Antibacterial Activities and Inhibitory Effects of Sitafloxacin (DU-6859a) and Its Optical Isomers against Type II Topoisomerases

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The in vitro inhibitory effects of sitafloxacin (DU-6859a) and its three stereoisomers on bacterial DNA gyrase from *Escherichia coli*, topoisomerase IV from *Staphylococcus aureus*, and topoisomerase II from human placenta were compared. No correlation was observed between the inhibitory activities of quinolones against bacterial type II topoisomerases and those against human topoisomerase II. Sitafloxacin showed the most potent inhibitory activities against bacterial type II topoisomerases and the lowest activity against human type II topoisomerase.

Quinolone antibacterial agents have been used in therapy for various bacterial infections. The enzyme targets of quinolones are considered to be DNA gyrase and topoisomerase IV (6, 7), which are essential enzymes responsible for controlling the topological state of DNA in DNA replication and transcription (16). DNA gyrase has ATP-dependent DNA supercoiling activity (8) and is a primary target of quinolones in the gram-negative species, such as *Escherichia coli* and *Neisseria gonorrhoeae* (3, 9, 15). In contrast, topoisomerase IV has an essential role in partitioning replicated chromosomes (13) and is more sensitive than DNA gyrase to some quinolones, such as levofloxacin and ciprofloxacin, in the gram-positive species, such as *Staphylococcus aureus* and *Streptococcus pneumoniae* (5, 19, 22, 30).

Certain quinolones have been shown to interfere with the activity of eukaryotic type II topoisomerase (topoisomerase II) (23, 24), and their inhibitory potencies against topoisomerase II might be correlated with their cytotoxicities (11). Actually, Akahane et al. (1) reported that quinolones inhibited the proliferation of murine cells in a dose-dependent manner, and the cytotoxicities of quinolones correlated well with their inhibitory potencies against topoisomerase II. Therefore, it is justified to compare the inhibition by quinolones of the activity of DNA gyrase or topoisomerase IV with their inhibition of topoisomerase II activity. Our previous study demonstrated that levofloxacin, the L-isomer of ofloxacin, possessed a higher selectivity than ofloxacin and its d-isomer (11).

Sitafloxacin (also known as DU-6859a) (12, 23, 24) is a quinolone synthesized at Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan. The in vitro activities of sitafloxacin, its optical isomers (DU-6856, DU-6857, and DU-6858), levofloxacin, ofloxacin, and ciprofloxacin against a variety of reference strains and clinical isolates were determined. The MICs were determined by the microbroth dilution method recommended by the National Committee for Clinical Laboratory Standards (21). The inoculum size was approximately 105 CFU/ml. The MIC was defined as the lowest drug concentration that prevented visible bacterial growth of the inoculum after incubation for 18 h at 37°C.

In in vitro activities of sitafloxacin, its optical isomers (DU-6856, DU-6857, and DU-6858), levofloxacin, ofloxacin, and ciprofloxacin against a variety of reference strains and clinical isolates are shown in Table 1. Among the quinolones tested, sitafloxacin had the greatest activity against members of the family Enterobacteriaceae, *Pseudomonas aeruginosa*, *S. aureus*, *Staphylococcus epidermidis*, and *Bacteroides fragilis*.

Subunits A and B of DNA gyrase were purified separately with maltose-binding protein by the method described previously (12, 30). The specific activities of purified subunits A and B of DNA gyrase were 106 U/mg of protein. Subunits A and B of *S. aureus* topoisomerase IV were purified as fusion proteins separately with maltose-binding protein by the method described previously (30). Human topoisomerase II was purchased from Topogen, Inc. (Columbus, Ohio). Inhibitory activities of quinolones against type II topoisomerases were assayed electrophoretically as described previously (12, 30) with minor modifications. The gel was

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against DNA gyrase. The IC50s against topoisomerase IV were determined as the drug concentrations that reduced the decatenation activity seen with drug-free controls by 50%. Additionally, for the relaxing assay of topoisomerase II, a reaction mixture (20 μl) containing 1 U of topoisomerase II (which brought 50% of the pBR322 plasmid to the relaxed form), drug solution, 50 mM Tris hydrochloride (pH 7.5), 100 mM KCl, 10 mM MgCl2, 1 mM ATP, 0.5 mM dithiothreitol, 0.5 mM EDTA, 30 μg of bovine serum albumin per ml, and 0.2 μg of supercoiled pBR322 was incubated at 37°C for 1 h. The IC50 of each quinolone against the relaxing activity of topoisomerase II was calculated by the same method as that for DNA gyrase.

