Factors Governing the Emergence of Resistance to Nalidixic Acid in Treatment of Urinary Tract Infection.

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Cultures of Escherichia coli were exposed to nalidixic acid in an in vitro model in which the conditions of drug-organism interaction resembled those of bacterial cystitis treatment. Results obtained in this way suggested that emergence of bacterial resistance should not be a major problem in treatment of uncomplicated urinary infection; such cases might indeed respond to a less intensive course of treatment than is usual. More prolonged, high-dosage therapy with nalidixic acid may be required for patients with more complicated infections if the risk of failure from the emergence of bacterial resistance is to be minimized.

The vast majority of enterobacteria isolated from infected urine are sensitive in vitro to nalidixic acid, and the compound has, consequently, been widely and successfully used in treatment of urinary tract infection. Nevertheless, there has been some concern about the use of nalidixic acid because it is very simple to convert bacteria to resistance in the laboratory (2, 4, 5), and because failure of therapy due to emergence of resistant bacteria has frequently been demonstrated (1, 2, 4, 6, 10, 11, 15, 19). On the other hand, the importance of conversion to resistance as a cause of treatment failure has been questioned (3), and the experience of some workers has led them to suggest that adequate dosage is the key to therapeutic success (18).

As a contribution to this debate, we have investigated the conditions governing the emergence of bacterial resistance to nalidixic acid by use of an in vitro model that mimics some important aspects of drug-bacterium interaction peculiar to the treatment of bacterial cystitis.

MATERIALS AND METHODS

Drug. Sodium nalidixate was kindly donated by Winthrop Laboratories. Suitable concentrations were freshly prepared in sterile, distilled water as required.

Organism. The strain of Escherichia coli (laboratory code ECSA 1), originally isolated from infected urine, was susceptible to 4 μg of nalidixic acid per ml, as judged by conventional titration in broth with a bacterial inoculum of ca. 10^6 bacteria per ml. Growth medium was a complete broth previously described (8).

Bladder model. (i) Design. The bladder model design was essentially the same as described elsewhere (7, 14), except that arrangements were made to deliver a varying concentration of drug in the "bladder" in simulation of the renal excretion pattern of nalidixic acid. This was achieved by use of an appropriately programmed gradient-forming device ("Mixograd"; Gilson Medical Electronics) linked to twin reservoirs of drug-containing and drug-free broth.

Bladder model. (ii) Experimental conditions. In all experiments, 20 ml of an overnight broth culture of bacteria was diluted with fresh broth at 1 ml/min (the normal diurnal rate of urine flow into a bladder), and at 1-h intervals a pump automatically emptied the model bladder, leaving behind a residual volume of 20 ml. In certain experiments, an 8-h "sleep" period was included in which the inflow rate was reduced to the nocturnal value of 0.25 ml/min, and "micturition" was stopped. Changes in the culture opacity were continuously monitored photometrically.

Susceptibility testing. The nalidixic acid susceptibility of surviving bacteria was tested by continuous turbidimetric monitoring, using a 12-channel bacterial growth monitoring device (13). Twenty-five-milliliter volumes of fresh broth containing graded concentrations of nalidixic acid were each inoculated with 1 drop of culture from the bladder model and incubated in the turbidimetric device for 20 h.

Control. Subcultures onto solid medium were made where appropriate to exclude the possibility that growth in the presence of nalidixic acid was due to contaminant organisms.

RESULTS

Two aspects of the therapeutic situation were investigated; the response of the bacterial culture to a single dose of the drug, subsequently repeated on relapse; and the response of the culture to multiple dosage aimed at achieving, and subsequently maintaining, a particular
drug level. The two types of experiments are depicted diagrammatically in Fig. 1, which also indicates that in the second type of experiment an 8-h "sleep" was included. The shaded areas in Fig. 1 refer to the nalidixic acid concentration in the diluent broth. The actual concentration achieved in the model bladder at any particular time was dependant (as it is in treatment) on the drug concentration entering the bladder and on the volume of urine encountered there. Previous studies (9) had established the response of cultures in this model to a pulse of nalidixic acid and that the response to nalidixic acid was relatively unaffected by variation in the conditions of "diuresis" and frequency of "micturition."

The form of response of *E. coli* to a single dose of nalidixic acid, repeated on "relapse," is shown in Fig. 2. In this experiment, the inflow contained drug-free broth for the first 4 h, after which the nalidixic acid concentration in the inflow increased linearly to reach a maximum of 250 μg/ml after 4 h; this level was held for 2 h, after which it declined linearly to 0 over a further 6-h period (12 h of antibiotic exposure in all). A second identical cycle of exposure was commenced after persisting bacteria had reestablished the bacterial population. After the first "dose," opacity of the culture fell as the concentration of drug reached an effective level, but regrowth occurred after the concentration of drug had declined. The second cycle of exposure to nalidixic acid had a much-reduced effect on the culture, and regrowth occurred sooner. Similar experiments were performed in which the peak concentrations achieved were 10 and 50 μg of nalidixic acid/ml. The time taken, after starting administration of each dose of the drug, for opacity of the culture to reattain an opacity level of 50% of maximum is shown for the three nalidixic acid concentrations in Table 1.

