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In Vitro and In Vivo Antibacterial Activities of AT-4140, a New Broad-Spectrum Quinolone

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AT-4140, 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid, showed broad and potent antibacterial activity. Its MICs for 90% of the strains tested were 0.1 to 0.78 μg/ml against gram-positive organisms, such as members of the genera *Staphylococcus*, *Streptococcus*, and *Enterococcus*, and 0.0125 to 1.56 μg/ml against gram-negative organisms, such as members of the family *Enterobacteriaceae* and the genera *Pseudomonas*, *Branhamella*, *Campylobacter*, *Haemophilus*, and *Neisseria*. Its MICs were 0.025 to 0.78 μg/ml against glucose nonfermenters, such as members of the genera *Xanthomonas*, *Acinetobacter*, *Alcaligenes*, *Moraxella*, *Flavobacterium*, and *Brucella*; 0.2 to 0.78 μg/ml against anaerobes, such as *Clostridium perfringens* and *Bacteroides fragilis*; 0.0125 to 0.05 μg/ml against *Legionella* spp.; 0.0125 to 0.2 μg/ml against *Mycoplasma* spp.; 0.031 to 0.063 μg/ml against *Chlamydia* spp.; and 0.1 to 0.3 μg/ml against *Mycobacterium* spp. The potencies of AT-4140 against gram-negative organisms were comparable to those of ciprofloxacin and higher than those of ofloxacin, enoxacin, and norfloxacin. The potencies of AT-4140 against gram-positive organisms, glucose nonfermenters, anaerobes, *Mycoplasma* spp., *Chlamydia* spp., and *Mycobacterium* spp. were generally higher than those of the quinolones with which AT-4140 was compared. AT-4140 showed good oral efficacy against systemic infections with *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* in mice. Its efficacy was better when a daily dose was given once than when it was given in two doses. Good efficacies of the orally administered drug were also observed in pulmonary, dermal, and urinary tract infection models in mice. The in vivo efficacies of AT-4140 were equal to or better than those of ciprofloxacin, ofloxacin, enoxacin, and norfloxacin.

New quinolones used clinically these days have a broad spectrum of activity against both gram-positive and gram-negative organisms. However, some important pathogens, such as streptococci, enterococci, *Mycoplasma* spp., *Chlamydia* spp., and *Mycobacterium* spp., are not sufficiently susceptible to the quinolones. To improve this point, we screened of this group of compounds and found a new compound called AT-4140 (Fig. 1) with a broader antibacterial spectrum. This paper describes the in vitro and in vivo antibacterial activities of AT-4140 compared with those of ciprofloxacin, ofloxacin, enoxacin, and norfloxacin.

(Parts of this study were presented previously [Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. nos. 1487 and 1488, 1988].)

MATERIALS AND METHODS

Compounds. AT-4140 (J. Matsumoto, T. Miyamoto, H. Egawa, and S. Nakamura, Chem. Abstr. **107**:236733v, 1987), ciprofloxacin (1), ofloxacin (2), enoxacin (6), and norfloxacin (4) were synthesized in our laboratories as previously reported. Other antimicrobial agents were purchased commercially. Erythromycin lactobionate was from Dainippon Pharmaceutical Co., Ltd.; streptomycin sulfate was from Meiji Seika Kaisha Ltd.; minocycline hydrochloride was from Sigma Chemical Co.; ethambutol hydrochloride was from Takeda Chemical Industries, Ltd.; isoniazid was from Wako Pure Chemical Industries Ltd.; rifampin was from Daiichi Seiyaku Co., Ltd.; kanamycin sulfate was from Banyu Pharmaceutical Co., Ltd.; and sodium *p*-aminosalicylic acid was from Maruwaka Kagaku Ltd. The compounds were dissolved in water with or without NaOH for determinations of MICs and dissolved or suspended in 0.2% carboxymethyl cellulose for oral administration.

Organisms. Anaerobes were obtained from the Institute of Anaerobic Bacteriology, Gifu University School of Medicine; *Legionella* spp. were from the Second Department of Internal Medicine, Nagasaki University School of Medicine; *Mycoplasma* spp. were from the Division of Animal Research, Faculty of Medicine, University of Tokyo and the Department of Pediatrics, Faculty of Medicine, Kurume University; *Chlamydia trachomatis* was from the Department of Urology, Gifu University School of Medicine; *Chlamydia psittaci* was from the Division of Respiratory Diseases, Department of Medicine, Kawasaki Medical School; and *Mycobacterium intracellulare* and *Mycobacterium fortuitum* were from the Department of Internal Medicine, Toneyama National Hospital. Clinical isolates were obtained from various hospitals in Japan. The other organisms used were stock strains of our laboratories.

