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# NOTES

## Comparative In Vitro Activities of the Investigational Fluoroquinolone DC-159a and Other Antimicrobial Agents against Human Mycoplasmas and Ureaplasmas[▽]

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**The in vitro susceptibilities of 151 unique clinical isolates of *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium*, and *Ureaplasma* species to DC-159a, an investigational fluoroquinolone, in comparison with those to other agents were determined. Macrolides were the most active agents against *M. pneumoniae* and *M. genitalium*, whereas clindamycin was most active against *M. hominis*. DC-159a MICs were ≤0.5 μg/ml for all *Mycoplasma* species and ≤4 μg/ml for ureaplasmas. DC-159a was the most active fluoroquinolone tested against *M. pneumoniae* and *M. fermentans*, and it was second to moxifloxacin against the other species. It was bactericidal against 10 *M. pneumoniae* isolates and demonstrated killing of ≥99.9% of the inoculum at 24 h for 2 isolates. The excellent in vitro activity of DC-159a demonstrates its potential for use in the treatment of infections due to mycoplasmas and ureaplasmas.**

Mycoplasmal species of human origin, including *Mycoplasma pneumoniae*, *M. genitalium*, *M. hominis*, *M. fermentans*, and *Ureaplasma* spp., can be responsible for infections in the respiratory and urogenital tracts. In some cases, these organisms can cause severe, systemic disease, especially in the setting of a debilitated or immunocompromised host (11). Current treatment alternatives are limited primarily to drugs in the macrolide, lincosamide, tetracycline, and fluoroquinolone classes, and various agents in these classes exhibit differential in vitro activities against these organisms.

Fluoroquinolones are useful treatment options for mycoplasmal and ureaplasmal infections because they have the advantages of being well tolerated in oral formulations; they have long half-lives, allowing once-daily dosage; they possess bactericidal activity; they are not affected by resistance mechanisms that affect other drug classes, such as tetracyclines and macrolides; and documented in vitro resistance is uncommon (3, 8, 9, 11). The broad spectrum of activity against many other microorganisms that may cause clinically similar illnesses makes fluoroquinolones attractive for empirical treatment since most mycoplasmal and ureaplasmal infections are rarely confirmed by microbiological testing.

DC-159a is an investigational fluoroquinolone being developed by Daiichi Sankyo Pharmaceuticals Co., Ltd., Tokyo, Japan. This agent has been shown previously to have potent in vitro activity against other bacterial pathogens causing diseases in the respiratory tract, including *Streptococcus pneumoniae*, *S. pyogenes*, *S. agalactiae*, *Haemophilus influenzae*, *H. parainfluenzae*, *Moraxella catarrhalis*, and *Bordetella pertussis* (5, 6). The only susceptibility data available for DC-159a thus far are those in a report providing MICs for 10 strains of *M. pneumoniae* (4). The present study was undertaken to investigate the comparative in vitro inhibitory and bactericidal activities of DC-159a against a collection of mycoplasma and ureaplasma species that are known to cause disease in humans.

(Results of this work were presented at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, September 2007.)

The 49 *M. pneumoniae* isolates tested were collected from the respiratory tracts of persons with pneumonia over a several-year period. Six *M. genitalium* isolates were obtained from the mycoplasma collection at the National Institutes of Health (NIH) and from Jorgen Jensen at the Staten Seruminstitut, Copenhagen, Denmark. Twenty-five *M. hominis* isolates were derived from clinical specimens from the urogenital tract or wound cultures. Fifteen of these *M. hominis* isolates were known to be resistant to tetracycline (MICs ≥ 8 μg/ml). Twenty *M. fermentans* isolates included organisms derived from clinical specimens and others obtained from the NIH. *Ureaplasma* isolates, including 25 *U. urealyticum* isolates and 25 *U. parvum* isolates, were derived from cultures of specimens from the urogenital tracts of adults or the lower respiratory tracts of neonates. Three *U. parvum* isolates were known to be resistant to tetracycline (MICs ≥ 8 μg/ml). One isolate of *U. parvum* was known to be resistant to fluoroquinolones (levofloxacin MIC = 16 μg/ml) (2).

