Mechanisms of resistance to quinolones and clinical perspectives

The primary target for the action of the quinolones is the A sub-units of DNA gyrase, although the actual mechanism of bactericidal action may involve a cascade of cellular events (Piddock & Wise, 1987a). Mutants of Escherichia coli with high-level resistance to nalidixic acid can easily be selected in the laboratory at a frequency of approximately $10^{-7}$, and have been shown to possess an altered DNA gyrase (Gellert et al., 1976). DNA gyrase has been shown to exist in all bacterial species examined to date (Gellert, 1981). In several species, including Pseudomonas aeruginosa, Haemophilus influenzae, and Citrobacter freundii, mutations occur which code for a DNA gyrase subunit that is less susceptible to inhibition by nalidixic acid, and over recent years there have been several reports of this mechanism of quinolone resistance (Rella & Haas, 1982; Setlow et al., 1985; Hirai et al., 1987; Inoue et al., 1987; Aoyama et al., 1988) (Table I). As can be seen in many of the mutants, such as nalA, norA, nfxA, cfxA, ofxB, appear very similar; all have been shown to possess a DNA gyrase with A subunits that are less susceptible to quinolone inhibition, and have been described as alleles of gyrA. The gyrA mutations encode resistance to quinolones, but there is no cross resistance to chemically unrelated antimicrobial agents observed.

Other DNA gyrase laboratory mutants of Esch. coli have also been described, nal-24 (formerly nalD), nal-31 (formerly nalC) (Inouye et al., 1978; Yamagishi et al., 1981) (Table I). The nal-24 and nal-31 mutations cause changes in the overall electrical charge of DNA gyrase, nal-24 causing a decrease in negative charge and nal-31 an increase. Nal-31 is therefore hypersusceptible to compounds that are positively charged (Smith, 1984). There is also some evidence that nal-24 confers decreased permeability to nalidixic acid. There have been several reports of decreased uptake of quinolones causing decreased susceptibility, although more than one mechanism appears to be implicated. The first 'permeability' mutant described was nal B (Hane & Wood, 1969) which is thought to have decreased permeability of nalidixic acid, as EDTA treatment of whole cells enhanced the measured DNA synthesis inhibition (i.e. DNA gyrase inhibition) whereas similar treatment of a gyrA mutant was ineffective (Bourguignon, Levitt & Sternglanz, 1973). Esch. coli nal-D, resistant to nalidixic acid at 37°C, is susceptible at 30°C (Hrebenda et al., 1985) and shows a decrease in the uptake of tritiated nalidixic acid and glycerol, but no change in the outer membrane protein (OMP) profile or cross resistance to chemically unrelated agents is observed. It has therefore been thought that this strain contains a mutation causing a phospholipid bilayer alteration. Recently mutants such as nfxB, norB, cfxB, ofxB in Esch. coli have been described (Hirai et al., 1986; Hooper et al., 1986, 1987); all have similar phenotypes (Table I) and have been shown to have decreased expression of OMP F and decreased uptake of quinolone. Chromosomal mapping of the mutants has shown that the nfx B gene occurs close to the structural gene for OMP F, whereas norB, cfxB, ofxB all map close to the marA gene which encodes multiple antibiotic resistance (George & Levy, 1983). All mutants demonstrate cross resistance to cephalosporins, tetracycline and chloramphenicol, the activity of which has been shown to be affected by the absence of OMP F (Reeve & Suttie, 1968; Pugsley & Schnaitman, 1978; Jaffe, Chabbert & Semonin, 1982). Ps. aeruginosa nalB is similar to Esch. coli norB with decreased susceptibility to quinolones and β-lactams (but not tetracycline); however the OMP profile of nalB has not been examined (Rella & Haas, 1982). Ps. aeruginosa nfxB is not similar to the nfxB mutant of Esch. coli, and has decreased susceptibility to quinolones and hypersusceptibility to β-lactams and aminoglycosides (Hirai et al., 1987), and expresses an extra OMP with a molecular weight of 54,000 and decreased uptake of norfloxacin. Esch. coli norC (not similar to nfxC) has altered expression of OMP F, but no change in norfloxacin uptake or susceptibility to cefoxitin or chloramphenicol. The norC mutant is hypersusceptible.
gyrase A subunits were less susceptible to floxacin MIC 100 mg/l) and the DNA urine expressed high level resistance (norfloxacineristant strains emerging during therapy with new quinolones; two strains of novofloxacin-resistant Esch. coli isolated from urine expressed high level resistance (norfloxacin MIC 100 mg/l) and the DNA gyrase A subunits were less susceptible to quinolone inhibition (Sato et al., 1986; Aoyama et al., 1987). The second strain also lacked OMP F.

