In vitro antimycobacterial activity of the new quinolone OPC-17116

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A newly synthesized quinolone, OPC-17116, was examined for its in vitro antimycobacterial activity. The MIC₉₀ of OPC-17116 against various mycobacteria measured by the agar dilution method using 7H11 agar plates, were as follows: Mycobacterium tuberculosis, 0.78 µg/ml; Mycobacterium kansasii, 25 µg/ml; Mycobacterium marinum, 100 µg/ml; Mycobacterium scrofulaceum, >100 µg/ml; Mycobacterium intracellulare, 12.5 µg/ml; Mycobacterium fortuitum, 25 µg/ml; Mycobacterium chelonae subsp. abscessus, >100 µg/ml; Mycobacterium chelonae subsp. chelonae, 100 µg/ml. The activity of OPC-17116 against M. tuberculosis was 2 to 4 times higher than that of fleroxacin, comparable to that of ofloxacin and ciprofloxacin, but 4 times lower than that of sparfloxacin. Against M. avium complex, OPC-17116 showed 4 times higher activity than ofloxacin and ciprofloxacin, and was comparable to ciprofloxacin and sparfloxacin. The antimicrobial activity of OPC-17116 against M. tuberculosis or M. intracellulare phagocytosed in murine peritoneal macrophages was slightly higher than that of ofloxacin. OPC-17116 exhibited less in vitro activity than ofloxacin against mycobacteria other than M. tuberculosis and M. avium complex.

Key words: Antimycobacterial activity, OPC-17116, Mycobacterium tuberculosis, Mycobacterium intracellulare

Introduction

A newly synthesized quinolone, OPC-17116, with the chemical structure (±)-1-cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid, possesses strong antimicrobial activity against various bacteria. The in vitro activity of OPC-17116 against Gram-positive bacteria such as Staphylococcus aureus, including methicillin-resistant organisms, Streptococcus pneumoniae, Streptococcus pyogenes, and enterococci is higher than those of ofloxacin (OFLX) and ciprofloxacin (CPFX)1-3. It exhibits more potent activity than OFLX against Gram-negative bacteria, including Pseudomonas aeruginosa, but somewhat lower activity than CPFX4-9. In experimental murine infections due to S. aureus, S. pneumoniae, Escherichia coli, Klebsiella pneumoniae, or P. aeruginosa, OPC-17116 exerted greater therapeutic efficacy than OFLX and CPFX11. In this study, we compared the in vitro antimycobacterial activity of OPC-17116 with those of quinolones including OFLX, CPFX, sparfloxacin (SPFX), and fleroxacin (FLRX), which possess appreciable in vitro and in vivo activity against mycobacteria12-19.

Materials and Methods

Organisms. Mycobacterium tuberculosis (20 strains), Mycobacterium kansasii (19 strains), Mycobacterium marinum (10 strains), Mycobacterium scrofulaceum (19 strains), Mycobacterium avium (20 strains), Mycobacterium intracellulare (20 strains); Mycobacterium fortuitum (20 strains), Mycobacterium chelonae subsp. abscessus (15 strains), and Mycobacterium chelonae subsp. chelo-
nae (20 strains) were used. The organisms were grown in 7H9 broth (Difco Laboratories) at 37°C (33°C for M. marinum and M. chelonae subsp. chelonae) for 3 to 7 days. All of the M. avium complex strains produced smooth and transparent colonies (SmT variants).

Mice. Female BALB/c mice (8 to 10 weeks old), purchased from Japan SLC Co., Shizuoka, Japan, were used.

Drugs. OPC-17116 was obtained from Otsuka Pharmaceutical Co., Tokyo. OFLX, CPFX, SPFX, and FLRX were obtained from Daiichi Pharmaceutical Co., Tokyo, Bayer Pharmaceutical Co., Tokyo, Dainippon Pharmaceutical Co., Osaka, and Kyorin Pharmaceutical Co, Tokyo, respectively.

Minimal inhibitory concentration (MIC) determination. MICs of test drugs for mycobacteria were measured by the agar dilution method, using 7H11 agar medium as previously described16). Five μl of the test bacterial suspension (about 10⁶ CFU/ml) was spotted onto drug-containing agar medium. After 7 days (rapid growers) or 14 days (slow growers) of cultivation at 37°C (33°C for M. marinum and M. chelonae subsp. chelonae) in a CO₂ incubator, growth of the organisms was observed. MIC was defined as the minimum concentration which completely inhibited the growth of the organism or which allowed no more than five colonies to grow.

