NALIDIXIC ACID IN INFECTIONS OF URINARY TRACT

LABORATORY AND CLINICAL INVESTIGATIONS

BY

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Nalidixic acid (1-ethyl-7-methyl-1,8-naphthyridine-4-one-3-carboxylic acid) is one of a new series of 1,8-naphthyridine derivatives with antibacterial properties which have been synthesized by Lesher *et al.* (1962). The data on previous bacteriological, toxicological, and pharmacological studies obtained at the Sterling Winthrop Research Institute have been summarized by Lishman and Swinney (1963). This work suggested that the drug might be of particular value in the treatment of infections of the urinary tract associated with Gram-negative organisms.

The following report shows the results of laboratory and clinical studies with nalidixic acid (" negram ").

Antibacterial Activity

Materials and Methods

The laboratory investigations were made on organisms recently isolated from infections of the urinary tract.

The minimum inhibitory concentration (M.I.C.) of nalidixic acid was determined on 236 organisms, using a serial-dilution-tube technique. Serial doubling dilutions of nalidixic acid solution were made in 1-ml. amounts of an assay broth composed of nutrient broth (" oxoid No. 2 ") with 0.2% glucose and 0.5% Andrade's indicator. The tubes were inoculated with 0.02 ml. of a 1 in 1,000 dilution of an overnight broth culture of the test organism and incubated for 18 hours at 37° C. The M.I.C. was recorded as the minimum concentration of nalidixic acid which prevented visible growth. In addition, a disk-sensitivity technique was carried out, using 5-mm. blotting-paper disks impregnated with 5, 30, and 60 μg. of nalidixic acid respectively.

An indication of the bactericidal activity of nalidixic acid was obtained by subculturing from the dilution tubes containing ×1, ×2, ×4, and ×8 M.I.C. for each organism. These subcultures were incubated at 37° C. for 24 hours, and the least concentration of the drug from which no organism could be isolated was taken as the minimal bactericidal concentration (M.B.C.).

A further 524 organisms were tested by the disk-sensitivity technique to compare the sensitivity of the organisms to nalidixic acid with their sensitivity to other antibacterial agents.

An attempt was made to induce resistance by inoculating a broth containing a subinhibitory concentration of nalidixic acid and subculturing the organism into a fresh nalidixic acid-broth mixture at daily intervals until a significant change in the M.I.C. of the organism had been obtained. Ten strains of *Escherichia coli* were tested by this technique.

Results

M.I.C. determinations are presented in Table I. The Gram-positive organisms tested had relatively high M.I.C.s; some strains of *Streptococcus faecalis* were not inhibited by a concentration of 2,400 μg./ml. With the exception of strains of *Pseudomonas pyocyanea*, 92% of the Gram-negative organisms tested had M.I.C.s of 16μg./ml. or less, and fell into the range which was considered to indicate a sensitive strain. *E. coli* with 111 out of 114 strains (97%) and *Klebsiella–Aerobacter* with 16 out of 17 strains (94%) within this range were more sensitive than *Proteus*, of which 41 out of 51 strains (80%) had M.I.C.s of 16 μg./ml. or less. The disk technique consistently showed zones of inhibition greater than 15 mm. in diameter with the 60-μg. nalidixic acid disk from organisms having M.I.C.s of 16 μg./ml. or less, and with the 30 μg. disk from organisms having M.I.C.s of 8 μg./ml. or less. The 5 μg. disk gave inconsistent results with organisms having M.I.C.s of 4 μg./ml. or less, while with M.I.C.s greater than this the zones were always less than 15 mm. in diameter.

TABLE I.—*Distribution of the Minimum Inhibitory Concentrations of Nalidixic Acid by Species*

Organism	No. of Strains Tested	No. of Strains having M.I.C. (μg./ml.) of									
		>256	256	128	64	32	16	8	4	2	1
E. coli ..	114	1		1	1		3	14	40	39	15
Proteus sp. ..	51			6	3	1	1	5	16	11	8
Ps. pyocyanea	16	12	1	1	1		1				
Klebsiella ..	13			1				2	2	3	5
Aerobacter ..	4						2	1		1	
Staph. pyogenes	30	1		15	13	1					
Str. faecalis ..	8	5		3							

The M.B.C. of nalidixic acid was identical with the M.I.C. in 76.5% of Gram-negative organisms and twice the M.I.C. in the remainder.