Strains of Ps. aeruginosa isolated after enoxacin therapy from the sputum of patients suffering from chronic obstructive airways disease showed a similar phenotype to that observed for a strain expressing a nalA mutation (Piddock & Wise, 1987b), and one post-enoxacin-therapy isolate showed a decrease in susceptibility to norfloxacin and some β-lactams, absence of OMP F and a decrease in the uptake of 14C enoxacin (Piddock, Wijnands & Wise, 1987) and was presumed to be a nalB mutation. Alterations in OMPs have also been implicated in causing a decrease in susceptibility to ciprofloxacin in a strain of Serratia marcescens isolated from the sputum of a patient suffering from a fatal bacteraemia, although the patient was treated with ticarcillin and tobramycin (Sanders & Watanakunakorn, 1986).

There have also been clinical reports (without biochemical or genetic analysis) of quinolone-resistant strains (Panhotra & Desai, 1983; Chapman, Speller & Reeves, 1985; Crook, Selkon & McLardy-Smith, 1985; Humphreys & Mulivhili, 1985; Panhotra, Desai & Sharma, 1985; Roberts, Batten & Hodson, 1985; Smith, Cashmore & Leyland, 1985; Wagenvoort et al., 1986; Glupczynski et al., 1987; Wise et al., 1987). Susceptibility determination of all strains except one Klebsiella pneumoniae (Chapman et al., 1985)
Table II. Pharmacology of quinolones in humans (administered by the oral route)

<table>
<thead>
<tr>
<th>Agent</th>
<th>dose (mg)</th>
<th>Serum C&lt;sub&gt;max&lt;/sub&gt; (mg/l)</th>
<th>Urine C&lt;sub&gt;max&lt;/sub&gt; (mg/l)</th>
<th>Sputum C&lt;sub&gt;max&lt;/sub&gt; (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalidixic acid</td>
<td>1000</td>
<td>—</td>
<td>50–500&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>500</td>
<td>1.9–2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>0.8–1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>400</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98–114&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Enoxacin</td>
<td>600</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>288&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>400</td>
<td>11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>200</td>
<td>—</td>
<td>216&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Floxacin</td>
<td>400</td>
<td>3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>400</td>
<td>6.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50–300&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.4&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50–300&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data from Hooper & Wolfson (1985); <sup>•</sup>R. Wise, personal observations; <sup>•</sup>Bronchial mucosal concentrations.

has implicated a gyrA mutation (L. J. V. Piddock, unpublished observations). The K. pneumoniae demonstrated cross-resistance to β-lactams but no apparent change in the OMP profile.

There has been one clinical report of plasmid-mediated nalidixic acid resistance in Shigella dysenteriae, but no cross-resistance to new quinolones was demonstrated (Munshi et al., 1987). The mechanism of resistance has not yet been determined.

It has long been realized that nalidixic acid displays little or no useful antibacterial activity against Gram-positive bacteria. With the development of newer more active quinolones the Gram-positive activity has been improved (Wolfson & Hooper, 1985). However, whilst the susceptibility of most Gram-positive bacteria is within the therapeutically achievable concentrations of many new quinolones the susceptibility of such species as Staphylococcus aureus and Streptococcus pneumoniae is far less than that of the Enterobacteriaceae, and resistant mutants can be selected easily in experimental infections (Kaatz et al., 1987). It is thought that this lower susceptibility is due to a DNA gyrase that is inherently less susceptible to the action of quinolones (N. Georgopapadakou, personal communication). Descriptions of the DNA gyrase of medically important Gram-positive species have yet to be published, but data are available for the enzymes from Micrococcus luteus and Bacillus subtilis (Liu & Wang, 1978; Sugino & Bott, 1980). A mutation at gyrA in B. subtilis caused decreased susceptibility to nalidixic acid (MIC 300 mg/l) (Vazquez-Ramos & Mandelstam, 1981). Two other mutations oxr-1 and oxr-2 (oxolinic acid-resistance) were also described and may code for a mutation affecting the uptake of nalidixic acid and oxolinic acids.

