Effect of Antibacterial Therapy on Renal Lysozyme Levels in Rats Developing Bacterial Pyelonephritis

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Received for publication 20 November 1971

In animals developing unilateral *Proteus mirabilis*-induced pyelonephritis, the total soluble renal lysozyme (SRL) of both kidneys undergoes a biphasic elevation. The second phase of elevated SRL is associated with the onset of chronicity in the infected kidney. To discover whether effective antibacterial therapy altered the second elevation of SRL, levels of SRL were determined in rats developing unilateral chronic pyelonephritis with and without effective regimens of antibacterial agents. Therapeutic doses of ampicillin and nitrofurantoin caused elevations of SRL in both kidneys of infected animals, but these differences were not statistically significant ($P > 0.05$). Both agents produce elevations of SRL in uninfected animals which were significant ($P < 0.05$) when compared with normal animals. Kanamycin sulfate at a therapeutic dose induced great elevations of SRL in kidneys of both infected and uninfected animals. It is concluded that infection per se is not the cause of the elevated SRL.

An experimental pyelonephritis model was described in a previous report from this laboratory (2). Using this model infection, we have demonstrated that soluble renal lysozyme (SRL) levels in both kidneys undergo synchronous, biphasic elevations after unilateral infection with *Proteus mirabilis* (5). The first elevation is associated with an acute inflammatory response to the trauma of infection. The second elevation is associated with the onset of chronicity in the infected kidney. The uninfected kidneys show no pathological abnormalities. Although the role (if any) of SRL in the pathogenesis is unknown, increases of this enzyme have been reported to accompany various aspects of the immune response (13, 15). In experimental unilateral pyelonephritis in rabbits, Lehmann et al. (7) demonstrated a significant production of immunoglobulin in pyelonephritic kidneys. The immunoglobulin synthesis correlated with the degree of mononuclear cell infiltration. If the elevated SRL resulted from an inflammatory or immune response to persistent foci of infection in the renal parenchyma, one would expect efficacious antibacterial therapy to reduce the inflammatory stimulus and thus repress the SRL to normal values. To test this hypothesis, the effect of clinically effective antibacterial agents on the levels of SRL in animals developing chronic pyelonephritis was determined. This report deals with the effect

of three clinically effective urinary-tract antibacterial agents on levels of SRL in animals developing chronic pyelonephritis.

MATERIALS AND METHODS

Experimental animals. CFN rats of either sex weighing 160 to 184 g were used. Animals were housed five per wire-mesh cage (38 by 35 by 18 cm) and were maintained on Purina Laboratory Chow and water *ad libitum*. Experimentally induced *P. mirabilis* (Eaton Laboratories strain Pr-91) unilateral pyelonephritis was established by methods previously described (2).

Therapy. Nitrofurantoin, ampicillin, and kanamycin were chosen for this study because they have different modes of action and because their antibacterial activities can readily be demonstrated in the pyelonephritis model infection. Drugs were administered orally or intraperitoneally at the specified doses twice daily. Nitrofurantoin (Macrochantin) was obtained from The Norwich Pharmacal Co. Ampicillin (Polycillin) and kanamycin sulfate (Kantrex) were products of Bristol Laboratories. Rats were routinely infected with *P. mirabilis*, and therapy was started 3 days after infection. Ampicillin and nitrofurantoin were administered orally, and kanamycin sulfate was administered intraperitoneally.

Determination of in vitro and urinary antibacterial activities. Antibacterial activities were measured on urine collected during the 4-hr intervals after administration of a single oral or intraperitoneal dose of antibiotic or antibacterial agent to groups of five rats which had been fasted overnight. The minimal inhibitory concentration (MIC) and the urinary antibacterial activity were determined by two-fold dilution end-

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point assay in Brain Heart Infusion broth inoculated with about 10^4 viable *P. mirabilis* cells per ml from an overnight broth culture. MIC values were expressed in micrograms per milliliter, and urinary antibacterial activity was expressed as the reciprocal of the highest inhibitory dilution (RID) of urine obtained.