The nalidixic acid susceptibility of cultures growing after each of the two cycles of exposure to drug is compared with that of a previously unexposed culture in Fig. 3. The susceptibility profile after a single dose was similar whether the maximum concentration to which the culture had been exposed was 10, 50, or 250 μg of nalidixic acid per ml (Fig. 3B). A further dose, achieving a peak concentration of 50 μg of nalidixic acid per ml, did not alter this profile significantly, but further increased resistance was observed after a second dose, achieving a peak of 250 μg of the drug per ml (Fig. 3C). This increase in resistance was found to remain stable through 12 passages in drug-free broth in all cases.

In experiments in which the peak nalidixic acid concentration, once achieved, was maintained (Fig. 1, experiment B), regrowth was detectable within 24 h of first exposure to nalidixic acid when the plateau level was 50

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**TABLE 1. Comparison of recovery times of cultures of* E. coli* after exposure to consecutive doses of nalidixic acid**

<table>
<thead>
<tr>
<th>Peak conc of nalidixic acid (μg/ml)</th>
<th>Time (h) to recovery* after dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>50</td>
<td>19.5</td>
</tr>
<tr>
<td>250</td>
<td>25</td>
</tr>
</tbody>
</table>

* Time after beginning of exposure to drug for opacity to reattain 50% level.

b NE, No effect on normal growth.

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**FIG. 1. Two types of experiments performed with the bladder model. (A) Single cycle of exposure to nalidixic acid, repeated after regrowth of the culture; (B) continuous exposure to nalidixic acid after attainment of peak level.**

**FIG. 2. Continuous opacity record obtained with the bladder model in the type of experiment, depicted in Fig. 1A, in which the peak level achieved after each dose was 250 μg of nalidixic acid per ml.**

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μg of drug per ml, but no regrowth was detected during a 36-h period of observation when the concentration achieved a plateau of 250 μg of nalidixic acid per ml. The culture growing in the presence of 50 μg of nalidixic acid per ml had a susceptibility profile similar to that shown in Fig. 3B.

Essentially similar results were obtained with three other E. coli strains.

**DISCUSSION**

These results may throw some light on the controversy surrounding the failure of nalidixic acid therapy caused by emergence of resistant bacteria: a relatively transient nalidixic acid concentration, reaching no more than 10 μg/ml, had a marked depressant effect on the bacterial population, lasting 15 h (Table 1)—much longer than the usual interval between doses—showing the high activity of this compound against the majority of the bacterial population. Increasing the peak level achieved to 50 or 250 μg of nalidixic acid per ml led to a further prolonged effect.

However, survivors of exposure to nalidixic acid showed a striking increase in resistance to the drug and, in the conditions of the model, eventual regrowth of a resistant population occurred within 24 h of first exposure in the presence of a plateau level of 50 μg of nalidixic acid per ml. The relatively high residual volume (20 ml) of the model, which is greatly in excess of the normal human value of about 1 ml (16), no doubt promoted the emergence of resistance in the presence of drug by reducing the efficiency of clearance of inhibited bacteria by dilution and periodic discharge. This underlines the importance of residual bladder volume in the maintenance of infection (12, 17).

These considerations encourage the view that the requirements for successful therapy of urinary tract infection differ markedly among different groups of patients: those with uncomplicated-infections may respond to minimal therapy for a short period of time, whereas higher and more prolonged dosage may be necessary for more intractable infection. The success of nalidixic acid seems to depend almost entirely on the very effective initial onslaught, which, in patients with uncomplicated cystitis, tips the balance in favor of intrinsic clearance mechanisms. Such success could probably be achieved by relatively low drug levels. Failure to eradicate the infection entirely at this stage, however, sets the scene for conversion to nalidixic acid resistance.

Emergence of resistance in the difficult patient represented by the model was not observed when the peak concentration was held at 250 μg of nalidixic acid per ml, but such a high concentration of active drug could only be reliably maintained in the most favorable circumstances in vivo. Low or irregular dosage or pharmacokinetic variability that leaves urine free of adequate drug levels may rapidly lead to establishment of a highly resistant population against which levels of nalidixic acid achievable in urine are ineffectual.

These results broadly support Stamey’s view (18) that high-dose nalidixic acid therapy is necessary for “poor responders” as, for example, in retreatment of relapse; even so, the emergence of resistance would be expected to be a significant cause of treatment failure in such cases. Minimal therapy, as with other agents, may be all that is required in the initial therapy of uncomplicated infection.

**LITERATURE CITED**