Antagonism of Wild-Type and Resistant *Escherichia coli* and Its DNA Gyrase by the Tricyclic 4-Quinolone Analogs Ofloxacin and S-25930 Stereoisomers

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The mechanism of action of the quinolone analogs ofloxacin and S-25930, which are unusual because of the presence of a third ring with an asymmetric carbon, was studied. Drug-resistant strains of *Escherichia coli* were selected by serial passage in the presence of ofloxacin, and a mutation was mapped near the *gyrA* gene of DNA gyrase. DNA gyrase containing the A subunit purified from this strain as compared with the isogenic wild-type strain exhibited increased resistance to ofloxacin, proving that the mutation was located in the *gyrA* gene. For S-25930, the S stereoisomer was more potent than the R isomer in inhibiting wild-type *E. coli* and DNA gyrase containing an A subunit isolated from this strain. Both isomers had decreased potency against the isogenic ofloxacin-resistant (gyrA) strain and its purified enzyme, but the S isomer remained more potent than the R isomer. These studies, using a combined genetic and biochemical approach, demonstrate (i) that DNA gyrase is a target of the tricyclics ofloxacin and S-25930, (ii) that serial exposure to ofloxacin can select resistance to tricyclic quinolone agents by mutation in the *gyrA* gene, and (iii) that the more potent antibacterial activity of S relative to R S-25930 correlates with increased activity against DNA gyrase for both wild-type and ofloxacin-resistant (gyrA) isogenic strains.

A primary target of the 4-quinolone antimicrobial agents is the A subunit of the bacterial enzyme DNA gyrase (3, 7, 9, 14, 16–18). Ofloxacin, S-25930, and flumequine are 4-quinolone analogs that are unusual because they (i) have a tricyclic rather than bicyclic (quinolone) core ring structure and (ii) contain in this third ring an asymmetric carbon (4, 13–15, 17, 20) (Fig. 1). For ofloxacin, the levorotatory (−) optical isomer has been found to be more potent than the dextrorotatory (+) isomer in inhibition of growth of *Escherichia coli* and antagonism of purified DNA gyrase (5, 10). In contrast to ofloxacin, the absolute structures of the stereo-isomers of S-25930 have been determined (4). The S isomer has been found to be more potent than the R isomer in inhibition of growth of different bacterial species (4; D. C. Hooper and J. S. Wolfson, Rev. Infect. Dis., in press) and of DNA gyrase isolated from wild-type *E. coli* (Hooper and Wolfson, in press).

To characterize the relationship of ofloxacin, R S-25930, and S S-25930 to DNA gyrase, we selected and characterized *E. coli* KL16 strains resistant to ofloxacin and determined the potency of these tricyclic and other quinolone agents against DNA gyrase isolated from isogenic wild-type and resistant strains. (These studies were presented in part at the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 28 September to 1 October 1986 [J. S. Wolfson, D. C. Hooper, E. Y. Ng, K. S. Souza, G. L. McHugh, and M. N. Swartz, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 950, 1986].)

Bacterial strains and methods for determination of MICs, transduction, partial purification of DNA gyrase, and assay of introduction of superwits into relaxed plasmid pBR322 DNA molecules were done as previously described (8, 9; Hooper and Wolfson, in press). MICs were determined on agar for all agents except R and S S-25930, for which broth was used because of limited quantities of the drug. Ofloxacin, S-25930 isomers, and norfloxacin were gifts from Ortho Pharmaceutical Corp. (Raritan, N.J.), Riker Laboratories (St. Paul, Minn.), and the Merck Institute for Therapeutic Research (Rahway, N.J.), respectively. Nalidixic acid was obtained from Calbiochem-Behring (San Diego, Calif.). Chloramphenicol was obtained from Sigma Chemical Co. (St. Louis, Mo.), and tetracycline hydrochloride was obtained from Lederle Laboratories (Pearl River, N.Y.). For DNA gyrase assays, the smallest amount of DNA gyrase A protein (2 U) that in the presence of excess DNA gyrase B protein fully superwirtped 0.3 μg of relaxed pBR322 DNA in 60 min at 25°C was used. The half-inhibitory concentration (IC₅₀) was defined as the concentration of drug that, when added to assays containing 2 U of DNA gyrase A protein and an excess of DNA gyrase B protein, reduced the intensity of the most superwirtped band by one half, as determined by visual inspection of photographs of gels or by scanning negatives of photographs of gels with a scanning densitometer.