Antimicrobial activity against organisms phagocytosed in macrophages. As described previously16), peritoneal exudate cells (7.5×10⁶ cells) induced with zymosan A (1 mg, 4 days before cell harvest) were incubated in 1 ml of RPMI 1640 medium containing 10% fetal bovine serum (FBS) (M. A. Bioproduct Co., Walkersville, MD., U.S.A.) in the culture wells (24-cell well; Corning Glass Works, Corning, NY, U.S.A.) at 37°C in a CO₂ incubator (5% CO₂-95% humidified air) for 2 h, and the wells were rinsed with 2% FBS-Hanks balanced salt solution (HBSS) to remove nonadherent cells. The resultant macrophage (MΦ) monolayer was overlaid with 1 ml of 10% FBS-RPMI 1640 medium containing 5.3×10⁶ CFU/ml of M. tuberculosis H₃₇Rv or 4.2×10⁶ CFU/ml of M. intracellulare N-260, incubated at 37°C for 1 h, and then washed with 2% FBS-HBSS to remove the non-phagocyted bacteria. The MΦs were further cultivated in 1 ml of 10% FBS-RPMI 1640 medium in the presence or absence (control) of 1 or 10 μg/ml of OPC-17116 or OFLX at 37°C for up to 5 days in a CO₂ incubator with daily changes of drug-containing medium. At intervals, the MΦ monolayer was rinsed and MΦs were lysed with distilled water containing 0.1% Tween 80 by sonication with a Handy Sonic (Tomy Seiko Co., Tokyo) for 10 s. CFUs in the cell lysate were counted on 7H11 agar plates. There was no difference in the number of attached MΦs on the wells between the drug-free and drug-containing media. Antimicrobial activity was calculated as follows: growth index =CFUs per M4 at each experimental period/CFUs per M4 at time 0. For statistical analysis, Student’s t-test was performed.

Results

MICs of OPC-17116 for various mycobacteria.

Table 1 compares MIC₅₀ and MIC₉₀ of OPC-17116 for M. tuberculosis, M. avium and M. intracellulare with those of OFLX, CPFX, SPFX, and FLRX. In the case of M. tuberculosis, the activity of OPC-17116 (MIC₅₀ and MIC₉₀ =0.78 μg/ml) was 2- to 4-
Antimycobacterial activity of OPC-17116

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fold higher than that of FLRX, essentially equal to those of OFLX and CPFX, and 4 times lower than that of SPFX. For M. avium, OPC-17116 was 4 times more active than OFLX, as active as CPFX, and 2 times less active than SPFX. The activity of OPC-17116 against M. intracellulare was 2 to 4 times greater than those of OFLX, CPFX, and FLRX, and comparable to that of SPFX.

Table 2 compares the in vitro antimicrobial activity of OPC-17116 against various mycobacteria other than M. tuberculosis and M. avium-intracellulare complex with that of OFLX. OPC-17116 was 2 to 8 times less active for all test organisms, including M. kansasii, M. marinum, M. scrofulaceum, M. fortuitum and M. chlonae, when compared with ofloxacin, in terms of MIC50 and MIC90 values.

OPC-17116 had a level of in vitro antimicrobial activity against M. tuberculosis and M. avium complex similar to those of OFLX and CPFX, but showed considerably lower activity against the other slowly growing mycobacteria.

Table 2. MICs of OPC-17116 and ofloxacin against various mycobacteria

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of strains</th>
<th>MIC (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>OPC-17116</td>
</tr>
<tr>
<td></td>
<td>MIC50</td>
<td>MIC90</td>
</tr>
<tr>
<td>M. kansasii</td>
<td>19</td>
<td>6.25</td>
</tr>
<tr>
<td>M. marinum</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>M. scrofulaceum</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>M. fortuitum</td>
<td>20</td>
<td>6.25</td>
</tr>
<tr>
<td>M. chelonae (abscessus)</td>
<td>15</td>
<td>&gt;100</td>
</tr>
<tr>
<td>M. chelonae (chelonae)</td>
<td>20</td>
<td>25</td>
</tr>
</tbody>
</table>

Antimicrobial activity of OPC-17116 against M. tuberculosis and M. intracellulare phagocytosed in MΦs. Table 3 compares the antimicrobial activities of OPC-17116 and OFLX against M. tuberculosis or M. intracellulare phagocytosed in MΦs. In this experiment, 1 and 10 µg/ml concentrations were selected, since these are nearly identical to the serum Cmax values of OPC-17116 and OFLX administered to humans in single oral doses of 400 and 600 mg (clinical dose), respectively1,7).

In the case of M. tuberculosis, the organisms grew steadily in MΦs during 5 days of incubation, when M. tuberculosis-phagocytizing MΦs were cultured in a drug-free medium. A significant decrease in the number of intracellularly surviving organisms from that at time zero, that is, bacterial killing in MΦs, was seen at days 3 and 5, when the MΦs were cultured in medium containing 10 µg/ml of OPC-17116 or OFLX. The bacterial killing efficacy was appreciably (but not significantly) greater in OPC-17116 than in OFLX. Moreover, only OPC-17116

Table 3. Antimicrobial activities of OPC-17116 and ofloxacin against Mycobacterium tuberculosis or Mycobacterium intracellulare phagocytosed in murine MΦs

