Activity of Tetracyclines, Nalidixic Acids, and Nitrofurantoin in Two Experimental Models of *Escherichia coli* Urinary Tract Disease in Rats (35435)

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Several methods are available for producing experimental *Escherichia coli* urinary tract disease in rats. For example, acute descending pyelonephritis can be induced by the intravenous injection of the microorganism after temporary ureter occlusion. In another model, acute ascending pyelonephritis can be produced by the injection of the organism directly into the bladder followed by induced reflux. Although there are similarities in pathology between these infection models, differences are apparent regarding the invasive route during the initial stages of infection and subsequent lesion distribution within renal tissue (1, 2).

It is common to utilize only a single infection model in the evaluation of agents useful in urinary tract disease. As therapeutic efficacy can be influenced by pharmacodynamic qualities, differences in drug performance might occur dependent upon the model system utilized. This possibility was investigated by comparative studies of 4 established compounds, having quite different pharmacodynamic properties, in *E. coli* urinary tract disease produced by two different methods in the rat.

Materials and Methods. Oxytetracycline (OTC) and α-6-deoxyoxytetracycline (DOO TC) were used as the hydrochloride salt. Nitrofurantoin, 1-(5-nitrofuryfurylidene amino) hydantoin, was used as the neutral compound, and nalidixic acid as the acid. All compounds were suspended in a diluent consisting of carboxymethylcellulose, water, and Tween 80 for oral administration at 100 or 50 mg/kg once daily to rats.

E. coli 028 was used in all experiments. Virulence was maintained by routine reisolation from the infected, nontreated group of rats.

Female albino rats of Sprague Dawley descent (Blue Spruce Farms, Inc., Altamont, N.Y.), weighing 200 g were used. The procedure for inducing descending pyelonephritis in the rat was based upon the studies of Guze and Beeson (3). Briefly, *E. coli* 028 was injected into the tail vein after the temporary occlusion of the left ureter for 20 min. The inoculum, which had been diluted 1:100, consisted of 0.5 ml of an 18-hr culture in brain heart infusion broth. Such an inoculum contained $51 \pm 0.3 \times 10^6$ viable cells. This number of cells as inoculum produced kidney counts in the range of 10^6 to 10^7 cells/g of kidney tissue under the experimental conditions utilized.

Ascending pyelonephritis was induced by administering *E. coli* 028 directly into the urinary bladder. Catheterization was readily accomplished with the use of a No. 4 Coude ureteral catheter (C. M. Bard Co., Summit, N.J.) described by Cohen and Oliver (4). The inoculum for the catheterization experiments was 1.0 ml of an 18-hr brain heart infusion culture consisting of $36 \pm 5 \times 10^7$ cells. After administration of the inoculum, the uretheral orifice was held closed and reflux was produced by gentle pressure on the rat's bladder. Drug administration started on the day after infection; treatment was once a day for 5 days. The rats were sacrificed 4 hr after the last treatment.

At the termination of the experiment, both kidneys were removed and macerated. Decimal dilutions were prepared and 3 pour plates were made in triple sugar iron agar. After incubation, typical *E. coli* colonies were counted, the counts of 5 to 8 rats was averaged, and the data were expressed as logarithmic means with their standard errors. The experiments were replicated 4 to 7 times.

TABLE I. Viable Count of *E. coli* 028 in Rat Kidneys.

	Log_{10} viable bacteria/g of kidney tissue			
	Ascending infection		Descending infection	
	100 mg/kg	50 mg/kg	100 mg/kg	50 mg/kg
Infected controls	6.67 ± 0.05	6.28 ± 0.26	6.53 ± 0.10	6.96 ± 0.17
Oxytetracycline	3.20 ± 0.14	3.83 ± 0.14	3.07 ± 0.29	4.82 ± 0.57
α-6-Deoxyoxytetracycline	2.62 ± 0.14	3.49 ± 0.30	2.48 ± 0.41	4.05 ± 0.38
Nitrofurantoin	2.76 ± 0.11	3.49 ± 0.39	2.57 ± 0.14	3.38 ± 0.31
Nalidixic acid	2.79 ± 0.18	3.77 ± 0.14	4.46 ± 0.16	5.56 ± 0.20

Results. High numbers of *E. coli* 208 were demonstrated in the kidneys of the nontreated, control rats infected by either the ascending or descending routes of infection (Table I). The infections produced were of comparable severity based on the number of viable organisms as the indicator of disease.

