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In Vitro and In Vivo Antichlamydial Activities of Newly Developed Quinolone Antimicrobial Agents

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The in vitro and in vivo activities of three newly developed quinolone antimicrobial agents (sparfloxacin, tosufloxacin, and OPC-17116) were investigated. All three agents showed potent in vitro activities against *Chlamydia psittaci*, *C. trachomatis*, and *C. pneumoniae* with MICs that ranged from 0.031 to 0.125 μg/ml. These values were higher than those of minocycline (0.0075 to 0.015 μg/ml) but lower than those of erythromycin (0.25 to 0.5 μg/ml) and ofloxacin and ciprofloxacin (0.5 to 1.0 μg/ml). Mice were challenged with 10^5 inclusion-forming units of *C. psittaci* each by nasal instillation. All untreated control animals died within 7 days. The survival rates of mice treated with 40 mg of sparfloxacin, OPC-17116, or tosufloxacin per kg of body weight every 12 h for 7 days were 73, 73, and 60%, respectively, 7 days after the challenge. The survival rate of mice treated with ofloxacin at the same dosage was 53%. On the basis of the above results, we concluded that these three new quinolones might be useful in the treatment of chlamydial respiratory infections.

Sparfloxacin (SPFX), OPC-17116 (OPC), and tosufloxacin (TFLX) are three newly developed quinolone antimicrobial agents characterized by potent and broad antibacterial activities. They have been reported to show potent in vitro activities not only against common gram-positive and -negative bacteria but also against mycoplasmal, mycobacterial, and chlamydial species, and they may be considered drugs of first choice for the treatment of respiratory tract infections. In this study, we investigated the in vitro activities of these agents against three chlamydial species and compared them with those of other antimicrobial agents. Most previous studies have been concerned with genitourinary tract infections caused by *Chlamydia trachomatis*. We also studied the in vivo therapeutic effects of these compounds using a mouse experimental model of *C. psittaci*-caused pneumonia.

MATERIALS AND METHODS

Antimicrobial agents. SPFX and OPC were obtained from Dainippon Pharmaceuticals and Otsuka Pharmaceuticals, respectively; TFLX was obtained from Toyama Chemicals; ofloxacin (OFLX) was purchased from Daiichi Seiyaku; ciprofloxacin was obtained from Bayer Yakuhin; norfloxacin was obtained from Kyorin Pharmaceuticals; minocycline was provided by Lederle Japan; doxycycline was purchased from Pfizer Pharmaceuticals; erythromycin was a gift from Shionogi Seiyaku.

Chlamydial strains. We used three strains of *C. trachomatis* (serovars D/UW-3/Cx, E/UW-5/Cx, and L_2/434/Bu [Washington Research Foundation, Seattle, Wash.]), three strains of *C. psittaci* (Budgerigar no. 1 [National Institute of Health, Tokyo, Japan], California 10 [Department of Microbiology, Kawasaki Medical School, Kurashiki, Japan], and the wild-type Izawa strain), and one strain of *C. pneumoniae* (TW-183 [Washington Research Foundation]) for in vitro studies. These strains were propagated on HeLa-229 cells. Culturing was performed on HeLa cell monolayers in 24-well plates.

MIC determination. In vitro determination of chlamydial susceptibility (MIC) was by the method of Japan Society of Chemotherapy (7). Briefly, HeLa-229 cells were seeded into 24-well plates 24 h prior to chlamydial inoculation. Cell monolayers were examined for confluency and were inoculated with 10^4 inclusion-forming units (IFU) of each chlamydial species per well. After the inoculation, a culture medium, consisting of Eagle's minimal essential medium (Nissui Pharmaceuticals) with fetal bovine serum (GIBCO Laboratories Inc.), and 1 μg of each cycloheximide (Nakarai Tesque)-diluted test agent per ml was applied, and the plates were incubated at 37°C in 5% CO_2 for 72 h (*C. trachomatis*), at 35°C in 5% CO_2 for 72 h (*C. pneumoniae*), or at 37°C in 5% CO_2 for 48 h (*C. psittaci*). Following incubations, Cultureset (Ortho Diagnostics), a genus-specific fluorescein isothiocyanate-conjugated monoclonal antibody to *Chlamydia* lipopolysaccharide, was used to stain inclusions. An average of 100 inclusions per field (×100) was observed. MIC was defined as the lowest concentration at which complete inhibition of inclusion formation was observed.

