THE JOURNAL OF INFECTIOUS DISEASES • VOL. 139, NO. 2 • FEBRUARY 1979
 0022-1899/79/3902-0007$00.75

Prevention of Pyelonephritis Due to *Escherichia coli* in Rats with Gentamicin Stored in Kidney Tissue

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Although gentamicin is known to accumulate and persist in the kidneys after systemic administration, its antibacterial activity at this site has not been determined. In the present study the accumulation of gentamicin in rat kidneys before infection prevented obstructive pyelonephritis due to *Escherichia coli* despite heavy urinary tract infection in the obstructed pelvis; thus the kidneys were protected against infection in the absence of effective levels of gentamicin in the urine. Stored gentamicin also protected pyelonephritic rats from relapse after complete obstruction of the kidneys. The level of antimicrobial activity of gentamicin in whole kidney tissue was 95% less than that anticipated on the basis of levels measured after dilution of kidney tissue homogenates; this low level of activity apparently was due in part to high concentrations of solutes. In view of these results in rats, the possibility must be considered that despite reduced activity, gentamicin storage might be useful in the prophylaxis of kidney infection in patients with abnormalities of the urinary tract. In the treatment of established kidney infection, the dose of gentamicin could be reduced and the interval of its administration increased for minimal toxicity.

Accumulation and storage of gentamicin in the kidneys has been reported recently [1] and described in rats [2], dogs [3, 4], rabbits [5], and normal human kidneys [3, 6–8]. These observations dealing primarily with the nephrotoxicity of gentamicin prompted the present study of (*1*) the antibacterial activity of the stored drug and (*2*) the possibility that its accumulation in the kidneys would prevent pyelonephritis if the drug was given before bacterial infection and would prevent relapse if the drug was given as therapy. These studies were done with a rat model of retrograde *Escherichia coli* pyelonephritis. Relapse was induced by obstruction of urinary flow at different times after gentamicin therapy. Prevention of relapse in rats receiving gentamicin was compared with that in rats receiving ampicillin, an antibiotic that does not accumulate in the kidneys.

Received for publication March 13, 1978, and in revised form July 6, 1978.

This work was supported by research grant no. 5R01-AI-12476-07 from the U.S. Public Health Service, by the Franz Joseph Foundation, and by the American-Swiss Foundation for Scientific Exchange.

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Materials and Methods

Bacteria. *E. coli* O6 (Williams), the strain used to infect the rats, has been described previously [9]. It is inhibited by 2.5 μg of ampicillin/ml or by 1.2 μg of gentamicin/ml.

Production of pyelonephritis. Retrograde pyelonephritis was produced in male Wistar-Lewis rats weighing 225–250 g (Charles River Breeding Laboratories, Wilmington, Mass.) as previously described [9]. In brief, after a suprapubic incision was made, 10^3 *E. coli* organisms were gently infused into the bladder until urine appeared at the urethral meatus, whereupon the inoculum consistently refluxed up the ureter. A ligature was then tied loosely around the left ureter with a silk suture through the left flank, and the abdominal wall was closed. The ligature was carefully removed from the outside 20 hr later. This procedure produces partial obstruction of urine flow and severe gross unilateral pyelonephritis. Pyelonephritic kidneys are greatly

enlarged and display numerous small abscesses over the cortex that extend through the medulla and riddle the interstitial areas with suppuration [9].

Prophylactic administration of drug. Before ligation, three groups of 24–36 rats each were given im injections, each of which contained a dose of 4 mg of gentamicin/kg of body weight in a total volume of 0.1 ml. The injection schedules for the three groups were as follows: (*1*) one injection 72 hr before ligation; (*2*) three injections, one every 12 hr, with the last injection 72 hr before ligation; and (*3*) seven injections, each at 12-hr intervals, with the last injection given 12 hr before ligation. The first two regimens allowed the study of prophylaxis of pyelonephritis three days after administration of gentamicin, while the third regimen allowed the study of prophylaxis shortly after administration of doses of gentamicin similar to those used in the treatment of pyelonephritis (see below). A control group was given an im injection of a 0.1-ml volume of 0.9% NaCl in place of each dose of gentamicin given to treated rats.