MIC determinations. MICs were determined by the agar dilution method with inocula of about 10^3 CFU per spot (the details were described previously [8, 10]), except for the following organisms. The MICs for anaerobes, *Legionella* spp., *Branhamella catarrhalis*, and *Campylobacter pylori* were determined by the twofold agar dilution method with GAM agar (Nissui Pharmaceutical Co., Ltd.), reconstituted BCYEα agar lacking charcoal (filtered [pore size, 1 μm] charcoal-yeast extract agar [Oxoid, Ltd.] plus BCYEα growth supplement [Oxoid] and 1.3% agar), heart infusion agar (Difco Laboratories) supplemented with 5% hemolyzed

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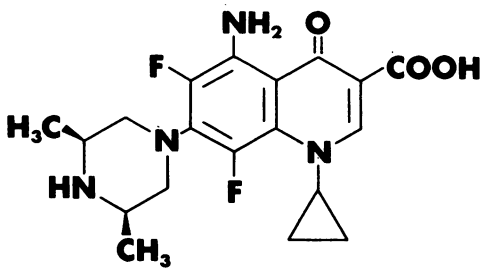

FIG. 1. Chemical structure of AT-4140.

horse blood, and brucella agar (Difco) supplemented with 5% hemolyzed horse blood, respectively. Anaerobes and *B. catarrhalis* were precultured overnight, and *Legionella* spp. were precultured for 2 days in the corresponding broths. The precultures were diluted appropriately with the each broth. *C. pylori* grown on brucella agar supplemented with 5% hemolyzed horse blood at 37°C for 3 days was suspended in physiological saline at a McFarland no. 1 cell density (about 3×10^8 CFU/ml) and diluted appropriately with saline. One loopful (about 10^3 CFU) of each organism suspension was spotted onto 10 ml of drug-containing agar in a petri dish by using a multiple inoculator (Cathra International). The petri dishes were incubated at 37°C overnight (anaerobes and *B. catarrhalis*) or for 4 days (*Legionella* spp. and *C. pylori*). Anaerobes were cultured anaerobically with the GasPak anaerobic system (BBL Microbiology Systems), and *C. pylori* was cultured under microaerophilic conditions with the CampyPak system (BBL). The MIC was defined as the lowest drug concentration at which no organism growth was detected.

All *Mycoplasm* spp. except *M. pneumoniae* were precultured for 2 days in Chanock broth (pleuropneumonia-like organism enrichment broth without crystal violet [Difco] with 20% horse serum and 10% fresh yeast extract solution [25%, wt/vol]). *M. pneumoniae* was precultured for 3 days in Chanock broth supplemented with 1% glucose and 0.002% phenol red. Each preculture was diluted with preculture medium, and one loopful (about 10^3 CFU) of the organism dilution was spotted onto 10 ml of drug-containing Chanock agar (pleuropneumonia-like organism agar [Difco] with 20% horse serum and 10% fresh yeast extract solution [25%, wt/vol]) in petri dishes with a multiple inoculator. For *M. pneumoniae*, petri dishes were packed in a polyethylene bag with water-dipped paper and incubated at 37°C for 7 days. For the other *Mycoplasma* spp., petri dishes were incubated anaerobically with the GasPak anaerobic system (BBL) at 37°C for 2 days. The growth of the organisms was observed under a dissecting microscope, and the lowest drug concentration at which no organism growth was detected was defined as the MIC.