Measurement of IFU. A serially diluted suspension containing chlamydiae was inoculated onto HeLa-229 confluent monolayer cells. After incubation, the number of inclusion bodies was counted. The number of IFU per ml was calculated from the number of inclusion bodies, inoculum size, and dilution of the suspension.

Therapeutic effects in mice with *C. psittaci*-caused pneumonia. Five-week-old male MCH mice (CLEA Japan Inc.) weighing 25 to 30 g were used for the following experiments. Each of the animals was infected with 10^5 IFU of *C. psittaci* California 10. The cells infected with *C. psittaci* were disrupted by ultrasonic waves for 40 s and diluted with a sucrose-phosphate-glutamic acid (SPG) medium to an appropriate titer, which was confirmed by fluorescein staining. Then, the animals were infected with the diluted cell solution by nasal instillation using a micropipette. Five compounds (SPFX, OPC, TFLX, OFLX, and minocycline) were used for treatment. All agents were suspended in 5% gum arabic and two doses, a low dose (20 mg/kg of body weight) and a high dose (40 mg/kg), were prepared. The animals were divided into groups of 15 each. Each animal received 0.2 ml of the antibiotic suspension orally every 12 h for 7 days. The

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TABLE 1. MICs against chlamydiae

Drug	MIC (μg/ml)[a]						
	C. pneumoniae TW-183	*C. psittaci*			*C. trachomatis*		
		BUD	CAL	Izawa	D	E	L_2
SPFX	0.063	0.063	0.063	0.063	0.063	0.063	0.063
TFLX	0.125	0.063	0.063	0.125	0.125	0.125	0.125
OPC	0.063	0.063	0.063	0.063	0.063	0.125	0.031
OFLX	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ciprofloxacin	1.0	1.0	0.5	0.5	0.5	1.0	0.5
Norfloxacin	16	16	16	16	32	16	16
Erythromycin	0.25	0.25	0.25	0.25	0.5	0.5	0.5
Minocycline	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Doxycycline	0.031	0.031	0.031	0.031	0.015	0.015	0.015

[a] BUD, Budgerigar no. 1; CAL, California 10; D, D/UW-3/Cx; E, E/UW-5/Cx; L_2, L_2/434/Bu.

control group received 5% gum arabic. The treatment was begun 24 h after infection. The survival rate on day 7 was used to evaluate the efficacies of the test compounds.

Concentrations in plasma. Four compounds (SPFX, OPC, TFLX, and OFLX) were each given orally to 5-week-old male MCH mice at a dose of 40 mg/kg. Four animals from each group were sacrificed, and blood samples were collected at 0.5, 1, 2, 4, and 6 h after administration. Concentrations in plasma were measured by high-pressure liquid chromatography.

Statistical analysis. Data were analyzed statistically by the Wilcoxon Mann-Whitney method.

RESULTS

Susceptibility studies. The MIC results for the various compounds are summarized in Table 1. The MIC of SPFX against each of seven chlamydial strains was 0.063 μg/ml, the MICs of OPC ranged from 0.031 to 0.125 μg/ml, and the MICs of TFLX ranged from 0.063 to 0.125 μg/ml. These MICs were lower than those of erythromycin (0.25 to 0.5 μg/ml) and other new quinolones, such as OFLX (0.5 μg/ml) and ciprofloxacin (0.5 to 1 μg/ml), but higher than those of minocycline (0.0075 to 0.015 μg/ml) and doxycycline (0.015 to 0.031 μg/ml).