Kidney bacterial titers. Kidneys were removed from rats under CO_2 anesthesia. After the pelvic tissue had been teased away, the kidneys were processed individually by washing in a stream of sterile 0.85% saline and were subsequently homogenized in 9.0 ml of sterile saline with a glass triturator. To determine the viable titer of each kidney, the dilution end point of the kidney brei in Brain Heart Infusion broth was measured with an Aminco automatic diluter. Viable titers of the experimentally infected kidneys were expressed as the $\log_{10}$ of the geometric mean of the titers of five kidneys.

Assay of SRL. Lysozyme was extracted from kidneys and assayed as previously described (5).

Statistics. Means were compared by Student's *t* test (14).

RESULTS

In vitro activities. The MIC and the urinary antibacterial activity (RID) of the three drugs are shown in Table 1. The MIC of ampicillin was the lowest, and, at the doses employed in the animal studies, kanamycin sulfate had the greatest urinary activity.

Efficacy of therapy. The three antibacterial agents employed reduced the bacterial titers of kidneys infected with *P. mirabilis*. Data from the efficacy studies (Table 2) showed that all three produced the expected dose-response relationship.

Effects of therapy on SRL. At selected times, groups of five rats were killed and the kidneys were assayed for total SRL. Uninfected rats on therapy, and infected and uninfected rats without therapy, served as controls. Kidneys of animals on ampicillin therapy tended to have slightly higher SRL levels than their respective unmedicated controls (Table 3). The differences were not significant ($P > 0.05$) when infected animals and treated infected animals were compared, but they

TABLE 1. *In vitro* antibacterial activities against *Proteus mirabilis* (Pr-91)

Drug ^a	MIC ($\mu\text{g}/\text{ml}$)	RID ^b
Kanamycin sulfate...	30	>64 at 18 mg/kg
Ampicillin.....	3.1	16 at 20 mg/kg
Nitrofurantoin.....	200	2 at 30 mg/kg

^a Kanamycin was administered intraperitoneally; ampicillin and nitrofurantoin were administered orally.

^b Urinary antibacterial activity expressed as the reciprocal of the highest inhibitory dilution of urine obtained.

TABLE 2. *Efficacy*^a of drugs used in experimental rat *Proteus pyelonephritis*

Drug ^b	Dosage (mg/kg) ^c	$\log_{10}$ of geometric mean of kidney viable titer ^d
Ampicillin	2.0	3.5
	7.0	2.7
	20.0	1.0 ^e
Nitrofurantoin	10	4.0
	30	3.0
Kanamycin sulfate	0.6	2.5
	1.8	2.4
	6.0	1.8
	18.0	1.7
	60.0	1.0 ^e
None	---	4.96 ± 0.94

^a An antibacterial agent is considered efficacious if it results in a 2σ reduction in the $\log_{10}$ of the geometric mean of the kidney viable titer (1).

^b Ampicillin and nitrofurantoin were administered orally; kanamycin was administered intraperitoneally.

^c The indicated dosage was administered twice daily.

^d Titer determined after 2 weeks of therapy.

^e An arbitrary $\log_{10}$ of 1.0 was assigned to any kidney titer of less than 10^2 .

