Antimicrobial Effects of a New Carboxyquinolone Drug, Q-35, on Five Serogroups of *Leptospira interrogans*

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New carboxyquinolone drugs, including the recently developed Q-35, were evaluated for their in vitro potency against five serogroups of *Leptospira interrogans*. Q-35, ofloxacin, ciprofloxacin, and tosufloxacin showed MICs (0.05 to 0.20 μg/ml) comparable to those of tetracycline. However, MBCs of these drugs varied between 10- and 100-fold above the MIC for most strains tested. Q-35 was shown to be active against *L. interrogans* in vitro as judged by the MICs obtained.

Leptospirosis is a zoonosis of worldwide distribution which causes an acute febrile illness in humans and in some domestic animals. *Leptospira* spp. are maintained asymptptomatically in the reservoir hosts, including a variety of wild and domestic animals (11). The antibiotics used primarily for the treatment of leptospirosis are penicillin or tetracycline (TC) (3, 6, 11); however, the effectiveness of such treatment is controversial, especially for patients with late-stage disease (9, 10). Other antimicrobial agents have been shown to be active against leptospires in vitro (1, 7), but they have not been used clinically. Recently, several new carboxyquinolones have been developed; these drugs are known to have a broad spectrum of antimicrobial activity and to be well absorbed by the oral route of administration (2). In addition, ciprofloxacin has been shown to be effective against *Leptospiira interrogans* in an animal model (8). However, that study was limited to only one serogroup strain, and there have been no studies comparing the antimicrobial effects of these new carboxyquinolones on multiple strains of leptospires. For this paper, several new carboxyquinolones including the recently developed Q-35 (5) were studied for their antimicrobial activity against five serogroups of *L. interrogans*.

The *Leptospira* strains used belong to five serogroups of *L. interrogans*: serogroup Icterohaemorrhagiae serovar *icterohaemorrhagiae* strain RGA, serogroup Hebdomadis serovar *hebdomadis* strain Hebdomadis, serogroup Australis serovar *australis* strain Akiyami C, serogroup Autumnalis serovar *autumnalis* strain Akiyami A, and serogroup Canicola serovar *canicola* strain Hond Utrecht IV. All of the strains were supplied by H. Kida, Department of Veterinary Hygiene and Microbiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan. The leptospires were grown in 0.2% tryptose phosphate broth (TPB) (Difco Laboratories, Detroit, Mich.) containing 10% heat-inactivated rabbit serum (4). Antimicrobial agents tested were Q-35 [1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylamino-piperidin-1-yl)-4-oxoquinoline-3-carboxylic acid dihydrate] (Chugai Pharmaceutical Co., Tokyo, Japan), norfloxacin (NFLX; Kyorin Pharmaceutical Co., Tokyo, Japan), ofloxacin (OFLX; Daiichi Pharmaceutical Co., Tokyo, Japan), ciprofloxacin (CPFX; Bayer Pharmaceutical Co., Osaka, Japan), and tetracycline (Lederle Co., Tokyo, Japan). The activity of each drug against these leptospires was examined by a broth macrodilution technique. Drugs tested were standard powders which were kept at 4°C. Dilutions of drugs were prepared as aqueous solutions in a minimum volume of distilled water and diluted with phosphate-buffered saline to a concentration of 1 mg/ml. On the basis of stability data, drug preparations were freshly prepared before use or were prepared as stock solutions and stored at −40°C. Serial twofold dilutions of each drug starting from 25 μg/ml were prepared in tubes containing 2 ml of TPB. Tubes were inoculated with leptospires (final inoculum, 5 × 10^6/ml) from logarithmic-phase cells and incubated at 30°C in air for 7 days. To study the effects of Q-35 on the growth of *L. interrogans* serogroup Icterohaemorrhagiae, leptospires were counted with a modified Neubauer chamber under dark-field microscopy on days 2, 5, and 7 of incubation with the drug. The MIC of each drug was defined as the lowest concentration which inhibited visible growth (turbidity) at the seventh day of incubation. The culture tubes were diluted 10-fold on the seventh day of incubation and observed under dark-field microscopy to estimate the number of leptospires. After quantitative determination of the MIC, 10 μl of the drug-exposed culture was