A broth microdilution method was performed as described previously to determine MICs (9, 10). Quality control strains used to validate MICs of comparator agents included *M. pneumoniae* ATCC 29342, *M. hominis* ATCC 43521, and *U. urealyticum* serovar 9 ATCC 33175.

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The antimicrobial agents tested over a range of 0.001 to 32 μg/ml were DC-159a, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, azithromycin, clindamycin, doxycycline, and tetracycline. Clindamycin was tested only against *M. hominis* and *M. fermentans*. Erythromycin and azithromycin were tested only against *M. pneumoniae*, *M. genitalium*, and *Ureaplasma* spp. The MIC was defined as the lowest concentration of a drug at which the metabolism of the organism was inhibited, as evidenced by a lack of color change in the medium at the time the drug-free control first showed color change.

Summaries of the in vitro activities of DC-159a and other antimicrobials are shown in Table 1. MICs obtained for currently available fluoroquinolones, macrolides, clindamycin, and tetracyclines were generally similar to those reported previously for these agents (1, 7, 9). DC-159a was the most active fluoroquinolone tested against *M. pneumoniae* and *M. fermentans* and inhibited all isolates of the four *Mycoplasma* species at concentrations of ≤0.5 μg/ml. All *Ureaplasma* isolates were inhibited at concentrations of ≤4 μg/ml, including the *U. parvum* isolate known previously to be resistant to fluoroquinolones (2). In vitro activities of fluoroquinolones were not affected by susceptibility or resistance to tetracycline. Macrolides were the most active agents tested against *M. pneumoniae* and *M. genitalium*, with all MICs of these agents being ≤0.016 μg/ml. Clindamycin was the most active agent tested against *M. hominis* ($MIC_{90}$ = 0.032 μg/ml).

Ten *M. pneumoniae* isolates were tested further to determine minimal bactericidal concentrations (MBCs) of DC-159a in comparison to those of moxifloxacin and levofloxacin. MBC testing was performed directly from microtiter plates used to determine MICs as described previously (9, 10). A bactericidal effect was identified when the MBC was within 1× to 4× MIC. DC-159a and moxifloxacin were bactericidal against all 10 *M. pneumoniae* isolates tested. Levofloxacin was bactericidal against 8 of 10 isolates tested. In contrast, erythromycin MBCs were ≥8 times the MICs for 9 of 10 isolates tested, indicating a bacteriostatic effect.

A time-kill study of DC-159a, moxifloxacin, and levofloxacin against two *M. pneumoniae* isolates was performed as described previously (9). One of these isolates was also tested in the same manner with erythromycin. The prolonged 6-h generation time of *M. pneumoniae* necessitates incubating time-kill assay mixtures for a full week and doing subcultures daily, as opposed to the shorter time intervals used in assays with conventional bacteria. A bactericidal effect (killing of ≥99.9% of or a 3-$log_{10}$ reduction in the inoculum population) was demonstrated for DC-159a after 24 or 72 h at the MICs for the two isolates tested. Moxifloxacin was bactericidal against both isolates after 48 h at 1× MIC. Levofloxacin was bactericidal after 72 h at the MIC for one isolate and after 48 h at 2× MIC for the second isolate. Erythromycin demonstrated bacterial killing only after 120 h of incubation and at a concentration of 8× MIC.

We have evaluated a large number of well-characterized clinical isolates of mycoplasmas and ureaplasmas against the investigational fluoroquinolone DC-159a and shown its in vitro activities to be comparable to those of moxifloxacin and generally superior to those of levofloxacin and ciprofloxacin, the most widely used agents of this class that are currently available commercially in the United States. An advantage of DC-159a and other fluoroquinolones over macrolides is that they are bactericidal against *M. pneumoniae.*

The excellent in vitro inhibitory and bactericidal activities of DC-159a shown in this study demonstrate its potential for use in the treatment of infections due to mycoplasmas and ureaplasmas. DC-159a deserves further study in a clinical setting.