Sacrifice of rats. All treated and control animals were killed with an overdose of ether 22 hr or three days after ligation as specified in Results and were bled from the heart. The abdominal wall was opened, and the pelvic contents were aspirated with tuberculin syringes. Kidneys were removed aseptically, examined for gross evidence of pyelonephritis [9], and homogenized in 1 ml of 0.1 M phosphate-buffered saline (PBS), pH 8.0; 0.5 ml of the homogenate was used for bacteriological study, and the remainder was stored at −20 C until antibiotic levels were determined.

Prevention of relapse by renal accumulation of gentamicin. We have shown in previous studies that as few as 46 live bacteria persisting after treatment in the pyelonephritic kidney may cause severe relapse when the ureter is reobstructed [10]. This phenomenon was used to determine the level of antibacterial activity of stored gentamicin given in the treatment of pyelonephritis. For comparative purposes, pyelonephritic rats were treated with ampicillin, an antibiotic that does not accumulate in the kidney.

Treatment of groups of 20–24 rats was started 30 hr after ligation, when ~75% of the rats had severe kidney infection. In one group of rats, sodium ampicillin (Bristol Laboratories, Syracuse, N.Y.) was given every 6 hr (160 mg/kg per day). A second group received gentamicin (Schering, Kenilworth, N.J.) at 12-hr intervals (8 mg/kg per day). Each drug was injected im (alternating thighs) in a volume of 0.1 ml. Treatment with each antibiotic was continued for 3.5 days (ampicillin given 14 times, gentamicin seven). As previously demonstrated, both of these dosages prevented further progression of grossly evident pyelonephritis while allowing a substantial degree of infection to remain in the kidney [10].

Eight days or 12 hr after treatment was ended, rats were either studied bacteriologically or ligated again to reestablish ureteral obstruction. Rats that were religated were anesthetized with ether, and a left paramedial incision into the abdominal wall was made. The left kidney was inspected for the presence of pyelonephritic cortical lesions. The left ureter was then religated with a silk ligature through the flank. The ligature was left in place for 48 hr. Animals were killed 24 hr after removal of the ligature (i.e., three days after religation) and examined for kidney infection and pyelonephritis. Relapse of pyelonephritis with severe recurrent infection was determined by exacerbation of gross suppurative lesions and by an increase in the number of *E. coli* cultivated from the kidneys over the number of *E. coli* found in kidneys of treated control animals killed at time of religation. Only animals exhibiting gross evidence of pyelonephritis were evaluated since kidneys without gross evidence were uniformly sterile.

Bacteriological cultures. The presence or absence of pyelonephritis was determined by macroscopic examination of the kidneys and by the culture of samples of kidney homogenates. Tenfold dilutions of a 0.5-ml aliquot of the homogenates were made in trypticase soy broth (Baltimore Biological Laboratories [BBL], Cockeysville, Md.), and 0.5 ml was plated onto trypticase soy agar. After the plates had been incubated overnight aerobically at 37 C, the number of cfu was counted. Cultures from animals treated with gentamicin were incubated in anaerobic jars (GasPak® catalyst; BBL) for 48 hr to decrease the level of gentamicin activity [11]. Urine samples obtained from the pelvis were cultured in the same manner.

Determination of antibiotic levels. The level

of gentamicin was assayed by the agar diffusion method described by Sabath and Matsen [12] with *Bacillus subtilis* ATCC 6633 (Difco Laboratories, Detroit, Mich.). Bio-Assay steel plates (Lab-Line Instruments, Melrose Park, Ill.) were used as sample holders on the surface of the agar plates, which contained 10 ml of antibiotic medium 5 (Difco). Kidney homogenates were assayed either without further dilution (undiluted homogenates) or diluted 1:50 in 0.1 M PBS, pH 8.0 (diluted homogenates). Samples (0.05 ml) were placed in the wells, allowed to diffuse overnight at room temperature (about 21 C), and then incubated for 2–4 hr at 37 C. The zone of inhibition was measured with a vernier caliper, and the level of drug was determined by comparison with a standard curve of gentamicin (gentamicin sulfate; Schering) diluted in 0.1 M PBS, pH 8.0. In some experiments, gentamicin was diluted in homogenates of normal kidney tissue. Ampicillin was assayed similarly, except that *Sarcina lutea* ATCC 9341 (American Type Culture Collection, Rockville, Md.), antibiotic medium 3 (Difco), and 0.1 M PBS, pH 7.4, were used. Each specimen was assayed in duplicate.