To summarize, there have been essentially two types of quinolone resistance in Gram-negative bacteria described to date, mutations affecting DNA gyrase and mutations affecting OMP F production and/or permeability. By comparing the in-vitro susceptibility data with the maximum achievable concentrations (C<sub>max</sub>) in selected body fluids (in healthy human volunteers) certain conclusions about the clinical relevance of characterized mechanisms of resistance can be made (Tables I and II). For ciprofloxacin, ofloxacin and pefloxacin a sufficient concentration can be achieved in the serum and urine to inhibit even the 'high-level' resistance exhibited by most laboratory derived gyrA mutants and it might be anticipated that in a patient such bacterial resistance would not occur. However, a gyrA mutant of Esch. coli selected in vivo had an MIC of ciprofloxacin and ofloxacin of 25 and 100 mg/l, respectively, which is well above the clinically achievable concentrations in serum and sputum (Sato et al., 1986). Comparison of the susceptibility of the gyr-A phenotype displayed by strains of Enterobacteriaceae other than Esch. coli suggests that certain species can have MICs greater than the achievable blood levels of norfloxacin and enoxacin; also such mutants might not be inhibited by the achievable concentrations of the more active ciprofloxacin in sputum, even allowing for the fact that concentration in bronchial mucosa occurs. It may be prudent to suggest that if a strain of a species such as Enterobacter cloacae is resistant to 64 mg/l nalidixic acid then it may well be clinically resistant to norfloxacin,
enoxacin and ciprofloxacin at certain body sites where concentration does not occur (e.g. skin and subcutaneous tissue). If one allows greater than four times the MIC as the required clinically achievable concentration (as argued by many clinicians and as suggested in the recent report of a working party of the British Society for Antimicrobial Chemotherapy (BSAC, 1988)) then the majority of the new quinolones would not retain their therapeutic activity against a gyr-A type mutation in species such as K. pneumoniae infecting the lungs or blood.

It is of interest to note that the species of bacteria that are predominant among the clinically resistant variants isolated to date (e.g. Staph. aureus, Ps. aeruginosa, K. pneumoniae) are species that are inherently less susceptible to most quinolones. Such strains commonly cause chronic infections (for example infection in cystic fibrosis, osteomyelitis). A mutation in such organisms such that the MIC is raised 4–8 fold may often be associated with clinical resistance. This, coupled with the fact that the bacteria may be causing infection at a somewhat sequestered part of the body, might also contribute to the occurrence of clinical resistance, and recent clinical reports would appear to confirm this.

It may be that for serious infections a quinolone will be combined with another antimicrobial agent which would be active against any variants expressing a gyr-A type mutation. Perhaps more worrying is a mechanism of resistance that causes decreased susceptibility to several chemically unrelated agents, such as the multi-resistant phenotype observed in certain Enterobacteriaceae, which is similar to the Esch. coli norB, the MIC of which is often above the achievable concentrations of ciprofloxacin and enoxacin in sputum, and norfloxacin and enoxacin in serum.

Many consider that, as quinolones (such as ofloxacin and ciprofloxacin) achieve concentrations in body fluids such as serum that are up to one hundred fold excess of the MICs for many bacterial species, the selection of resistant organisms and hence failure of therapy is extremely unlikely. This, with the suggestion that the susceptibility of strains of Esch. coli expressing gyrA mutations is still within the range of quinolone activity, may make the likelihood of emergence of resistant variants very remote. However, evidence is appearing that what may be the case for laboratory strains of Esch. coli, certainly is not the case for other species. The organisms isolated during or after therapy all expressed a more pronounced decrease in susceptibility to all quinolones than one might anticipate from laboratory studies (L. J. V. Piddock & J. M. Diver, unpublished observations). It is worrying that for this class of antimicrobial agents laboratory-derived resistant variants may not be emulating their counterparts isolated from patients undergoing treatment. This is perhaps a facet of the properties of quinolones that is not seen in other chemical classes of antimicrobial agent.

The actual number of nalidixic acid-resistant clinical isolates that are cross resistant to newer quinolones found to date is still low (Piddock et al., 1986) and the available statistics of the occurrence of clinical resistance to new quinolones confirm this. However, as quinolones are used to treat a wider range of infections in a larger number of patients, isolates of certain species expressing resistance to certain agents at certain sites will probably increase. From the evidence to date, the species will include Ps. aeruginosa, Staph. aureus, K. pneumoniae and Ent. cloacae infecting not only the blood, but other sites. Perhaps clinicians should be particularly cautious about the use of quinolones in the therapy of chronic infections caused by such pathogens. Here is a dilemma: these are just the situations in which we should wish to use them, and avoid injectable agents. If they are to be used in such infections close bacteriological monitoring will be very important.

L. J. V. PIDDock
R. WISE
Antimicrobial Agents Research Group,
Department of Medical Microbiology,
The Medical School,
University of Birmingham,
Birmingham B15 2TT, UK

References


Leading articles


Prevention of infection after vascular reconstruction

Wound infection after vascular reconstruction may cause considerable delay in hospital discharge and therefore be expensive in resources (Johnson et al., 1988). However, the real hazard is that the infection may involve prosthetic material. Graft infection is