The results of the comparative sensitivity tests on 524 organisms are shown in Table II. More strains of *E. coli* (90%) and *Proteus* sp. (81.2%) showed a " sensitive " inhibitory zone to nalidixic acid than to any of the other antibacterial agents tested. Of these organisms, 82 (19.7%) out of 416 strains of *E. coli* and 12 (18.8%) out of 64 strains of *Proteus* sp. were sensitive only to nalidixic acid. Resistance to nalidixic acid was shown by 42 (10%) strains of *E. coli* and 12 (18.8%) strains of *Proteus* sp. Of these, 22 strains of *E. coli* and 3 strains of *Proteus* sp. were resistant to all the antibacterial agents tested.

TABLE II.—*Distribution of Sensitive Strains of 524 Organisms to Nalidixic Acid and Other Common Antibacterial Agents as Assessed by Disk-sensitivity Technique*

Organism	No. of Strains Tested	No. of Strains Sensitive to Each Antibacterial Agent					
		N	C	T	S	Sulph.	F
E. coli ..	416	374	291	158	181	20	135/352*
Proteus sp. ..	64	52	37	1	28	0	15
Ps. pyocyanea	25	1	0	0	1	0	0
Klebsiella ..	3	3	3	3	3	0	—
Staph. pyogenes	16	4	12	5	6	0	—

N=Nalidixic acid (30 μg.). C=Chloramphenicol (25 μg.). T=Tetracycline (25 μg.). S=Streptomycin (10 μg.). Sulph.=Sulphadimidine (200 μg.). F=Nitrofurantoin (200 μg.).
* Only 352 strains of *E. coli* were tested against nitrofurantoin.

Resistance to nalidixic acid was rapidly induced in the 10 strains of *E. coli* tested. Table III shows the increase in M.I.C. with serial subcultures. Six strains showed single-step increases into the resistant range, four of them after two transfers, one after three transfers, and one after eight transfers. In the remaining four strains the M.I.C.s increased by one or two steps before a resistant strain developed. Further subculture of eight of the ten strains showed increasing resistance of the multiple-step pattern.

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TABLE III.—*Development of Resistance to Nalidixic Acid by Strains of E. coli During Serial Daily Subculture Into Subinhibitory Concentrations of Nalidixic Acid*

No. of Transfers	0	1	2	3	4	5	6	7	8	9
M.I.C. (μg./ml.)	4	4	4	256						
	4	4	256	512						
	8	8	32	512						
	2	2	128	128	128	>2,048				
	8	8	64	512	2,048	>2,048				
	4	4	8	128	512	2,048	>2,048			
	8	8	8	16	16	64	64	256		
	2	2	2	8	8	8	128	512		
	16	16	16	16	16	16	16	16	128	128
	4	4	4	4	8	16	16	128	128	256

Clinical Trial

Nalidixic acid was used to treat infections of the urinary tract in 57 patients (21 male, 36 female), with an age range of 18–89 years. The primary diagnosis is shown in Table IV. There were 27 patients with acute and 30 patients with chronic or recurrent infections. Patients were included in the trial only when a catheter or mid-stream urine specimen contained numerous pus cells and organisms sensitive to nalidixic acid as assessed by the disk technique. The drug was given orally as 0.5-g. tablets. A daily dose of 4 g. was given to 56 patients, the remaining patient receiving 8 g. daily. A single course of five days' duration was given to 51 patients; three others received the drug for 3, 8, and 10 days respectively; two were given two separate courses; and one patient received three separate courses—two of 5 days' and one of 10 days' duration.

TABLE IV

Clinical Diagnosis	No. of Patients
Post-operative urinary infections:	
Prostatectomy	7
Colporrhaphy	5
Hysterectomy	2
Caesarean section	5
Abdomino-perineal resection	2
Mid-thigh amputation	1
Post-partum urinary infection	2
Pyelitis in pregnancy	4
Chronic pyelonephritis (without obstructive urinary-tract abnormality)	9
Chronic urinary infection associated with:	
Carcinoma of penis	1
,, ,, prostate	1
,, ,, rectum with hydronephrosis	1
Hemiplegia	7
Disseminated sclerosis	1
Acute urinary infection	9
Total	57

Urine samples were taken after five days' therapy: if pus cells were present the drug was continued, unless culture of the urine revealed a resistant organism. From 35 of the 43 patients with a sterile post-treatment urine a further urine sample was obtained after four to six weeks.