Statistics. The number of cfu/g in relapsing kidney tissue was compared with the number in control tissue by Student's unpaired *t*-test. Statistical comparison of the attack rates of acute pyelonephritis in the prophylaxis experiments and of the rates of relapse in the religation experiments was performed by the χ^2 method with Yates's correction.

Results

Prophylaxis of pyelonephritis. In a series of prophylaxis experiments, the susceptibility of rats to ascending obstructive pyelonephritis was tested at various intervals following prophylactic administration of different quantities of gentamicin. There was no protection against pyelonephritis in rats infected 72 hr after a single injection of gentamicin; 22 (79%) of 28 rats had macroscopic evidence of pyelonephritis three days after infection, a proportion similar to that seen among untreated control rats (13 [76%] of 17). However, when antibiotic-treated rats were challenged with *E. coli* 72 hr after the last of three injections of gentamicin, the proportion was lowered to four (20%) of 20 ($P < 0.05$).

Thus it appeared that three injections of gentamicin were sufficient to prevent pyelonephritis in some animals, even when serum levels of gentamicin were not detectable. This effect might have been due either to an accumulation of antibiotic in the kidney or to the presence of a sufficient amount of antibiotic in the urine to destroy the infecting bacteria. To eliminate the latter possibility, animals that received three injections of gentamicin were killed and studied bacteriologically 22 hr after ligation (1–2 hr after removal of the ureteral ligature). The pelvic fluid was cloudy in eight of 10 rats and contained a mean of 6.6×10^7 viable *E. coli*/ml. In the previously cited experiment, when rats were killed three days after ligation, all of the protected animals had sterile pelvic urine. Thus it was evident that there was not enough gentamicin in the pelvic urine during the phase of acute obstruction to prevent the bacterial inoculum from reaching the obstructed renal parenchyma. The inhibition of renal parenchymal infection three days after ligation must have resulted from antimicrobial activity effective at the level of the renal parenchyma and not at that of the pelvic urine.

Pyelonephritis was prevented in all eight animals that were given seven injections of gentamicin and were ligated 12 hr (rather than 72 hr) after the last injection. No lesions were found in any of these animals.

Prevention of relapse by gentamicin accumulation. It has been shown that antibiotic treatment of ascending obstructive pyelonephritis for 3.5 days prevents the progression of acute pyelonephritis while allowing a substantial number of bacteria to survive in the renal parenchyma [10]. Thus studies were conducted to examine whether the accumulation of antibiotic in the kidney, as was seen after gentamicin treatment, influenced the potential pathogenicity of these remaining bacteria when the ureter was religated.

Religation of the left ureter 12 hr after completion of therapy: bacteriologic study three days later. When the left ureter was religated 12 hr after the last injection of antibiotic, none of eight pyelonephritic rats treated with gentamicin had a recurrence of gross lesions. The mean number of viable bacteria ± SD recovered from their kidneys ($1.39 \pm 0.86 \times 10^5$) had not

increased significantly over that found in the kidneys of treated controls killed at the time of religation ($2.43 \pm 2.51 \times 10^4$).

In contrast, when pyelonephritic rats treated with ampicillin were religated, eight of 10 developed recurrent destructive pyelonephritis involving the entire kidney; the number of bacteria in the kidney increased significantly (from $1.16 \pm 1.7 \times 10^5$ cfu to $1.57 \pm 1.65 \times 10^8$ cfu; $P < 0.01$).

Evidently, in contrast to the ease with which infection was reestablished in ampicillin-treated animals, prior treatment with gentamicin prevented the redevelopment of acute renal infection after ureteral ligation despite the persistence of 10^5 viable bacteria in the obstructed kidneys. This protection was impressive because we have previously shown that under these experimental conditions, as few as 46 live *E. coli* can cause severe recurrent pyelonephritis in the absence of accumulated antibiotic [10].

