Antimycoplasmal Activity of Ofloxacin (DL-8280)

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Received 23 November 1982/Accepted 5 January 1983

Ofloxacin (DL-8280; (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid) showed a broader spectrum and a greater potency of antimycoplasmal activity than did pipemidic acid, norfloxacin, tetracyclines, and lincomycin, but was inferior to erythromycin. Its mycoplasmalic potency against clinical isolates of Mycoplasma pneumoniae was also greater than that of other quinolones and tetracyclines.

Ofloxacin (DL-8280; Fig. 1), a new quinolone derivative, has a broader and more potent antimicrobial activity than do the related agents pipemidic acid (PPA) and norfloxacin (NFLX) (11). Not only against facultative anaerobes, but also against obligate anaerobes, ofloxacin is more active than PPA and NFLX. This paper deals with the in vitro antimycoplasmal spectrum and potency of ofloxacin in comparison with those of PPA, NFLX, and other antibiotics which have been widely used for treatment of Mycoplasma infections in humans or animals (5, 9, 10, 12–14, 17, 18).

Ofloxacin, PPA, and NFLX were synthesized at the Research Institute, Daiichi Seiyaku Co., Ltd., Tokyo, Japan. Doxycycline, tetracycline hydrochloride (TC), minocycline hydrochloride, erythromycin lactobionate (EM), oleandomycin phosphate, leucomycin tartrate (LM), josamycin tartrate (JM), lincomycin hydrochloride (LCM), and clindamycin hydrochloride were purchased commercially. Chlortetracycline hydrochloride and spiramycin hydrochloride were kindly provided by H. Azechi, National Veterinary Assay Laboratory, Ministry of Agriculture and Forestry, Japan. The quinolones (ofloxacin, PPA, and NFLX) and the other antibiotics were dissolved in 0.1 N sodium hydroxide and distilled water, respectively, at a concentration of 2,000 μg/ml and diluted serially twofold with sterile distilled water to 0.02 μg/ml. Portions (1 ml) of each dilution were poured into test tubes containing 9 ml of modified Chanock medium (4, 8) consisting of 7 parts PPLO broth (Difco Laboratories, Detroit, Mich.), 2 parts uninactivated horse serum, 1 part 25% fresh yeast extract, 0.002% phenol red, and 1% glucose or 0.2% L-arginine hydrochloride. The media with glucose and L-arginine were adjusted to pH 8.2 and pH 6.8, respectively. A 0.1-ml aliquot of each medium containing the antibiotics was poured into a well of a U-plate (Sumitomo Bakelite Inc., Tokyo, Japan), a microplate consisting of 96 wells.

The laboratory Mycoplasma strains M. bovis genitalium PG-11, M. canis PG-14, M. gallisepticum PG-31, M. gallinarum PG-16, M. arthritidis PG-6, M. meleagridis 17529, M. bucale CH-20247, M. salivalium PG-20, M. hominis PG-21, and M. pneumoniae FH-Liu were donated by M. Ogata, Department of Microbiology, Faculty of Agriculture, Tokyo University. Forty-eight clinical isolates of strains of M. pneumoniae and a standard strain, M. pneumoniae Mac, were obtained from T. Motohiro, Department of Pediatrics, Kurume Medical College. Of these strains, PG-11, PG-14, PG-31, FH-Liu, Mac, and the M. pneumoniae isolates were cultivated in the medium supplemented with glucose, and their growth was determined by a color change of the medium from red to yellow. Other strains were cultured in the medium with L-arginine, which indicated growth by changing from orange to red. The cultures were each diluted 10^−1 to 10^−2 with the appropriate medium (to 10^8 CFU/ml), and dilutions were used for the inocula. The number of CFU was measured by the agar plate method. By using a multichannel pipette (Titertek; Flow Laboratories, Inc., Rockville, Md.), 0.1 ml of each inoculum was poured into a well of a microplate previously charged with the same volume of the medium containing antibiotics; thus, final concentrations of the antibiotics were made serially from 0.001 to 100 μg/ml. The plates, sealed with plate sealers (Dynatech Industries, Alexandria, Va.), were then incubated for 2 to 10 days at 37°C. In each case, when the color of the medium of the drug-free control changed from red to yellow or from orange to red, the minimal concentration of drug preventing the color change was defined as the initial minimal inhibitory concentration (MIC). After measurement of the MICs, the plates were incubated for a further 14 to 20 days to determine the final MIC (FMIC).
Against the reference strains of each *Mycoplasma* species, EM was the most active antibiotic, followed by ofloxacin, clindamycin, TC, NFLX, spiramycin, and chlorotetracycline; the MICs ranged from 0.012 to 0.39, 0.10 to 3.13, 0.10 to >25, 0.39 to 25, 0.78 to 25, 1.56 to >25, and 1.56 to >25 μg/ml, respectively. PPA was hardly active against the test strains, its MICs being over 25 μg/ml. Ofloxacin had a broader spectrum of antimycoplasmal activity than did the other antibiotics. Table 1 shows the susceptibilities of 48 *M. pneumoniae* isolates to quinolones, tetracyclines, macrolides, and LCM. Of the quinolones, ofloxacin was the most active, followed by NFLX and PPA in that order. The IMICs of ofloxacin were almost equivalent to those of the tetracyclines, the concentrations which inhibited 90% of the strains being 1.56 and 0.78 μg/ml, respectively. The activity of LCM was equivalent to or less than that of ofloxacin and the tetracyclines, whereas the macrolides were more potent. Of the latter, EM was the most active, followed by LM, oleandomycin, and JM; the 90% IMICs of EM, LM, oleandomycin, and JM were 0.006, 0.025, 0.10, and 0.10 μg/ml, respectively. On the other hand, a few strains were highly resistant to LM and JM, the IMICs being over 12.5 μg/ml. Ofloxacin also inhibited these resistant strains at 1.56 μg/ml. By a further incubation of the plates, the MIC values of each antibiotic increased by one to three wells; thus, the FMICs became two to eight times higher than the IMICs. Nevertheless, all of the strains were also inhibited by 1.56 μg of ofloxacin per ml; no increase in the concentrations which inhibited 50% and 90% of the strains was found. With the tetracyclines, on the other hand, complete inhibition was barely achieved with 6.25 μg/ml, indicating that the mycoplasmacidal activity of ofloxacin was greater than that of tetracyclines.