The susceptibility of *Chlamydia* spp. was determined by the cell culture method. McCoy cells freshly cultured in Eagle minimum essential medium supplemented with 4% fetal bovine serum and 0.03% L-glutamine were trypsinized and suspended in the same culture medium at a cell concentration of 1×10^5 to 2×10^5/ml. One milliliter of the suspension was pipetted into a flat-bottom plastic tube (14-mm diameter) containing a glass cover disk (12-mm diameter) and incubated at 37°C in humidified 5% CO_2 in air for 20 h. One-half milliliter of a chlamydial suspension containing 1×10^3 to 4×10^3 inclusion body-forming units was added to each tube, which was then centrifuged at 1,500 $\times$ *g* for 1 h and incubated at 37°C for 1 h. The medium was changed with 1 ml of Eagle minimum essential medium supplemented with 8% fetal bovine serum (Flow Laboratories, Inc.)–0.03% L-glutamine–1 μg of cycloheximide (Nakalai Tesque) per ml–0.5% glucose (only for *C. trachomatis*) and drugs at various concentrations. After further incubation at 37°C for 40 to 48 h, the cells on the glass cover were fixed with methanol and stained with a Giemsa solution. Inclusion bodies in the cells on the whole glass covers were observed with a microscope at a magnification of ×200 to ×400. The MIC was defined as the lowest drug concentration at which no inclusion bodies were observed throughout the glass covers.

The MICs for *Mycobacterium* spp. were determined by the broth dilution method. Organisms precultured at 37°C for 1 week in Dubos broth (Difco) were adjusted with the same medium to an optical density of 0.3 at 530 nm. One-drop (about 10^3 minimal growing units) volumes of appropriate dilutions of *Mycobacterium* cultures were inoculated into test tubes (7-mm diameter) with 3 ml of the medium containing drugs at various concentrations. The test tubes were incubated at 37°C for 3 weeks. The MIC was defined as the lowest drug concentration which prevented visible organism growth.

Assessment of in vivo activities. In vivo activities of compounds were assessed in four infection models in mice, i.e., systemic, pulmonary, dermal, and urinary tract infections. Each dosing group consisted of eight Std-ddY mice weighing 18 to 22 g. Systemic infection with *S. pneumoniae* was induced by inoculating male mice intraperitoneally with 3×10^3 CFU (100 times the 50% lethal dose) of the organism suspended in 0.4 ml of brain heart infusion. Medications were performed four times, immediately after infection and 6, 24, and 30 h later. Efficacy was evaluated in terms of the percentage of survivors at 2 weeks postinoculation. All of unmedicated mice died within 4 days postinoculation. Assessment of the other infections was performed as reported previously (9, 11). In brief, systemic infections were induced by inoculating male mice intravenously or intraperitoneally with 4 to 180 times the 50% lethal dose of an infecting organism. Unless otherwise stated, compounds were administered orally twice, immediately after challenge and 6 h later. Therapeutic success, in terms of the percentage of survivors, was evaluated 1 or 2 weeks postinoculation. Survival rates of untreated controls rarely exceeded 10%. Pulmonary infections were induced in female mice by instilling 3 to 20 times the 50% lethal dose of infecting organisms intranasally. More than 97% of unmedicated mice died, usually with pulmonary hemorrhage or consolidation. Compounds were given orally twice, immediately after challenge and 6 h later, or three times, immediately after challenge and 1 and 3 h later. Survival for 5 or 14 days postchallenge was used as the endpoint for judging therapeutic efficacy. Dermal infections were induced by subcutaneous inoculation of female mice in a depiliated area of the back with 16 times the 50% infective dose of *P. aeruginosa*. In unmedicated mice, skin abscesses usually formed in 2 days after inoculation. Compounds were administered orally six times, immediately after challenge and 3, 6, 24, 27, and 30 h later. Absence of skin abscesses at 4 days after inoculation served as the indicator of therapeutic success. Urinary tract infections were induced in female mice by injecting 10 to 100 times the 50% infective dose of infecting organisms directly into their bladders. Compounds were given orally six times, at 3, 8, 24, 30, 48, and 50 h postchallenge. Kidneys were removed 5 days after inoculation and bisected, and the two halves were pressed on agar medium for cultivation. In the kidneys of

TABLE 1. Antibacterial activities of AT-4140 and related compounds against aerobic and facultatively anaerobic organisms