Religation of the left ureter eight days after completion of therapy: bacteriologic study three days later. When ureters were religated eight days after completion of treatment with ampicillin or gentamicin, there was no significant difference between the effect of ampicillin and that of gentamicin on the incidence of relapse: eight of 10 rats in the ampicillin-treated group and nine of 17 rats in the gentamicin-treated group relapsed. Eight days after treatment with either drug, the kidneys of animals that experienced a relapse contained significantly more bacteria than the kidneys of treated control rats killed at the time of religation ($2.85 \pm 4.51 \times 10^7$ cfu vs. $7.7 \pm 7.0 \times 10^2$ cfu, $P < 0.05$ with gentamicin; $1.26 \pm 1.02 \times 10^8$ cfu vs. $4.52 \pm 1.10 \times 10^4$ cfu, $P < 0.0025$ with ampicillin).

Determination of antibiotic activity in kidneys. No ampicillin activity was detected either 12 hr or eight days after completion of therapy. In the prophylaxis experiments (table 1), protection against infection was a function of the level of gentamicin activity measured in the undiluted kidney homogenates. At 72 hr after the last of three injections of gentamicin when only 20% of the rats involved had acute pyelonephritis, a level of activity equivalent to 1.26 μg of gentamicin/ml was measured in the undiluted homogenates; this concentration is similar to the MIC for the infecting strain of *E. coli*. At 72 hr after a single injection of gentamicin, there was no protection against pyelonephritis, and the level of activity in the undiluted kidney homogenates was well below the MIC for the organism.

Similar relations between infection and the concentration of gentamicin in the undiluted homogenates were seen in the religation experiments (table 1). The undiluted kidney homogenates of rats that were protected from recurrent pyelonephritis 12 hr after the last of seven injections of gentamicin contained a level of activity equivalent to 4.33 μg of gentamicin/ml, a concentration three to four times the MIC for the organism. Kidneys religated eight days after treatment were not protected, and the level of activity in the undiluted homogenates was equivalent to 1.66 μg of gentamicin/ml.

There is an obvious difference between the prophylaxis and the religation studies in the relative effectiveness of the quantity of gentamicin in the kidney and the drug's ability to prevent

Table 1. Activity of gentamicin in undiluted and diluted homogenates of kidneys from rats infected with *Escherichia coli* O6 (Williams).

Experiment	Time of sacrifice after last injection (no. of injections)*	Gentamicin activity (μg/ml)		Percentage activity in undiluted vs. diluted homogenate
		Undiluted	Diluted	
Prophylaxis of pyelonephritis	72 (1)	0.41 ± 0.29	14.2 ± 7.2	2.9
	72 (3)	1.26 ± 0.17	87.2 ± 24	1.44
	12 (7)	8.4 ± 2.0	291 ± 109	2.9
Protection against recurrent pyelonephritis	12 (7)	4.33 ± 1.3	203 ± 83	2.13
	8 days (7)	1.66 ± 0.47	65 ± 19	2.55

NOTE. Data represent the mean value ± SD of determinations from eight to 16 rats. Each im injection contained gentamicin at a concentration sufficient to give the rat a dose of 4 mg of gentamicin/kg of body weight in a volume of 0.1 ml.

*Time is given in hours unless indicated otherwise.

infection. The conditions for initiation of infection in the two studies were sufficiently different to make direct quantitative comparisons difficult.

Table 1 also shows that important differences were observed when the level of gentamicin activity in diluted renal homogenates was compared with that in undiluted renal homogenates. The experiments described in table 2 were designed to investigate why the level of gentamicin activity in the undiluted homogenates was lower than that in diluted samples. Gentamicin (8 and 64 μg/ml) was added to undiluted homogenates of normal kidney tissue and to homogenates diluted 1:50 in 0.1 M PBS, pH 8.0. There was no significant loss of activity in diluted homogenates. However, activity was reduced in undiluted kidney homogenates to the same degree as in the kidneys of gentamicin-treated rats. A 1:50 dilution of the undiluted normal kidney homogenates containing 8 or 64 μg of gentamicin restored full activity of gentamicin.