Therapeutic effects in mice with *C. psittaci*-caused pneumonia. All of the untreated control mice died within 7 days after the infection. Microscopic observation of lung tissues of the control animals showed mild interstitial infiltration of mononuclear cells at 48 h after the infection, and severe diffuse parenchymal pneumonia was observed at 96 h after the infection. Monoclonal antibody staining of lung homogenate confirmed 10^7 IFU of chlamydiae per lung. As shown in Fig. 1, the survival rates of mice treated with 20 mg of TFLX, SPFX, or OPC per kg every 12 h for 7 days were 80, 60, and 53%, respectively. These rates were better than that of mice treated with OFLX (13%). The survival rates of mice treated with a dose of 40 mg each of these drugs per kg are shown in Fig. 2. Survival rates of 60 to 73% were obtained with SPFX, OPC, and TFLX. Approximately half of the animals in the OFLX-treated group died within 7 days at this dosage, but no animals in the minocycline-treated group died with this regimen.

Concentrations in plasma. The peak level of OFLX in plasma following the administration of an oral dose of 40 mg/kg was significantly higher (5.29 μg/ml) than those of SPFX (1.17 μg/ml) and OPC (1.01 μg/ml) ($P < 0.05$; Table 2). The value of the area under the curve for OFLX also was extremely high (7.24 μg · h/ml). The peak level of TFLX in plasma was 0.37 μg/ml, and the area under the curve was 1.81 μg · h/ml.

DISCUSSION

Chlamydiae are now recognized as one of the most important groups of pathogens causing community-acquired respiratory infections. *C. pneumoniae*, a new chlamydial species, in particular, has shown worldwide epidemic spread (3), as indicated by numerous studies, including our own (9). Tetracycline and macrolides have been recommended for the treatment of chlamydial infection (1, 2, 6), and their effectiveness has been shown, since only a few resistant

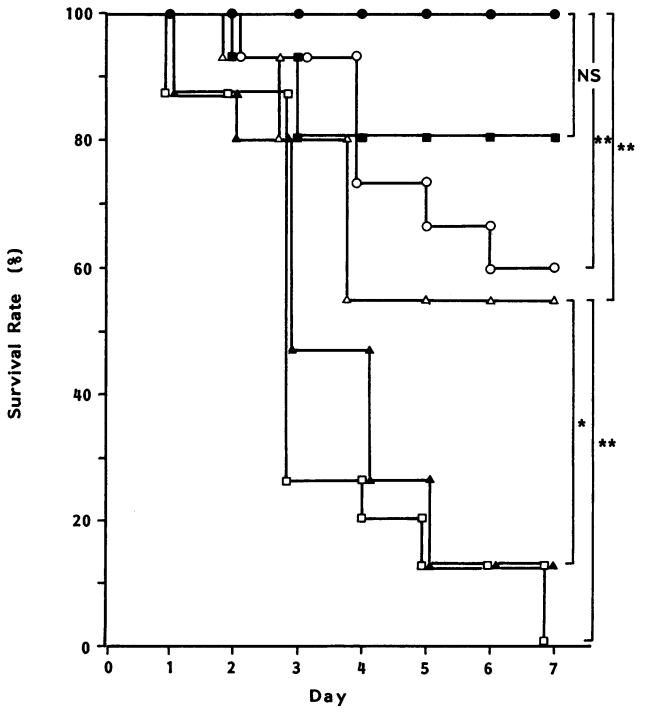

FIG. 1. Survival rates of infected mice treated with 20 mg of minocycline (●), SPFX (○), OPC (△), TFLX (■), or OFLX (▲) per kg every 12 h for 7 days. Control mice received 5% gum arabic (□). *, $P < 0.05$; **, $P < 0.01$.