TABLE 3. *Effect of ampicillin therapy at 20 mg/kg twice daily on lysozyme levels in rat experimental Proteus pyelonephritis model*

Kidney	Time (days after infection)	Mean total lysozyme		$P < 0.05$
		Treated	Untreated	
Normal kidney in normal rat	7	6.75	4.36	Yes
	17	6.74	4.36	Yes
Noninfected kidney in unilaterally infected rat	7	9.09	7.53	No
	17	8.28	7.02	No
Infected kidney in unilaterally infected rat	7	7.88	5.36	No
	17	5.14	6.76	No

were significant ($P < 0.05$) when normal animals were compared with treated normal animals. Nitrofurantoin produced similar results (Table 4). Kidneys of animals on nitrofurantoin tended to have slightly higher SRL levels than their respective unmedicated controls. The differences were not consistently significant ($P > 0.05$) when infected animals were compared with treated in-

TABLE 4. Effect of nitrofurantoin therapy at 30 mg/kg twice daily on lysozyme levels in unilateral *Proteus* pyelonephritis in rats

Kidney	Time (days after infection)	Mean total lysozyme		$P < 0.05$
		Treated	Untreated	
Normal kidney in normal rat	4	4.68	4.36	No
	5	4.52	4.36	No
	7	3.94	4.36	No
	10	6.34	4.36	Yes
	11	5.20	4.36	Yes
	12	6.33	4.36	Yes
	13	7.39	4.36	Yes
	14	5.14	4.36	Yes
	17	6.13	4.36	Yes
Noninfected kidney in unilaterally infected rat	4	11.90	10.54	No
	5	11.38	6.56	Yes
	7	6.23	7.53	No
	11	10.06	7.94	No
	17	9.37	7.02	Yes
Infected kidney in unilaterally infected rat	4	9.85	7.64	Yes
	5	9.16	6.40	No
	7	4.25	5.36	No
	11	7.33	5.17	Yes
	17	7.70	6.76	No

FIG. 1. Renal lysozyme levels of normal rats on intraperitoneal kanamycin sulfate therapy (18 mg/kg twice daily) during the normal 14-day course of therapy used in the standard small animal pyelonephritis model. Therapy was started on day 3. Total soluble renal lysozyme is represented by the open circles on the dashed line. The vertical bars represent ± 1 SD from the mean. The solid line represents the normal (4.3 ± 0.69) level of lysozyme in nonmanipulated rats. Kanamycin sulfate therapy caused an immediate and marked elevation of the renal lysozyme level in normal animals. All values were significantly different from normal values by Student's *t* test at the 95% confidence level.

FIG. 2. Renal lysozyme levels of experimentally infected kidneys (IK) of *Pr-91*-infected rats on intraperitoneal kanamycin sulfate therapy (18 mg/kg twice daily) during the normal 14-day course of therapy used in the standard small animal pyelonephritis model. Animals were infected on day 0, and therapy was started on day 3. The open circles on the dashed lines represent the levels of total renal lysozyme in the treated animals. The vertical bars represent ± 1 SD from the mean. The solid line is the level of total soluble renal lysozyme in the nonmedicated IK. These values were not significantly different by *t* test ($P > 0.05$) at days 5 and 7 but were at days 9 and 17 ($P < 0.05$).

ected animals, but, as with ampicillin, the differences were significant ($P < 0.05$) when normal animals were compared with treated normal animals.

In the case of kanamycin sulfate therapy, marked elevations of SRL were observed in all kidneys of medicated animals (Fig. 1-3). In normal animals on kanamycin sulfate therapy, SRL increased 245% above that of unmedicated controls in approximately 1 week. Although the increases were not as dramatic in the infected animals (Fig. 2 and 3), they were, nevertheless, significant ($P < 0.05$).

DISCUSSION

In this investigation, we determined the effects of three clinically effective urinary-tract antibacterial agents on levels of SRL in normal rats and in rats developing chronic, unilateral *P. mirabilis*-induced pyelonephritis. Our working hypothesis was that efficacious antibacterial therapy would repress the elevated SRL toward the values observed in normal treated rats.