The pH of the undiluted kidney homogenates was repeatedly measured as 7.2; that of the diluted homogenates, 8.0. When the kidney homogenates were diluted in buffer at pH 7.2 (table 2), the level of activity of gentamicin was lower than at pH 8.0 but was reduced less than in undiluted kidney homogenates. Thus while the difference in activity can be explained to some extent by differences in pH, the solute concentration in the kidney homogenate evidently is also an important factor in determination of the biologic activity of gentamicin.

To see whether pyelonephritis had an influence on the renal accumulation and persistence of gentamicin, the level of gentamicin activity after 3.5 days of treatment was measured in homogenates of right kidneys and compared with that found in homogenates of left kidneys. When rats were killed 12 hr after the last injection of gentamicin, the mean level of activity (± SD) in the undiluted homogenates was 4.33 ± 1.3 μg of gentamicin/ml in the left kidney, compared with 8.4 ± 2.0 μg/ml in the right kidney ($P < 0.01$). Similarly, in diluted homogenates, the level of gentamicin activity was 203 ± 83 μg/ml in the left kidney, compared with 291 ± 109 μg/ml in the right kidney ($P < 0.0025$). No such difference was seen when animals were killed eight days after completion of therapy. This difference might be related to the observation that gentamicin, like other antibiotics, is inactivated by pus in vitro [13]. Pyelonephritic left kidneys 12 hr after completion of therapy showed large areas of suppuration, but when rats were killed eight days thereafter, the kidneys displayed mainly retracted scars.

Discussion

This study of the pyelonephritic rat showed that when gentamicin accumulated and persisted in the kidney, it retained effective though reduced antimicrobial activity at that site. However, a significant discrepancy between the level of antibacterial activity in vivo and the level of total antibiotic activity in vitro, as measured in diluted kidney homogenates, was demonstrated. This finding was not surprising because of the following three points: (*1*) gentamicin activity depends on electrolyte concentration and on osmolality [14, 15]. Minuth et al. [16] showed that the drug was much less active in human urine or at osmolalities simulating concentrated urines. Gentamicin activity should be reduced in the renal medulla because solute concentration there is similar to that in urine. (*2*) Kunin [17] found a 90% reduction in level of activity of gentamicin when the drug was incubated in kidney homogenate and explained this reduction by tissue

Table 2. Effect of dilution and pH on the activity of gentamicin in homogenates of kidneys from uninfected rats.

Amount of gentamicin added (μg/ml)	Activity of gentamicin (μg/ml) incubated in homogenates of normal kidney tissue			Activity of gentamicin (μg/ml) incubated in buffer solution	
		Undiluted, pH 7.2			
	Diluted 1:50, pH 8.0	Initial	After 1:50 dilution	pH 8.0	pH 7.2
64	52	1.89	58.7	64	14.0
8	9.7	0.212	7.5	8.0	0.61

NOTE. The data represent the mean value of measurements for five different samples.

binding of gentamicin. We also found a reduction in the drug's level of activity when it was incubated with undiluted kidney homogenate, but this reduction was reversible because most of the activity was recovered by dilution (table 2). (*3*) The level of gentamicin activity demonstrable in pyelonephritic left kidneys 12 hr after completion of therapy was slightly but significantly lower than that in the intact right kidney. This difference, which might be due to inactivation of gentamicin by pus [13], was absent eight days after therapy when suppuration was almost over.

Protection against pyelonephritis was observed despite the presence of 10^7–10^8 bacteria/ml in pelvic urine and in the presence of obstruction. Both Stamey et al. [18] and McCabe and Jackson [19] have shown that to sterilize the urine of infected patients with kidney involvement, it is sufficient that antimicrobial drugs reach adequate urinary concentrations. The experiments presented here show that gentamicin persisting in the kidney prevents obstructive pyelonephritis even if there is not enough antimicrobial activity in the urine to abolish heavy pelvic infection.

These observations of the rat model suggest that despite an apparent reduction of activity in vivo, the accumulation and persistence of gentamicin in the kidneys might be used to advantage. It is possible that dosages of gentamicin currently recommended for the treatment of pyelonephritis in humans could be reduced, thereby diminishing the risk of toxicity while maintaining therapeutic effectiveness. Furthermore, it is interesting to speculate that in small doses gentamicin might be a useful means of preventing kidney involvement in patients with persistent urinary tract infections, especially in cases of irreparable urinary tract abnormalities.

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