<table>
<thead>
<tr>
<th>Organism</th>
<th>Incubation time (days)</th>
<th>Control (drug free)</th>
<th>Growth index*³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OPC-17116</td>
<td>ofloxacin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 µg/ml</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td>3</td>
<td>1.78±0.08</td>
<td>1.27±0.02</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.48±0.10</td>
<td>0.72±0.11</td>
</tr>
<tr>
<td>M. intracellulare</td>
<td>3</td>
<td>2.98±0.34</td>
<td>2.64±0.07</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.3±1.97</td>
<td>5.77±1.28</td>
</tr>
</tbody>
</table>

* Values are expressed as mean±SEM (n=3).

b) CFUs per 100 Mos of M. tuberculosis and M. intracellulare at time 0 were 17.2±0.60 (mean±SEM) and 9.89±0.28, respectively.

Significantly different from control (P<0.05).

Significantly different from control (P<0.01).
Caused a decrease in the survival of the organisms during 5 days of incubation of MФs when the drugs were added at the lower concentration of 1 μg/ml.

In the case of *M. intracellulare*, the organisms grew rapidly in MФs during 5 days of cultivation in the drug-free medium. The two quinolones significantly retarded the intracellular growth of the organisms (*P* < 0.01) when added at concentrations of 1 and 10 μg/ml, but failed to exhibit bactericidal activity even at 10 μg/ml. Growth inhibitory efficacy was considerably (but not significantly) higher in OPC-17116 than in OFLX. Thus, OPC-17116 seems to be more active than OFLX against *M. tuberculosis* and *M. intracellulare* phagocytosed in MФs.

**DISCUSSION**

This study revealed that OPC-17116 has relatively potent *in vitro* activity against *M. tuberculosis* and *M. avium* complex, compared with other new quinolones. Its anti-*M. tuberculosis* activity is similar to those of OFLX and CPFX but less than that of SPFX. Moreover, its anti-*M. avium* complex activity is more potent than those of OFLX and CPFX but somewhat lower than that of SPFX. FLRX is less effective than the other quinolones against both organisms.

Since the C<sub>max</sub> of OPC-17116 in the blood was reported to be 1.8 μg/ml when given orally to humans at a 400 mg dose (clinical dosage for other quinolones)<sup>3)</sup> and the MIC<sub>90</sub> of the drug for *M. tuberculosis* (0.78 μg/ml) (Table 1) is about 3 times lower than the C<sub>max</sub> value, proper therapeutic efficacy of OPC-17116 is expected in the clinical treatment of tuberculosis patients. This concept is supported by the observation (Table 3) that OPC-17116 exhibited bactericidal action against *M. tuberculosis* phagocytosed in MФs when added at the concentration of 1 μg/ml, which is lower than its C<sub>max</sub> in the blood. It is noteworthy that OFLX failed to exert such a killing effect when added at the same dose, presumably because its MIC (0.78 μg/ml) is 2 times higher than that of OPC-17116 (0.39 μg/ml) for the test organism, *M. tuberculosis* H<sub>37</sub>Rv, and its ability to penetrate into phagocytic cells is less<sup>17</sup>.

However, the therapeutic efficacy of OPC-17116 *in vivo* may not exceed that of OFLX, since the drug is greatly inferior to OFLX in terms of its absorption and tissue distribution, that is, its C<sub>max</sub> in blood is about 5 times lower than that of OFLX: C<sub>max</sub> in mice: OPC-17116 (50 mg/kg), 2.2 μg/ml; OFLX (50 mg/kg), 11 μg/ml; C<sub>max</sub> in humans: OPC-17116 (400 mg), 1.8 μg/ml; OFLX (600 mg), 11 μg/ml<sup>[14,19]</sup>. Although OPC-17116 has 2 to 4 times lower MICs for *M. avium* complex than those of OFLX and FLRX and MICs comparable to those of CPFX, the MIC<sub>90</sub> and MIC<sub>50</sub> values of the drug, ranging from 3.13 to 50 μg/ml, were much larger than its C<sub>max</sub> in blood, suggesting weak therapeutic efficacy against *M. avium* complex infections *in vivo*, even if a sub-MIC effect of this drug is provided. Nevertheless, it seems necessary to evaluate the therapeutic efficacy of OPC-17116 against *M. tuberculosis* and *M. avium* complex infections *in vivo*, if a sub-MIC effect of this drug is provided. Nevertheless, it seems necessary to evaluate the therapeutic efficacy of OPC-17116 against *M. tuberculosis* and *M. avium* complex infections induced in experimental animals by using adequate protocols and dosages, since intractable tuberculosis and *M. avium* complex infections are increasing in parallel to the recent increase in AIDS patients.

**ACKNOWLEDGEMENTS**


**LITERATURE CITED**


11) Tomioka H, Sato K, Saito H: Effect of ofloxacin combined with Lactobacillus casei against Mycobacterium fortuitum infection induced in mice.


ニューキノロン OPC-17116 の in vitro 抗マイコバクテリア活性

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