Of the mycoplasmas, *M. pneumoniae* is an important cause of bronchitis and atypical pneumonia in older children and young adults (1, 3, 5-7, 15, 18). Two antibiotics, EM and TC, have primarily been used and have been shown to be efficacious in reducing hospitalization time, days of fever, cough, and resolution of chest film infiltrates in patients with *M. pneumoniae* pneumonia (2, 5, 10, 12, 18). Recent isolates from patients in Japan were also susceptible to these antibiotics. EM was shown to have the most potent in vitro activity against these *M. pneumoniae* isolates. At the same time, an increased incidence of isolation of mutants resistant to EM or JM from the patients treated with or without these antibiotics has been found (8, 9, 16). Tetracyclines, including TC and minocycline, show no such problem (8, 16). Niitsu et al. (9) also stated that no difference in in vivo efficacy such as that in in vitro activity was found between EM and TC. This may be related to the differences in the pharmacokinetic properties of the antibiotics (13, 16). During a long incubation time in an enriched medium mixed with organisms, some antibiotics may be degraded by temperature, pH, or other factors in the medium.

### Table 1. Susceptibility of 48 *M. pneumoniae* isolates to quinolone derivatives, tetracyclines, macrolides, and LCM

<table>
<thead>
<tr>
<th>Drug</th>
<th>IMIC (μg/ml)</th>
<th>FMIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>For 50% of strains</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For 50% of strains</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>0.39–1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>NFLX</td>
<td>0.78–12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>PPA</td>
<td>3.13–&gt;100</td>
<td>50</td>
</tr>
<tr>
<td>TC</td>
<td>0.10–1.56</td>
<td>0.39</td>
</tr>
<tr>
<td>DOXY</td>
<td>0.10–0.78</td>
<td>0.39</td>
</tr>
<tr>
<td>MINO</td>
<td>0.05–0.78</td>
<td>0.19</td>
</tr>
<tr>
<td>EM</td>
<td>≤0.001–0.025</td>
<td>0.003</td>
</tr>
<tr>
<td>OM</td>
<td>≤0.001–0.39</td>
<td>0.05</td>
</tr>
<tr>
<td>LM</td>
<td>0.006–50</td>
<td>0.013</td>
</tr>
<tr>
<td>JM</td>
<td>0.006–&gt;100</td>
<td>0.05</td>
</tr>
<tr>
<td>LCM</td>
<td>0.05–12.5</td>
<td>1.56</td>
</tr>
</tbody>
</table>

*DOXY,* Doxycycline; MINO, minocycline hydrochloride; OM, oleandomycin phosphate. See text for other abbreviations.
and could also be inactivated by enzymes produced by the organisms. If the organisms have been killed by a certain level of an antibiotic before such a degradation reaction occurs, no further increase in the MIC levels should occur. Thus, the FMIC may be a representation of mycoplasmacidal potency of antibiotics. From this point of view, ofloxacin showed a greater mycoplasmacidal potency than did the tetracyclines, its FMIC being lower. This observation may lead to the possibility of the clinical application of ofloxacin.

LITERATURE CITED
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Volume 23, no. 3, table of contents: The title of the last article appearing in the “Susceptibility” section is incorrect as printed. It should appear as shown above.