MICS and inhibitory effects on topoisomerases are shown in Table 2. Sitafloxacin was twice as active against E. coli KL-16 as DU-6856, four times more active than DU-6857, and eight times more active than DU-6858. Against the supercoiling activity of E. coli DNA gyrase, the IC50s of sitafloxacin, DU-6856, DU-6857, and DU-6858 were 0.13, 0.18, 0.42, and 0.69 μg/ml, respectively. Thus, the anti-DNA gyrase activity of sitafloxacin was highest, followed by those of DU-6856, DU-6857, and DU-6858, in that order. There is a high correlation between the inhibitory effects of the quinolones on bacterial growth and the inhibition of DNA gyrase; the correlation coefficient, calculated by using a transformation of the data points of all the compounds tested, was 0.941.

Against S. aureus FDA 209-P, sitafloxacin was twice as active as DU-6856 and eight times more active than DU-6857 and DU-6858. Against the decatenation activity of topoisomerase IV, the inhibitory activity of sitafloxacin was about twice as potent as that of DU-6856. DU-6857 and DU-6858 were about three times less active than sitafloxacin. There was also a significant correlation between the inhibitory effects on S. aureus growth and the inhibition of topoisomerase IV (correlation coefficient, 0.986).

On the other hand, the IC50s of four isomers against topoisomerase II from human placenta ranged from 1,147 to 2,369 μg/ml, and the inhibitory potency was lowest for sitafloxacin, followed by DU-6857, DU-6856, and DU-6858 in increasing order of potency. There was no correlation between the IC50 for any bacterial topoisomerase and that for mammalian topoisomerase II.

Eukaryotic topoisomerase II is known as an essential enzyme for cell growth (4). Because of the similarities in the biochemical mechanisms and amino acid sequences between DNA gyrase and mammalian topoisomerase II (17), the inhibition of human topoisomerase II by quinolones would be an undesirable side effect of antibacterial chemotherapy, and information on the relative selectivity toward bacterial target enzyme versus human topoisomerase II would be of significance. Fortunately, the compounds tested required higher concentrations to inhibit human topoisomerase II than to inhibit DNA gyrase or topoisomerase IV, and all the selectivity values

![Figure 1. Structures of sitafloxacin and its stereoisomers.](http://aac.asm.org/)

### TABLE 1. Antimicrobial activities of quinolones

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STFX</td>
</tr>
<tr>
<td><em>E. coli</em> KL-16</td>
<td>0.008</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em> IID 976</td>
<td>0.008</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> 08601</td>
<td>≤0.004</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> 08103</td>
<td>0.008</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> type 1</td>
<td>0.031</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 03400</td>
<td>≤0.004</td>
</tr>
<tr>
<td><em>Serratia marcescens</em> 10100</td>
<td>0.031</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> PAO1</td>
<td>0.125</td>
</tr>
<tr>
<td><em>Bacterium anitratum</em> ATCC 19606</td>
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</tr>
<tr>
<td><em>H. influenzae</em> 038003 960614</td>
<td>0.004</td>
</tr>
<tr>
<td><em>S. aureus</em> FDA 209-P</td>
<td>0.008</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 56500</td>
<td>0.063</td>
</tr>
<tr>
<td><em>S. pyogenes</em> G-36</td>
<td>0.063</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 19433</td>
<td>0.25</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> J24</td>
<td>0.031</td>
</tr>
</tbody>
</table>

*a* STFX, sitafloxacin; LVFX, levofloxacin; OFLX, ofloxacin; CPFX, ciprofloxacin.
of the compounds tested were greater than 100 (Table 2). Sitafloxacin proved to possess the highest relative selectivity for bacterial target enzyme versus human topoisomerase II among the compounds tested in this study.

It has been proposed that quinolones bind to a specific site on DNA in the DNA-enzyme complex (26–28). According to this model, differences in enzyme inhibitory potency are mainly determined by the strength of drug binding to a DNA receptor site on the enzyme-substrate complex, while the interaction of the C-7 substituent with the enzyme plays a supporting role (18). Yoshida et al. (31) have provided evidence showing that C-7-7 cis-N-1 substituent, to C-7-7 (9 cis) is in close contact with the so-called quinolone pocket in the enzyme-substrate complex, while the interaction of the C-7 substituent with the enzyme, compared to C-7-7 (R)-isomers. In the stereoisomeric derivatives of the N-1 substituent, cis-isomers were more potent than trans-isomers, as previously reported (2). In this study, sitafloxacin (C-1-[(1R,2S)-cis-2-fluorocyclopropyl]) showed more potent antibacterial activities against the bacterial enzymes, DNA gyrase and topoisomerase IV, than its C-1-[(1S,2R)-cis-2-fluorocyclopropyl] isomer DU-6856. This result suggested that C-1-[(1R,2S)-cis-2-fluorocyclopropyl] moiety has interactive potential with the receptor site on the DNA-enzyme complex. We plan to pursue further study of the difference in the ratios of binding of the isomers to DNA-topoisomerase II complex.

REFERENCES