TABLE 1. Summary of MICs of DC-159a and other antimicrobial agents for mycoplasmas and ureaplasmas

| Organism (no. of isolates) | Drug | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *M. pneumoniae* (49) | DC-159a | 0.008–0.125 | 0.063 | 0.063 |
| | Levofloxacin | 0.25–1 | 0.5 | 1 |
| | Moxifloxacin | 0.063–0.25 | 0.125 | 0.125 |
| | Ciprofloxacin | 0.5–4 | 2 | 4 |
| | Azithromycin | ≤0.001–0.002 | ≤0.001 | ≤0.001 |
| | Erythromycin | ≤0.001–0.016 | 0.004 | 0.004 |
| | Tetracycline | 0.25–1 | 0.5 | 1 |
| | Doxycycline | 0.125–0.5 | 0.25 | 0.5 |
| *M. genitalium* (6) | DC-159a | 0.016–0.25 | NA[c] | NA |
| | Levofloxacin | 1–2 | NA | NA |
| | Moxifloxacin | 0.016–0.125 | NA | NA |
| | Ciprofloxacin | 0.5–4 | NA | NA |
| | Azithromycin | ≤0.001 | NA | NA |
| | Erythromycin | ≤0.001–0.008 | NA | NA |
| | Tetracycline | 0.25–2 | NA | NA |
| | Doxycycline | 0.032–0.5 | NA | NA |
| *M. hominis*[a] (25) | DC-159a | 0.063–0.5 | 0.125 | 0.25 |
| | Levofloxacin | 0.125–2 | 0.25 | 0.5 |
| | Moxifloxacin | 0.032–0.125 | 0.063 | 0.063 |
| | Ciprofloxacin | 0.25–2 | 0.5 | 1 |
| | Clindamycin | 0.008–0.063 | 0.016 | 0.032 |
| | Tetracycline | 0.25–32 | 0.5 | 16 |
| | Doxycycline | 0.016–8 | 2 | 8 |
| *M. fermentans* (20) | DC-159a | 0.004–0.063 | 0.016 | 0.032 |
| | Levofloxacin | 0.032–0.125 | 0.063 | 0.125 |
| | Moxifloxacin | 0.016–0.063 | 0.032 | 0.063 |
| | Ciprofloxacin | 0.032–0.125 | 0.063 | 0.125 |
| | Clindamycin | 0.008–0.125 | 0.032 | 0.125 |
| | Tetracycline | 0.125–2 | 0.5 | 1 |
| | Doxycycline | 0.063–1 | 0.25 | 1 |
| *U. parvum*[a,b] (25) | DC-159a | 0.125–4 | 0.5 | 2 |
| | Levofloxacin | 0.5–16 | 0.5 | 2 |
| | Moxifloxacin | 0.125–4 | 0.5 | 2 |
| | Ciprofloxacin | 0.125–32 | 2 | 16 |
| | Azithromycin | 0.063–4 | 1 | 2 |
| | Erythromycin | 0.5–4 | 1 | 4 |
| | Tetracycline | 0.063–32 | 0.5 | 8 |
| | Doxycycline | 0.032–16 | 0.125 | 2 |
| *U. urealyticum* (25) | DC-159a | 0.25–1 | 0.5 | 1 |
| | Levofloxacin | 0.5–4 | 1 | 1 |
| | Moxifloxacin | 0.25–1 | 0.25 | 0.5 |
| | Ciprofloxacin | 2–8 | 4 | 8 |
| | Azithromycin | 0.25–4 | 2 | 4 |
| | Erythromycin | 0.25–4 | 2 | 4 |
| | Tetracycline | 0.125 | 0.5 | 2 |
| | Doxycycline | 0.032–0.5 | 0.125 | 0.5 |

[a] No difference in DC-159a MICs between tetracycline-susceptible and tetracycline-resistant isolates was observed.

[b] One *U. parvum* isolate was known to be fluoroquinolone resistant (2). MICs for this isolate were as follows: ciprofloxacin, 32 μg/ml; levofloxacin, 16 μg/ml; moxifloxacin, 4 μg/ml; and DC-159a, 4 μg/ml.

[c] NA, not applicable due to the small number of isolates tested.

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