ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1987, p. 1640–1641
0066-4804/87/101640-02$02.00/0
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Vol. 31, No. 10

NOTES

Norfloxacin Resistance in a Clinical Isolate of *Escherichia coli*

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Received 4 May 1987/Accepted 24 July 1987

Analysis of DNA gyrase supercoiling and of norfloxacin uptake in *Escherichia coli* GN14176, a moderately norfloxacin-resistant clinical isolate, indicated that resistance was associated with both an altered drug target and a reduction in drug uptake.

Norfloxacin-resistant mutants obtained by spontaneous single-step mutations in *Escherichia coli* K-12 strains have been identified as having an alteration either in the A subunit of DNA gyrase (*nfxA* or *norA*, alleles of *gyrA*) or in outer membrane proteins (*nfxB*, *norB*, and *norC*) (7, 8). Although the MICs of norfloxacin against these mutants (0.2 to 0.6 μg/ml) were only four- to eightfold higher than that against the parent strain, those against norfloxacin-resistant *E. coli* strains isolated from clinical material ranged from 0.8 to more than 25 μg/ml. We reported earlier that one of the highly norfloxacin-resistant clinical isolates has an altered DNA gyrase (11). In the present study, we describe the resistance mechanisms of a moderately norfloxacin-resistant clinical isolate of *E. coli*, focusing both on DNA gyrase and norfloxacin uptake.

E. coli GN14176 was isolated from a urinary tract infection and identified by standard methods (2). *E. coli* K-12 strain KL-16 (Hfr *thi relA*) and its *gyrA* derivative, MH-5, were used (1, 5). Norfloxacin was synthesized by Kyorin Pharmaceutical Co. Ltd., Tochigi, Japan.

Susceptibility to norfloxacin was measured by agar dilution (7) by using Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) with an inoculum of 2.5×10^4 CFU per spot. The A and B subunits of DNA gyrases were purified by ammonium sulfate precipitation (3), followed by novobiocin-Sepharose (13) and heparin-Sepharose CL-6B (Pharmacia Fine Chemicals, Piscataway, N.J.) column chromatography as described previously (11). The assay system of ATP-dependent DNA gyrase activity was modified from previous reports (3, 4). The inhibitory effect of norfloxacin on the supercoiling activity of DNA gyrase was determined as described by Sato et al. (11). The uptake of norfloxacin by *E. coli* cells was measured by a bioassay with *E. coli* NIHJ JC-2 by the method described previously (6, 7). Outer membrane proteins were prepared by the method of Sawai et al. (12) and analyzed on a urea-sodium dodecyl sulfate-polyacrylamide gel as described by Uemura and Mizushima (14). To identify OmpF and OmpC porin proteins, the outer membrane proteins prepared from KE7 ($OmpC^-$) and KE11 ($OmpF^-$) (6) were used as references.

The susceptibility of GN14176, MH-5, and KL-16 to norfloxacin and the 50% inhibitory concentrations (IC_{50}s) of norfloxacin for the supercoiling activity of DNA gyrase from these strains are shown in Table 1. The MIC of norfloxacin against strain GN14176 was 8 times higher than that against MH-5 and 128 times higher than that against KL-16. The IC_{50} for DNA gyrase from GN14176 was 100-fold higher than that for DNA gyrase from KL-16. This study also suggests that DNA gyrase is a target of norfloxacin in *E. coli* GN14176. However, the IC_{50} for GN14176 was the same as that for MH-5. That is, the inhibitory effects of norfloxacin on the supercoiling activity of DNA gyrase from GN14176 and MH-5 strains were not in parallel with the antibacterial activities against these strains. The results suggest that other factors also contribute to norfloxacin resistance in GN14176.

