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 MIC_{90}s 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 avium*, 12.5 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 fleroxacin, 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 CPFX[1~3]. 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 CPFX[1]. 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 mycobacteria[4~15].

Materials and Methods

Organisms. *Mycobacterium tuberculosis* (20 strains), *Mycobacterium kansaii* (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 described[16]. Five μl of the test bacterial suspension (about 10^6 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 CO_2 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 previously[16], peritoneal exudate cells (7.5×10^5 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 CO_2 incubator (5% CO_2-95% 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 5.3×10^6 CFU/ml of *M. tuberculosis* $H_{37}Rv$ or 4.2×10^6 CFU/ml of *M. intracellulare* N-260, incubated at 37°C for 1 h, and then washed with 2% FBS-HBSS to remove the nonphagocytosed 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 CO_2 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 MΦ at each experimental period/CFUs per MΦ at time 0. For statistical analysis, Student's *t*-test was performed.

Results

MICs of OPC-17116 for various mycobacteria. Table 1 compares MIC_{50}s and MIC_{90}s 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 (MIC_{50} and MIC_{90} = 0.78 μg/ml) was 2- to 4-

Table 1. Antimicrobial activities of OPC-17116 and four other quinolones against *Mycobacterium tuberculosis*, *Mycobacterium avium* and *Mycobacterium intracellulare*

Species	Number of strains	MIC (μg/ml)									
		OPC-17116		OFLX		CPFX		SPFX		FLRX	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
M. tuberculosis	20	0.78	0.78	0.78	0.78	0.39	0.78	0.2	0.2	1.56	3.13
M. avium	20	3.13	12.5	12.5	50	3.13	12.5	1.56	6.25	12.5	50
M. intracellulare	20	6.25	12.5	25	50	12.5	25	6.25	12.5	25	50

OFLX: ofloxacin, CPFX: ciprofloxacin, SPFX: sparfloxacin, FLRX: fleroxacin

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 MIC_{50} and MIC_{90} 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.

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 C_{max} values of OPC-17116 and OFLX administered to humans in single oral doses of 400 and 600 mg (clinical dose), respectively[1,7].

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 signifcantly) greater in OPC-17116 than in OFLX. Moreover, only OPC-17116

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

Species	Number of strains	MIC (μg/ml)			
		OPC-17116		ofloxacin	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
M. kansasii	19	6.25	25	0.78	3.13
M. marinum	10	25	100	6.25	25
M. scrofulaceum	19	25	>100	6.25	25
M. fortuitum	20	6.25	25	0.39	0.78
M. chelonae (abscessus)	15	>100	>100	100	>100
M. chelonae (chelonae)	20	25	100	12.5	50

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

Organism	Incubation time (days)	Control (drug free)	Growth index[b]			
			OPC-17116		ofloxacin	
			1 μg/ml	10 μg/ml	1 μg/ml	10 μg/ml
M. tuberculosis	3	1.78±0.08	1.27±0.02[c]	0.22±0.04[d]	1.30±0.14	0.37±0.02[d]
	5	2.48±0.10	0.72±0.11[d]	0.13±0.01[d]	1.42±0.33[c]	0.28±0.06[d]
M. intracellulare	3	2.98±0.34	2.64±0.07	0.84±0.06[d]	1.94±0.23[c]	1.31±0.12[d]
	5	10.3±1.97	5.77±1.28[c]	1.40±0.32[d]	7.05±0.22	2.52±0.21[d]

[a] Values are expressed as mean±SEM (n=3).

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

[c] Significantly different from control ($P<0.05$).

[d] Significantly different from control ($P<0.01$).

caused a decrease in the survival of the organisms during 5 days of incubation of MΦs when the drugs were added at the lower concentration of 1 μg/ml.

In the case of *M. intracellulare,* the organisms grew rapidly in MΦs 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* phagosytosed in MΦs.

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_{max} 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)[3] and the MIC_{90} of the drug for *M. tuberculosis* (0.78 μg/ml) (Table 1) is about 3 times lower than the C_{max} 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 MΦs when added at the concentration of 1 μg/ml, which is lower than its C_{max} 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* $H_{37}Rv$, and its ability to penetrate into phagocytic cells is less[17].

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_{max} in blood is about 5 times lower than that of OFLX: C_{max} in mice: OPC-17116 (50 mg/kg), 2.2 μg/ml; OFLX (50 mg/kg), 11 μg/ml: C_{max} in humans: OPC-17116 (400 mg), 1.8 μg/ml; OFLX (600 mg), 11 μg/ml[1,6,15].

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_{80} and MIC_{90} values of the drug, ranging from 3.13 to 50 μg/ml, were much larger than its C_{max} 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 neccessary 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.

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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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ニューキノロン OPC-17116 の *in vitro* 抗マイコバクテリア活性を検討した。7H11 寒天平板を用いた寒天希釈法による OPC-17116 の諸種抗酸菌に対する MIC_{90} は次のようであった。*Mycobacterium tuberculosis* 0.78 μg/ml, *Mycobacterium kansasii* 25 μg/ml, *Mycobacterium marinum* 100 μg/ml, *Mycobacterium scrofulaceum* >100 μg/ml, *Mycobacterium avium* 12.5 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。*M. tuberculosis* に対する OPC-17116 の抗菌活性は fleroxacin の 2～4 倍高く，ofloxacin ならびに ciprofloxacin のそれに匹敵したが，sparfloxacin よりも 4 倍低かった。*M. avium* complex に対しては，OPC-17116 は ofloxacin ならびに fleroxacin よりも 4 倍高い抗菌活性を示し，ciprofloxacin と sparfloxacin のそれに匹敵した。マウス腹腔マクロファージ内被貪食 *M. tuberculosis* あるいは *M. intracellulare* に対する OPC-17116 の抗菌活性は ofloxacin におけるよりも若干高かった。OPC-17116 は，ofloxacin と比較した場合，*M. tuberculosis* ならびに *M. avium* complex 以外の抗酸菌に対しては低い *in vitro* 活性を示した。

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