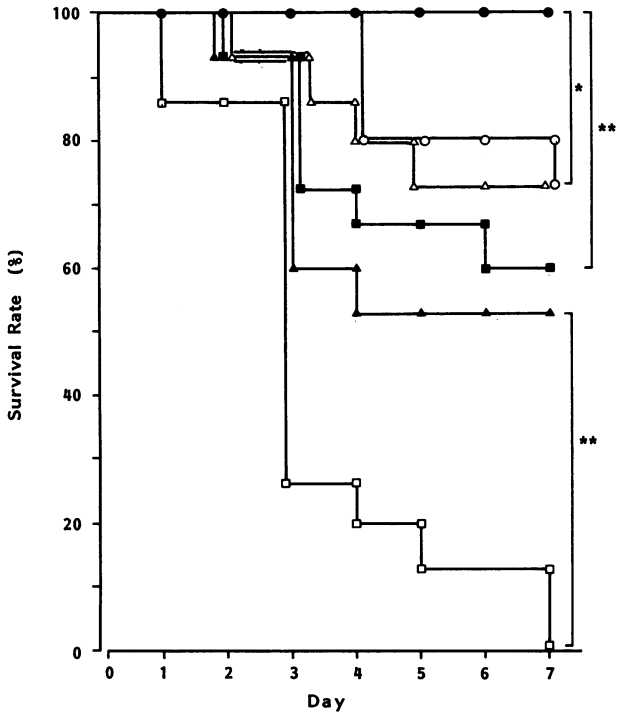

FIG. 2. Survival rates of infected mice treated with 40 mg of minocycline (●), SPFX (○), OPC (△), TFLX (■), or OFLX (▲) per kg every 12 h for 7 days. Control mice received 5% gum arabic (□). *, $P < 0.05$; **, $P < 0.01$.

strains of *C. trachomatis* (8) and no resistant strains of *C. pneumoniae* have been reported.

Recently, the antichlamydial activities of some new quinolone antimicrobial agents have been reported (5, 11). Among these agents, SPFX and TFLX have exhibited potent in vitro and in vivo activities against *C. trachomatis*. Nakata et al. (12) demonstrated that the 50% effective dose of SPFX for a *C. trachomatis* urinary tract infection in leukopenic mice was 3.4 mg/kg, which was lower than the 50% effective doses of minocycline and doxycycline.

In our study, SPFX, TFLX, and OPC (a fluoroquinolone produced by Otsuka Pharmaceuticals, undergoing clinical evaluation in Japan) showed in vitro antichlamydial activities that were comparable to those of tetracyclines and other new quinolones.

These new quinolones also showed good therapeutic effects in the treatment of mice infected with *C. psittaci*-caused pneumonia. With high-dose treatment groups, even OFLX showed good results because of its extremely high concentrations in plasma. However, only minocycline produced a 100% survival rate at the end of treatment.

One of the reasons for the discrepancy between the results with our respiratory infection model and those with the urinary tract infection model of Nakata et al. (12) is the difference in the pharmacokinetic features of quinolones and tetracyclines. Quinolones have been shown to be excreted in larger amounts in urine than tetracyclines are. Furthermore, in clinical use, the common daily dose of minocycline is 200 mg whereas those for the new quinolones are 300 to 600 mg, or as much as three times higher. Therefore, we conclude that these new quinolones, which have potent in vitro activities against chlamydiae, might be effective not only against urinary tract infections caused by chlamydiae but also against respiratory tract infections caused by these bacteria.

TABLE 2. Pharmacokinetic parameters of quinolones in mice after single oral administrations

Drug (40 mg/kg)	C_{max}[a] (μg/ml)	AUC[b] (μg · h/ml)
OFLX	5.29 ± 1.43	7.24
SPFX	1.17 ± 0.31	3.56
OPC	1.01 ± 0.27	3.05
TFLX	0.37 ± 0.15	1.81

[a] C_{max}, peak level in plasma. Values are means ± standard deviations (n = 4).
[b] AUC, area under the curve. Trapezoidal calculations were used.

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