio is 1 in normal rats.

Prophylactic administration of antibiotics. It has been established previously that gentamicin given intramuscularly to rats in a dosage of 4 mg/kg gives levels in serum similar to those achieved in humans. Furthermore, three doses at 12-h intervals (the last dose at 72 h before the operation and infection) provide significant protection against the development of AOPN. This interval of 3 days between the last injection and the operation was chosen to have levels of the drug in serum and urine far below the minimal inhibitory concentrations of the test organism for each given aminoglycoside. The dosages of gentamicin and tobramycin (three doses of 4 mg/kg), amikacin (three doses of 20 mg/kg), and netilmicin (three doses of 5 mg/kg) were chosen according to the amount given and levels in serum achieved in humans. All four aminoglycosides were given at 12-h intervals, the last dose at 72 h before the operation.

Sacrifice of rats. Animals were sacrificed 3 days or 3 months after the operation. The kidneys were examined for gross acute pyelonephritis at 3 days or for scars of chronic pyelonephritis at 3 months, weighed, and homogenized. The homogenate was diluted in saline before plating onto MacConkey agar and cultivated aerobically and anaerobically in anaerobic jars (GasPak catalyst; BBL Microbiology Systems) for 48 h to decrease the level of aminoglycoside activity (18).

Determination of antibiotic levels. Aminoglycoside levels in the homogenate were measured by the agar diffusion method as previously described (15), diluting known amounts of the aminoglycosides in normal kidney homogenate as a standard. Results are expressed in micrograms per gram of kidney.

Statistical evaluation. The number of colony-forming units per gram of kidney and the ratio of the left kidney weight to the right kidney weight of pre-treated and control rats were compared by Student’s unpaired-t test. A statistical comparison of the incidence of pyelonephritis between controls and treated rats was performed by the chi-square method with the Yates correction.

RESULTS

Prophylaxis of pyelonephritis. Table 1 is a comparison of the incidence of pyelonephritis in animals given prophylactic aminoglycoside antibiotics with that in those given saline. There was a significant reduction in the frequency of pyelonephritis after prophylactic treatment with the four aminoglycosides. In addition, no statistically significant differences were observed in the protective efficacy of the four different antibiotics.

This protection was conferred even though at the time of the operation no residual antimicrobial activity in the serum was detectable by bioassay and only traces of antibiotic were present in the urine. These traces in the urine were well below the minimal inhibitory concentrations of these antibiotics for the infecting organism. As previously described (9), protection was therefore probably due to the antimicrobial activity of the accumulated and persistent aminoglycoside antibiotics in the kidney parenchyma.

Table 1. Incidence of acute and chronic pyelonephritis after administration of prophylactic aminoglycoside antibiotics

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Rats without evidence of pyelonephritis*</th>
<th>Rats with evidence of pyelonephritis*</th>
<th>Overall incidence of pyelonephritis (%)</th>
<th>Protective value (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 3 days</td>
<td>At 90 days</td>
<td>Overall</td>
<td>At 3 days</td>
</tr>
<tr>
<td>Controls</td>
<td>32</td>
<td>18</td>
<td>50</td>
<td>56</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>32</td>
<td>17</td>
<td>49</td>
<td>10</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>18</td>
<td>13</td>
<td>36</td>
<td>10</td>
</tr>
<tr>
<td>Amikacin</td>
<td>21</td>
<td>19</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>14</td>
<td>20</td>
<td>34</td>
<td>16</td>
</tr>
</tbody>
</table>

* By 3 days after an operation, acute pyelonephritis manifests itself by gross kidney enlargement and innumerable small abscesses on the cortex surface. After 3 months, depressed scars on the cortex and more or less extended kidney destruction are characteristic of chronic pyelonephritis.

b The protective value is expressed by the ratio [(a - b)/a] × 100, where a is the overall incidence of pyelonephritis in controls, and b is the overall incidence of pyelonephritis in the respective aminoglycoside groups.

c Number of rats in each group.

P < 0.001 when compared with controls.
Reduction in the severity of pyelonephritis. Table 1 shows that protection against AOPN by prophylactic aminoglycoside antibiotics was not complete, and between 25 and 36% of rats given aminoglycoside prophylaxis developed AOPN. Table 2 is an analysis of the severity of acute and chronic pyelonephritis in the treated animals. When expressed by the kidney weight ratio, inflammation during the acute phase of the disease in the treated animals was markedly reduced compared with that in the control rats. In addition, this reduction in inflammation at 3 days was followed by a decrease in the development at 3 months of the destructions of chronic pyelonephritis. There was no significant difference among the four aminoglycosides in the reduction of inflammation during the acute phase and in the prevention of kidney damage during the chronic phase of the disease.

Infection, as expressed by the log$_{10}$ colony-forming units per gram of kidney, was also found to be diminished in all four groups of rats given prophylactic aminoglycoside antibiotics that were sacrificed during the acute phase of pyelonephritis. However, no reduction in infection was apparent in rats sacrificed 3 months after the operation.