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**FIG. 1.** Growth kinetics of *L. interrogans* serogroup Icterohaemorrhagiae RGA in the presence of Q-35. Leptospires at a concentration of 5 × 10^6/ml in TPB were inoculated with various concentrations of Q-35. The number of leptospires was determined by dark-field microscopy using a modified Neubauer chamber.
inoculated into 5 ml of drug-free TPB and incubated for an additional 3 weeks at 30°C. The MBC was determined by turbidity measurement and defined as the lowest concentration of drug allowing no growth after 3 weeks at 30°C. The MBC in our study was assumed to reflect >99.99% killing of leptospires since 10 μl of the drug-exposed culture (initial inoculum of 5 × 10^5/ml) was subcultured for the MBC determination. If the killing of the leptospires was less than 99.99% in the presence of 25 μg of these drugs per ml, MBCs were not determined.

Growth kinetics of *L. interrogans* serogroup Icterohaemorrhagiae were examined in the presence of several drugs. Q-35 inhibited the growth of leptospires at a concentration of 0.10 μg/ml after 7 days of incubation as judged by direct cell count and turbidity measurements (Fig. 1). Leptospires at a concentration greater than 5 × 10^5 cells per ml produced visibly turbid cultures. The optical density at 560 nm at day 7 in drug-free culture was 0.047, which corresponded to 1.4 × 10^8 leptospires per ml. The MIC of Q-35 determined from this experiment was 0.10 μg/ml for *L. interrogans* serogroup Icterohaemorrhagiae RGA. Growth kinetics of this serogroup were also tested in the presence of TC and OFLX (data not shown); the MICs of TC and OFLX were 0.10 and 0.20 μg/ml, respectively, for this strain.

MBCs and MICs of each drug for five serogroups of *L. interrogans* are shown in Table 1. MICs of Q-35 for five *Leptospira* strains were within the range of 0.05 to 0.10 μg/ml. Q-35, OFLX, CPFX, TFLX, and TC showed similar MICs, while NFLX was less active.

MBCs of each drug for five serogroups of *L. interrogans* are also shown in Table 1. MBCs of each drug varied depending on the strains used. In particular, *L. interrogans* serogroup Icterohaemorrhagiae was relatively resistant to the bactericidal activity of each drug, and the MBC of Q-35 was four times higher than that of TC for that serogroup. MBCs of Q-35 for the other four serogroups of *L. interrogans* ranged from 1.56 to 3.13 μg/ml, and similar values were obtained for CPFX, TFLX, and TC. NFLX and OFLX were found to be significantly less active compared with the other drugs.

Turbidity measurement and microscopic observation were used in this study to define the MIC for multiple *Leptospira* strains. The cell count under dark-field conditions in the presence of a test drug corresponded well with visible turbidity, which verified the method of defining the MIC endpoint. Furthermore, the additional monitoring of diluted cultures under dark-field microscopy made the MIC determination more reliable.

MBCs varied considerably depending on the strains and the drugs. Of the five carboxyquinolones tested, Q-35, CPFX, and TFLX showed similar MBCs. These were comparable to those of TC for *L. interrogans* serogroups, except for serogroup Icterohaemorrhagiae, for which the MBC of TC was considerably higher. NFLX and OFLX had MBCs of >25 μg/ml for most of the serogroups.

**L. interrogans** serogroup Icterohaemorrhagiae tended to be most resistant to the bactericidal action of these drugs. Our data showed that the MBC of CPFX against *L. interrogans* serogroup Icterohaemorrhagiae was >25 μg/ml, whereas Shalit et al. observed an MBC of CPFX for this serogroup of 0.6 μg/ml (8). This discrepancy might be due to the different experimental procedures employed in the two studies. In our study, a final inoculum of 5 × 10^6 cells per ml was used, which was 10 times higher than that of Shalit et al. This might produce the observed differences in our MBCs. Another possible explanation might be the difference in strains of serogroup Icterohaemorrhagiae used in the two studies.

The data presented in this paper show that new carboxyquinolone drugs including the newly developed Q-35 are active against *L. interrogans* as evaluated by MICs, although MBCs were usually 10- to 100-fold higher than MICs.

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### REFERENCES


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<th>Q-35 (μg/ml)</th>
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<th>OFLX (μg/ml)</th>
<th>CPFX (μg/ml)</th>
<th>TFLX (μg/ml)</th>
<th>TC (μg/ml)</th>
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