The uptake of norfloxacin by GN14176 was about one-third of that by KL-16 cells (Fig. 1). We found previously that quinolone compounds penetrate the outer membrane of *E. coli* K-12 through the OmpF porin (6, 7). The electrophoretic pattern demonstrated that the amount of OmpF protein in the cell envelope was decreased in GN14176 (Fig. 2). These results indicate that norfloxacin resistance in GN14176 was associated with alterations of the DNA gyrase and that decreased norfloxacin uptake was caused by the change of outer membrane proteins. The double-resistance mechanism of GN14176 could thus explain the resistance to norfloxacin that was eight times higher than that of MH-5, which appears to have a mutation only in DNA gyrase. We previously reported that the DNA gyrase of a clinical isolate of *E. coli* GN14181 highly resistant to norfloxacin (MIC, 100 μg/ml) is more resistant to norfloxacin (IC_{50}, >800 μg/ml) than the DNA gyrase of MH-5 (11).

In laboratory strains, norfloxacin-resistant mutants with the alteration in both DNA gyrase and the cell membrane have not been obtained in a single step (7, 8). However, highly norfloxacin-resistant clinical isolates have been isolated more frequently than expected (9). GN14176 showed

TABLE 1. Antibacterial activity (MIC) versus inhibitory concentrations (IC_{50}) of norfloxacin for the DNA gyrase supercoiling activities

E. coli strain	Concn of norfloxacin (μg/ml)[a]	
	MIC	IC_{50}
GN14176	6.25	47.0
KL-16	0.05	0.47
MH-5	0.78	46.3

[a] MIC was determined by the agar dilution method. IC_{50} was determined by measuring the supercoiled pBR322 DNA peak in an agarose gel by a densitometric assay.

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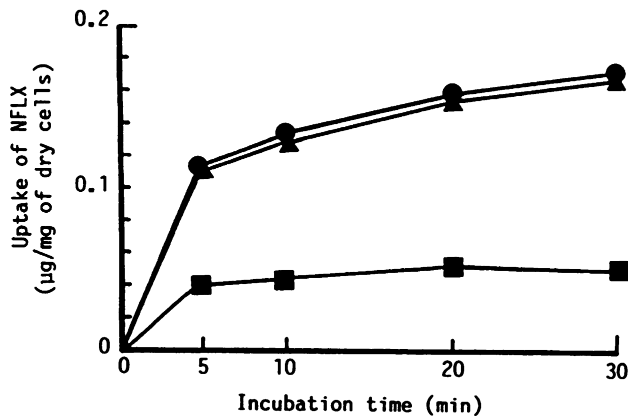

FIG. 1. Uptake of norfloxacin (NFLX) by strains of *E. coli*. Symbols: ■, GN14176; ▲, MH-5; ●, KL-16.

the low-level resistance to chloramphenicol and cefoxitin (data not shown) that is also seen in *nfxB*, *norB*, and *norC* mutants. This low-level multiple-antibiotic resistance with the alteration of outer membrane proteins has also been studied in clinical isolates of *Serratia marcescens* (10). Therapeutic use of agents other than quinolones, such as cephalosporins, might change the bacterial outer membrane so that low-level multiple-antibiotic-resistant clinical isolates

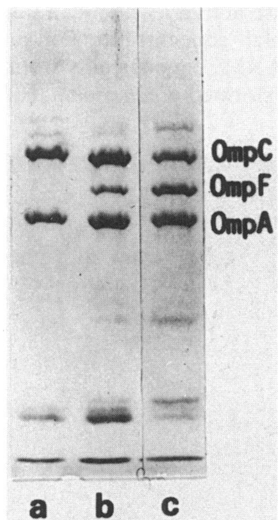

FIG. 2. Urea-sodium dodecyl sulfate-polyacrylamide gel electrophoresis of outer membrane proteins. Lanes: a, GN14176; b, MH-5; c, KL-16. Approximately 10 to 20 µg of protein was loaded onto the gel.

could be easily produced. This might increase the isolation frequency of quinolone-resistant strains from clinical sources. Further studies are planned to test this hypothesis.

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