In normal rats, all three antibacterial agents caused significant elevations of SRL. Nitrofurantoin and ampicillin evoked increases of approximately 40 and 50%, respectively, and kanamycin sulfate therapy resulted in increases of SRL of approximately 245% above control values. With

FIG. 3. Renal lysozyme levels of the experimentally uninfected kidneys (NIK) of Pr-91-infected rats on intraperitoneal kanamycin therapy (18 mg/kg twice daily) during the normal course of therapy used in the standard small animal pyelonephritis model. Animals were infected at time zero, and therapy was started on day 3. The open circles on the dashed lines represent the levels of total renal lysozyme in the NIK of the infected, dosed animals. The vertical bars represent ± 1 SD from the mean. The solid line represents the level of total soluble renal lysozyme in an unmedicated NIK. The differences seen at days 5 and 7 were not significant ($P > 0.05$), whereas those at days 9 and 17 were significantly different ($P < 0.05$). The mean for day 12 treated was compared with the mean for day 11 untreated, and these were different at a confidence level greater than the 95%.

all three agents, the levels of SRL tended to be higher in kidneys of infected animals on therapy than in infected animals without therapy.

Since our observations on both kidneys of infected rats were not consistent with the hypothetical predictions, it appears that the therapeutic agents employed have the pharmacological property of elevating SRL in the presence or absence of infection, and that infection per se is not the cause of elevated renal lysozyme. This conclusion is in agreement with our recent observation that sterile chronic pyelonephritis induced with heat-killed *P. mirabilis* produces an SRL response similar to that seen in bacterial pyelonephritis (6). Furthermore, the work of Lehmann et al. (7) indicated that the contralateral kidney in rabbits with unilateral pyelonephritis produces very little antibody to the renal pathogen and that this activity is correlated with the degree of infiltration of mononuclear cells. In the rat developing unilateral pyelonephritis, the contralateral kidney shows no inflammatory infiltration (6). It appears

that the elevated SRL of the contralateral kidney is not associated with the local production of antibody.

Two of the three compounds chosen for this study possess high in vitro antibacterial activity against *P. mirabilis*. Recent data from this laboratory indicate that in vitro antibacterial activity, expressed either as the RID of urine after a single oral dose or as the MIC, has no correlation to antibacterial action in vivo as reduction of viable kidney titers (3). Kanamycin sulfate possesses the best urinary antibacterial activity of the three drugs tested. Yet, in reduction of the kidney viable titer, ampicillin was clearly more effective. These results, although unexplained, are in agreement with other recently published data showing inconsistencies between in vitro antibacterial activities and in vivo efficacy (4, 8). Perhaps other pharmacological activities of these drugs may be responsible (at least in part) for their observed and unquestioned efficacies. In the case of nitrofurantoin, the efficacy may be attributed to the high concentrations of drug obtained in the kidney cortex as a result of the unusual cycling of the compound in the tubules of the nephron (17). It could also be argued that the increased lysozyme content of the treated kidney serves an antibacterial function. Results from this (5) and other laboratories (9) tend to void this argument. Another possibility is that these agents enhance other host defense mechanisms which operate in the kidney.

Finally, the effect of kanamycin sulfate in renal tissue is far more dramatic than that of either ampicillin or nitrofurantoin. The nephrotoxicity of kanamycin is generally acknowledged (1, 16). Renal toxins have been shown to produce lysozymuria (10, 11) and urinary excretion of other acid hydrolases (12). The elevated SRL produced by kanamycin may be the result of renal toxicity. This possibility is in general agreement with the conclusion of Raab (11), who demonstrated that a much higher single dose of kanamycin caused enzymuria which he interpreted as indicative of nephrotoxicity. The results reported here were obtained with therapeutic dosages of kanamycin, dosages below those considered toxic. Efforts are currently under way to investigate the effects of other nephrotoxic aminoglycoside antibiotics on SRL in an effort to clarify the relation between elevated SRL and nephrotoxicity.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Roberta Alexander, Carol Dubiel, Nancy Gallo, Patrick Moynihan, and Christina Kinney. The in vitro studies reported here were carried out under the supervision of Raymond Freedman. Statistical evaluations were conducted by C. T. Tu and J. L. Butterfield.

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