Results

The infecting organism was a pure growth of *E. coli* in 53 patients, *Proteus* sp. in two, and a mixed growth of *E. coli* and *Proteus* in two.

The degree of symptomatic relief was assessed as excellent in 25 patients, good in eight, and moderate in one. Symptoms continued without relief in nine patients from whose post-treatment urine a resistant organism was isolated. The degree of relief was not assessable in the remaining 14 patients, whose only symptom had been incontinence of urine, which remained whether or not the infection had been cleared. Of the 34 patients whose symptoms were relieved, 28 (82.5%) were symptom-free within 48 hours of the onset of therapy, and the remainder within 72 hours. Of 23 patients with a pretreatment pyrexia, 20 (87%) were apyrexial within 48 hours.

The post-treatment urine specimens were sterile and free from pus cells in 43 patients (75.5%); in six others the original infecting organism had been eliminated but reinfection with resistant organisms (*Ps. pyocyanea* 4, *Str. faecalis* 2) had occurred. These six patients had chronic urinary infections, and four of them had indwelling catheters. A five-day course of treatment was sufficient to eradicate the original infecting organism from the urine in 46 of these 49 patients; in one patient the urine was clear after three days' therapy, while two patients required up to 10 days' treatment. Urine specimens collected from the remaining eight patients after five days' therapy showed persistence of the infection with the original organism, which was now resistant to nalidixic acid. In each case this was a strain of *E. coli*. Three of these patients had chronic urinary infections associated with previous prostatic obstruction; in the other five the infection was acute.

Follow-up urine samples were obtained from 35 of the 43 patients with a sterile post-treatment urine. The results are shown in Table V.

TABLE V.—*Response of Acute and Chronic Urinary Infections to Treatment with Nalidixic Acid*

Type of Infection	No. of Patients	Sterile Post-treatment Urine		No. Reinfected During Treatment	No. Developing Resistant Strain	No. with Follow-up Urine	No. Reinfected at Follow-up	Follow-up Cure Rate (%)
		No.	%					
Acute	27	22	81·5	0	5	18	2	89
Chronic	30	21	70	6	3	17	12	29·5

Further courses of treatment with nalidixic acid were given to three patients. The first had a prostatic carcinoma with a two-months history of difficulty with micturition and scalding. An *E. coli* infection was successfully treated by the first course of nalidixic acid and the urine remained sterile for four weeks before reinfection with a *Proteus* sp. occurred. A second course of treatment eradicated this infection, and urine specimens remained sterile for the next two months. A dose of 4 g. daily for five days was used in both courses of treatment.

The second patient, an 89-year-old woman, had a chronic urinary infection with incontinence and scalding. Two courses of nalidixic acid 4 g. daily for five days were given at an interval of four weeks. After both treatments the symptoms persisted, and the originally sensitive strains of *E. coli* had developed resistance to the drug.

Three courses of treatment were given to the third patient, a 76-year-old man with acute retention of urine. After a transvesical prostatectomy he developed an *E. coli* infection which was successfully treated with a five-day course of 4 g. of nalidixic acid daily. The urine remained sterile, and he was symptom-free for two months until the obstructive symptoms recurred. After a transurethral resection of the bladder neck he developed a urinary infection with a different strain of *E. coli*, which was cleared after a second identical course of nalidixic acid. A further episode of urinary retention occurred three weeks later and an indwelling catheter was inserted. After catheterization the urine became infected with a strain of *E. coli* sensitive only to nalidixic acid. A third course of 4 g. daily for 10 days cleared the *E. coli* from the urine, but reinfection with a resistant *Str. faecalis* occurred, which persisted in spite of treatment with various antibacterial agents.