We first attempted to isolate strains of *E. coli* KL16 spontaneously highly resistant to ofloxacin in a single step but were unsuccessful, as has been the experience of others (1, 2, 11, 13). Highly resistant strains were isolated, however, by serial exposure of bacteria on quinolone-containing agar (11–13, 19). Cells were sequentially passed on agar medium containing twofold-increasing concentrations of ofloxacin (0.02 to 5.1 μg/ml) (8). One isolate, EN120, which exhibited 128-fold increased resistance to ofloxacin, was further characterized (Table 1). The isolate also had increased resistance to norfloxacin (64-fold), nalidixic acid (≥64-fold), chloramphenicol (4-fold), and tetracycline (2-
(fold). By P1 transduction, mutations conferring ofloxacin resistance were transferred back into wild-type KL16 selecting for ofloxacin resistance (9). Two classes of mutants were identified, one with low-level resistance to quinolone agents only (fourfold to eightfold), as represented by EN121, and the other with low-level resistance to both quinolone agents and the structurally unrelated drugs tetracycline and chloramphenicol, as represented by EN122 (Table 1). Mapping by P1 transduction demonstrated cotransduction (4 of 15) of the first class of mutations and the glpT gene, which is near the gyrA gene on the E. coli chromosome (9). Isolation of a DNA gyrase A subunit from one of these transductants, EN121, yielded an ofloxacin-resistant enzyme (Table 2). This result proves for the first time with a combined genetic and biochemical approach that the DNA gyrase A subunit is a target of ofloxacin and that drug resistance after exposure to ofloxacin may occur by mutation in the gyrA gene. The mechanism of increased resistance of the second class of mutants is yet not known, but the pleiotropic nature of the resistance suggests decreased permeation, as has been found for norfloxacin- and ciprofloxacin-resistant mutants of E. coli (6–9; Hooper and Wolfson, in press).

In contrast to ofloxacin, the absolute structures of the S-25930 stereoisomers have been determined (Fig. 1) (4). Therefore, we studied R and S S-25930 and found the S isomer to be at least 10-fold more potent than the R isomer in inhibition of growth of E. coli KL16 and antagonism of DNA gyrase containing the A subunit isolated from this strain (Table 2). The racemic form of S-25930 had MICs twofold higher than those of the S form, as expected. The mutation in the gyrA gene in strain EN121 also generated about 10-fold increased resistance in bacteria and their purified A subunits to both isomers, with the S isomer remaining more active (Table 2). These findings indicate that the S isomer of S-25930 is more potent against bacteria and purified DNA gyrase than the R isomer and that mutation in the A subunit selected with ofloxacin can generate substantially increased resistance to S-25930, a tricyclic quinolone agent that has an asymmetric center in the same position of the third ring as ofloxacin. The stereochemical differences between S and R S-25930 are likely to be useful for understanding the interactions of quinolones, DNA gyrase, and DNA.

With respect to the asymmetric carbon in S-25930, the desmethyl derivative has been shown to have antibacterial activity in mediating a selective advantage in the R isomers, a finding that raised the interesting possibility that the methyl group is needed in the S orientation to generate optimal inhibitory activity against DNA gyrase (4).

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**TABLE 1. Susceptibilities of ofloxacin-resistant mutants of E. coli to selected quinolones and other antimicrobial agents**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characteristic</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OFX</td>
<td>NFX</td>
</tr>
<tr>
<td>KL16</td>
<td>Wild type</td>
<td>0.08</td>
</tr>
<tr>
<td>EN120</td>
<td>Ofxr</td>
<td>10</td>
</tr>
<tr>
<td>EN121</td>
<td>gyrA Ofxr</td>
<td>0.32</td>
</tr>
<tr>
<td>EN122</td>
<td>Ofxr</td>
<td>0.32</td>
</tr>
</tbody>
</table>

* OFX, Ofloxacin; NFX, norfloxacin; NAL, nalidixic acid; CM, chloramphenicol; TC, tetracycline.

* ND, Not determined.

**TABLE 2. Effect of a gyrA Ofxr's mutation on the ability of ofloxacin, S S-25930, and R S-25930 to inhibit growth of E. coli (measured as MIC) and DNA supercoiling activity of DNA gyrase (measured as IC50)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Ofloxacin S S-25930</th>
<th>R S-25930</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>IC50</td>
<td>MIC</td>
</tr>
<tr>
<td>KL16</td>
<td>Wild type</td>
<td>0.08*</td>
<td>0.5</td>
</tr>
<tr>
<td>EN121</td>
<td>gyrA Ofxr</td>
<td>0.32</td>
<td>5.0</td>
</tr>
</tbody>
</table>

* All concentrations are in micrograms per milliliter.

**LITERATURE CITED**


a quinolone carboxylic acid compared to other quinolones and other antimicrobial agents. J. Antimicrob. Chemother. 16:563–574.