Organism	MIC (μg/ml)[a]				
	AT-4140	CPFX	OFLX	ENX	NFLX
Staphylococcus aureus 209P JC-1	0.05	0.1	0.2	0.39	0.39
Staphylococcus epidermidis 8	0.05	0.1	0.2	0.39	0.78
Streptococcus pyogenes A65	0.39	0.2	0.39	3.13	1.56
Streptococcus pneumoniae I Neufeld	0.39	0.78	1.56	6.25	3.13
Enterococcus faecalis 2473	0.78	1.56	1.56	6.25	3.13
Actinomyces pyogenes C-21	0.1	0.1	0.39	0.78	0.39
Bacillus subtilis PCI 219	0.025	0.05	0.1	0.2	0.2
Listeria monocytogenes LI-2402	0.78	0.78	1.56	6.25	3.13
Escherichia coli NIHJ JC-2	0.0125	0.0063	0.05	0.1	0.1
Salmonella typhimurium S-9	0.0125	0.0063	0.025	0.1	0.05
Salmonella typhi 901	0.0063	0.0063	0.05	0.1	0.05
Salmonella paratyphi 1015	0.0125	0.0125	0.05	0.1	0.05
Salmonella schottmuelleri 8006	0.0125	0.0031	0.025	0.05	0.05
Salmonella enteritidis 1891	0.0063	0.0031	0.025	0.05	0.05
Shigella flexneri 2a EW10	0.0125	0.0125	0.05	0.1	0.1
Shigella sonnei EW33	0.0063	0.0031	0.025	0.05	0.05
Yersinia enterocolitica MY-79	0.025	0.0125	0.1	0.1	0.1
Vibrio parahaemolyticus S-1	0.1	0.05	0.2	0.2	0.2
Morganella morganii Kono	0.1	0.0125	0.1	0.1	0.1
Proteus mirabilis IFO 3849-4	0.78	0.1	0.39	0.78	0.2
Proteus vulgaris OX-19	0.05	0.0125	0.05	0.1	0.1
Providencia sp. strain P-5415	0.025	0.0063	0.025	0.1	0.1
Providencia rettgeri IFO 3850	0.05	0.025	0.2	0.2	0.1
Klebsiella pneumoniae P-5709	0.025	0.025	0.1	0.2	0.1
Klebsiella oxytoca P-5708	0.025	0.0125	0.1	0.1	0.1
Enterobacter aerogenes ATCC 13048	0.05	0.0125	0.1	0.1	0.2
Enterobacter cloacae 963	0.05	0.0125	0.1	0.2	0.2
Citrobacter freundii P-6802	0.025	0.0125	0.1	0.1	0.1
Serratia marcescens S-9	0.2	0.05	0.2	0.2	0.2
Pseudomonas aeruginosa 12	0.39	0.1	0.78	0.78	0.78
Pseudomonas putida P-5522	1.56	0.39	6.25	3.13	3.13
Xanthomonas maltophilia P-5523	0.2	0.78	1.56	6.25	12.5
Acinetobacter calcoaceticus P-6901	0.025	0.2	0.39	0.78	3.13
Alcaligenes faecalis P-7001	0.39	0.78	0.78	1.56	6.25
Moraxella bovis P-7101	0.39	3.13	1.56	3.13	3.13
Moraxella lacunata P-7102	0.78	6.25	6.25	12.5	25
Flavobacterium sp. strain P-7201	0.05	0.39	0.39	1.56	3.13
Brucella abortus Kusayanagi	0.78	3.13	3.13	6.25	12.5

[a] CPFX, Ciprofloxacin; OFLX, ofloxacin; ENX, enoxacin; NFLX, norfloxacin.

unmedicated mice, bacterial growth was always detected. The absence of organisms in both kidneys was the sole endpoint of therapeutic success. Experiments were repeated at least twice to confirm the reproducibility of the results. Accumulated data were used for calculation of the 50% effective doses (ED_{50}s) and 95% confidence limits, which were calculated by probit analysis (7) and the method of Litchfield and Wilcoxon (5), respectively.

RESULTS

Activities against aerobic and facultatively anaerobic organisms. The antibacterial activities of AT-4140 against aerobic and facultatively anaerobic organisms were compared with those of ciprofloxacin, ofloxacin, enoxacin, and norfloxacin (Table 1). The MICs of AT-4140 ranged from 0.05 to 0.78 μg/ml for gram-positive organisms, such as staphylococci, streptococci, *Enterococcus faecalis*, *Actinomyces pyogenes*, and *Listeria monocytogenes*; from 0.0063 to 1.56 μg/ml for gram-negative organisms, such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Vibrio parahaemolyticus*, *Morganella morganii*, *Proteus* spp., *Providencia* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii*, *Serratia marcescens*, and *Pseudomonas* spp.; and from 0.025 to 0.78 μg/ml for glucose nonfermenters, such as *Xanthomonas maltophilia*, *Acinetobacter calcoaceticus*, *Alcaligenes faecalis*, *Moraxella* spp., *Flavobacterium* sp., and *Brucella abortus*. The antibacterial potencies of AT-4140 against gram-positive organisms and glucose nonfermenters were generally higher than those of the quinolones with which it was compared, and its potencies against gram-negative organisms were comparable to those of ciprofloxacin and higher than those of ofloxacin, enoxacin, and norfloxacin.