The ascending infection in rats was quite amenable to drug therapy. Dosage of 100 or 50 mg/kg of each drug studied markedly reduced the number of viable organisms in the kidneys. For example, the number of *E. coli* isolated from rats receiving 100 mg/kg of drug was represented by about log 2.7. In contrast, the kidney count of organisms isolated from the nontreated control rats approximated log 6.7. Rats which received a drug at 50 mg/kg demonstrated log 3.5 numbers of organisms in their kidneys; the nontreated controls, log 6.3.

Three of the 4 drugs studied, *i.e.*, OTC, DOOTC, and nitrofurantoin, were very active also in rats infected by the descending route. The activity of these 3 was nearly equivalent at each dosage level. In addition, each of these 3 agents was as effective against disease produced by the ascending as by the descending route of administration. The minor differences between log counts of viable organisms, for example, between OTC administered at 100 or 50 mg/kg in the descending and ascending infections, are not significant at the 95% level (*t* test).

Although nalidixic acid administered at 100 and 50 mg/kg was able to reduce the number of organisms in the kidneys of rats infected by the descending route when compared to the nontreated controls (Table I), this agent was less active than OTC, DOOTC, and nitrofurantoin. In general, the number of organisms in the kidneys of rats administered nalidixic acid were higher by 1 to 2 logs than with other agents. In addition, nalidixic acid was less effective in the descending than in the ascending type of infection. For example, the count in the kidneys was log 3.77 in the ascending infection after dosage of 50 mg/kg. This value increased to log 5.56 in the descending type of infection. The values for nalidixic acid at both dosages in the descending infection type are significantly less active at the 99% level than in the ascending infection type.

Discussion. It is well recognized that pharmacodynamic properties of drugs markedly influence therapeutic efficacy. One would expect, therefore, that the tetracyclines, which appear in active form in serum, urines, and tissues, would be efficacious in both types of infections. This was confirmed by the experimental findings. In contrast, nalidixic acid and nitrofurantoin appear in active form in urine but neither produces effective serum concentrations of biologically active material after oral dosage. Therefore, one would predict these compounds to have greater efficacy in the ascending infection, where the primary site of infection is in the efferent urinary pathway, rather than in the descending infection (1, 2). Nalidixic acid, in agreement with prediction, was active in the ascending infection type but was considerably less active (about a 2 log difference) in the descending infection. These findings are similar to those of Prat *et al.* (5) for nalidixic acid in a study of *E. coli* pyelonephritis in rabbits. In contrast, nitrofurantoin was as active in the descending as in the ascending infection. As

discussed by Cockett *et al.* (6) and Rocha *et al.* (7), the activity of nitrofurantoin is probably the result of localization within medullary tubular lumen and interstitial spaces, in concentrations inhibitory for many urinary pathogens. In addition, several workers have reported that nitrofurantoin is found in much higher (3-fold) concentration in renal lymph than in the blood (9, 10).

The assessment of therapeutic efficacy in urinary tract drugs is incomplete, as the data for nalidixic acid indicates, when evaluated in a single model infection system. A 2-model system, which encompasses differences in the invasive route of the organism during the initial stages of infection and subsequent lesion distribution within renal tissue would provide a more critical assessment of antibacterial activity than can be obtained in the use of a single model.

Summary. Oxytetracycline, α-6-deoxyoxytetracycline, nitrofurantoin, and nalidixic acid were studied in two experimental *Escherichia coli* models of urinary tract infection in rats, *i.e.*, descending pyelonephritis induced by intravenous injection of the organism after temporary ureter occlusion and ascending pyelonephritis induced by administration of the organism into the bladder followed by manually produced reflux.

Oxytetracycline and α-6-deoxyoxytetracycline, agents which appear in active form in body fluids and tissues, had equal activity in both infection models. Although nitrofurantoin appears in active form only in the urine, equal activity was demonstrated in both infection models. Nalidixic acid, an agent primarily active in the urine, was markedly more active in the ascending than in the descending urinary infection model. A more critical and complete appraisal of chemotherapeutants for use in urinary tract disease would be gained by evaluation in 2 different experimental urinary tract models rather than in a single infection model.

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