Aminoglycoside levels in kidney homogenates. Rats were sacrificed 3 days after completion of the aminoglycoside prophylaxis (at the time of the operation) to determine the amount of antibiotic that had accumulated and persisted in the kidney parenchyma. Table 3 shows that gentamicin accumulated and persisted significantly more than did the three other antibiotics, with tobramycin accumulating the least. Amikacin, which was given in a dosage five times greater than those of gentamicin and tobramycin, did not show a corresponding increase in the degree of accumulation. Therefore, its relative accumulation was the least of the four aminoglycosides studied. There was no apparent relation between the level of accumulation of the four aminoglycoside antibiotics in kidneys and the protection against pyelonephritis, as expressed either by the severity of pyelonephritis or by the intensity of infection.

DISCUSSION

Renal accumulation of aminoglycoside antibiotics has been established in both animals and humans and related to their nephrotoxicity. In the present experiments, it was confirmed that amikacin accumulated the least when the dosage administered is considered and that tobramycin accumulated less than did gentamicin and netilmicin (1, 10, 16). Although often thought of as a therapeutic advantage, this accumulation has only recently been shown to confer protection in experimental renal ascending infection (9). Based on this study, the observation made with gentamicin, which showed that gentamicin per-

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Ratio of left kidney wt to right kidney wt$^a$</th>
<th>Log$_{10}$ CFU per g of kidney$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APN$^c$</td>
<td>CPN$^c$</td>
</tr>
<tr>
<td>Controls</td>
<td>1.97 ± 0.3</td>
<td>0.56 ± 0.3</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1.57 ± 0.2$^{d}$</td>
<td>0.96 ± 0.1$^{d}$</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>1.40 ± 0.2$^{d}$</td>
<td>0.93 ± 0.1$^{d}$</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1.60 ± 0.3$^{d}$</td>
<td>0.89 ± 0.1$^{d}$</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>1.57 ± 0.2$^{d}$</td>
<td>0.88 ± 0.1$^{d}$</td>
</tr>
</tbody>
</table>

$^a$ Mean ratio of left kidney weight to right kidney weight ± standard deviation: at 3 days, this ratio is an expression of the intensity of the inflammation of the left pyelonephritic kidney; at 3 months, this ratio is a measurement of the severity of the parenchymal destructions of the left pyelonephritic kidney.

$^b$ Mean log$_{10}$ colony-forming units (CFU) per gram of pyelonephritic kidney homogenate ± standard deviation.

$^c$ The numbers of rats from which the mean values ± standard deviations are calculated in the respective groups are given in Table 1.

$^d P < 0.001$ when compared with controls.
sisting in the kidney parenchyma could confer protection against the occurrence of AOPN (9), may be extended to other aminoglycosides. As previously suggested, this protection is probably due to the accumulation and persistence of aminoglycoside antibiotics in the kidney parenchyma rather than to antimicrobial activity in the urine (9). In the present experiments, only traces of the different aminoglycosides were measured in the urine at the time of the operation and inoculation of the test bacteria into the bladder. It has been shown previously that gentamicin, when given 12 h before an operation, provides complete protection against AOPN that is due to sufficient antibacterial activity in the urine so as to kill the infecting inoculum (9).

One could have expected complete protection from developing disease because the levels of each aminoglycoside antibiotic in kidneys, as measured in these experiments, were much higher than the minimal inhibitory concentrations of the test organism. However, these levels were measured in whole kidney homogenates, and previous observations have shown that aminoglycosides accumulate to a lesser extent in the medulla and papilla of the kidney (2, 11, 13), where ascending pyelonephritis starts to develop (3, 17). Furthermore, accumulated aminoglycosides are located mostly inside the tubular cells, whereas infection starts in the interstitium (5). Lastly, we have shown previously that the level of gentamicin, as measured after dilution of kidney tissue homogenate, does not correlate with in vivo protection (9), probably because the high concentrations of urea and other solutes in the papilla and medulla decrease the antibacterial activity of aminoglycoside antibiotics in the kidney (14). Therefore, it is likely that in these experiments the antibacterial activity of the four different aminoglycoside antibiotics in the kidney parenchyma was not sufficient so as to completely suppress AOPN, as can be achieved if the antibiotics are given 12 h before infection (9).

The aminoglycoside-treated rats that developed pyelonephritis had a much less severe infection during the acute phase of the disease, as expressed by the number of bacteria recovered from kidneys, resulting in less kidney inflammation. This reduction of infection, however, was no longer apparent in the rats that were sacrificed late in the course of the disease. Previous experiments have suggested that the formation of kidney scars results less from persisting infection than from suppurrative necrosis during the acute phase of pyelonephritis (7). In the present experiments, next to diminished infection, aminoglycoside prophylaxis afforded a significant reduction of inflammation in those treated animals that developed AOPN; this was reflected 3 months later by a striking reduction in kidney scars. This observation underlines the critical role of acute inflammation in the development of parenchymal damage. This is supported further by recent experiments which show that colchicine, given during AOPN, diminishes inflammation without influencing infection, with a resulting decrease in kidney scarring, when animals are sacrificed late in the course of pyelonephritis (J. Bille and M.-P. Glauser, Clin. Res. 28:364A, 1980).

These experiments in rats suggest that the unique pharmacokinetic properties of the aminoglycoside antibiotics may prove to be of specific therapeutic value.

LITERATURE CITED