Toxic effects in this series of patients were slight. A maculopapular rash on the trunk and lower limbs occurred

in two patients. The rash appeared three and eight days respectively after treatment; in both it cleared two days after stopping the drug. Other drugs which the patients were receiving had been continued, and the prompt fading of the rash after cessation of nalidixic acid administration strongly suggested that this drug was the causal agent. No other toxic effects were noted. Estimation of blood urea and microscopy of urine for casts did not show any changes which might indicate that the drug produced renal damage. Similarly, regular blood counts failed to show any changes attributable to the drug, at least over the short periods of its administration.

Four patients were pregnant at the time of treatment. Two were at 34 weeks' gestation, one at 20 weeks', and one at 16 weeks'; all four had normal deliveries of healthy infants.

Discussion

The laboratory investigations show that nalidixic acid has antibacterial activity against Gram-negative organisms commonly isolated from infections of the urinary tract. The exception is *Ps. pyocyanea*, of which 15 out of 16 strains tested had minimum inhibitory concentrations greater than 16 μg./ml. Gram-positive organisms also were relatively resistant to the drug. These results are in agreement with those of previous workers (Lesher *et al.*, 1962; Buchbinder *et al.*, 1962). In 76.5% of the Gram-negative organisms the drug was bactericidal in the same concentration as that required to inhibit growth, and in the remainder only slightly higher concentrations were required. With the disk-sensitivity technique, nalidixic acid compared favourably with other drugs commonly used to treat infections of the urinary tract at least with regard to its activity against coliforms and *Proteus* sp. Similar results were obtained by Lishman and Swinney (1963), using the same technique, and by Buchbinder *et al.* (1962), who used the tube-dilution technique.

In the clinical trial eradication of bacteria from the urine was achieved in 45 out of 61 treatments (74%). Reinfection with either *Str. faecalis* or *Ps. pyocyanea* was a problem only in chronic infections, but the development of resistant strains occurred in both acute and chronic infections. The demonstrations *in vitro* of rapidly increasing resistance to the drug by strains of *E. coli* and its occurrence during treatment on nine occasions in eight patients suggests that the development of drug-resistant strains may become a problem if nalidixic acid is widely used. Buchbinder *et al.* (1962) obtained similar results on *in vitro* testing of four strains of *E. coli* and after treatment in 3 out of 15 patients.

The good follow-up cure rate in acute infections without urinary-tract abnormality (89%) and the rather poor follow-up response of chronic infections (29.5%) are similar to the results previously reported on treatment of urinary infections using various agents (Rhoads *et al.*, 1952; Garrod *et al.*, 1954; Kass, 1955; Turck *et al.*, 1962).

Adverse reactions to the drug occurred in only 2 of the 57 patients. In each case an irritant rash developed after three and eight days respectively which cleared on cessation of therapy. Lishman and Swinney (1963) report drowsiness in one patient, "light-headedness" in a second, and a transient irritant rash in a third, out of 60 patients treated with the drug. Buchbinder *et al.* (1962), using a higher daily dosage for a longer period, reported toxic reactions, mostly nausea and vomiting, in 6 out of 15 patients.

As stated, all four pregnant patients in this series were of 16 weeks' gestation or longer, and each of them had a normal delivery of a healthy infant. The drug has not been administered in the early months of pregnancy, so no information about possible teratogenic effects is available.

The evaluation of any new antibacterial agent in the treatment of infections of the urinary tract is made more difficult by the spontaneous cures which may occur in the absence of antibacterial treatment, particularly in patients without a previous history of urinary infection, and also after correction of abnormalities of the urinary tract (Kass, 1955; Turck *et al.*, 1962). From the results obtained in this trial, however, nalidixic acid would seem to be of value in the treatment of infections of the urinary tract, particularly those associated with coliform organisms and *Proteus* sp. A dose of 1 g. every six hours for five days has proved adequate and safe in most cases, and is the recommended regime.

Summary and Conclusions

Nalidixic acid is a new synthetic antibacterial agent active against coliform organisms and *Proteus* sp. It is clinically effective in urinary infections due to Gram-negative organisms, with the exception of those associated with *Ps. pyocyanea*, but the follow-up cure rate in chronic infections is as disappointing as with other antibacterial agents. Resistant strains of *E. coli* are rapidly developed *in vitro*, and they occurred during treatment in 15% of patients. Toxic effects of the drug were insignificant.

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