The susceptibilities of clinical isolates of representative pathogenic organisms to AT-4140 were compared with their susceptibilities to ciprofloxacin and ofloxacin (Table 2). Against gram-negative organisms, the antibacterial activity of AT-4140 was comparable to that of ciprofloxacin and generally higher than that of ofloxacin. The MICs of AT-4140 for 90% of the strains tested were lower than those of ciprofloxacin for *B. catarrhalis*, *Campylobacter jejuni*, and *Neisseria gonorrhoeae*; equal to those of ciprofloxacin for *E. coli*, *Haemophilus influenzae*, and indole-positive *Proteus* spp.; and higher than those of ciprofloxacin for *K. pneumoniae*, *P. aeruginosa*, *S. marcescens*, and *P. mirabilis*. Against gram-positive organisms, such as *S. aureus*, *S.*

TABLE 2. Susceptibilities of clinical isolates to AT-4140 and related compounds

Organism (no. of strains)	Compound	MIC (μg/ml)[a]		
		Range	50%	90%
Escherichia coli (57)	AT-4140	0.0016–0.2	0.0125	0.1
	Ciprofloxacin	0.0031–0.39	0.0063	0.1
	Ofloxacin	0.0125–0.78	0.05	0.2
Klebsiella pneumoniae (53)	AT-4140	≦0.0031–0.2	0.05	0.1
	Ciprofloxacin	≦0.0031–0.1	0.0125	0.025
	Ofloxacin	0.025–0.78	0.1	0.2
Pseudomonas aeruginosa (51)	AT-4140	0.2–12.5	0.78	1.56
	Ciprofloxacin	0.05–1.56	0.2	0.78
	Ofloxacin	0.39–12.5	1.56	6.25
Serratia marcescens (47)	AT-4140	0.05–6.25	0.39	1.56
	Ciprofloxacin	0.025–1.56	0.1	0.78
	Ofloxacin	0.1–6.25	0.39	1.56
Branhamella catarrhalis (32)	AT-4140	0.0063–0.025	0.0125	0.0125
	Ciprofloxacin	0.0125–0.05	0.025	0.05
	Ofloxacin	0.05–0.1	0.1	0.1
Campylobacter jejuni (13)	AT-4140	0.0125–0.2	0.025	0.1
	Ciprofloxacin	0.1–0.39	0.1	0.39
	Ofloxacin	0.1–1.56	0.2	0.78
Campylobacter pylori (6)	AT-4140	0.05–0.39	0.2	
	Ciprofloxacin	0.0125–0.78	0.2	
	Ofloxacin	0.05–0.78	0.39	
Haemophilus influenzae (20)	AT-4140	0.0031–0.0125	0.0063	0.0125
	Ciprofloxacin	0.0063–0.0125	0.0125	0.0125
	Ofloxacin	0.025–0.05	0.05	0.05
Neisseria gonorrhoeae (22)	AT-4140	≦0.0031–0.0125	0.0063	0.0125
	Ciprofloxacin	0.0063–0.025	0.0063	0.025
	Ofloxacin	0.0125–0.1	0.025	0.05
Proteus mirabilis (30)	AT-4140	0.2–3.13	0.39	0.78
	Ciprofloxacin	0.025–0.39	0.05	0.1
	Ofloxacin	0.1–6.25	0.2	0.78
Indole-positive *Proteus* spp. (56)	AT-4140	0.0063–3.13	0.2	0.39
	Ciprofloxacin	0.0063–0.78	0.1	0.39
	Ofloxacin	0.1–3.13	0.39	0.78
Staphylococcus aureus (55)	AT-4140	0.05–0.2	0.1	0.1
	Ciprofloxacin	0.1–1.56	0.39	0.78
	Ofloxacin	0.2–0.78	0.39	0.39
MRSA[b] (42)	AT-4140	0.025–0.1	0.05	0.1
	Ciprofloxacin	0.1–1.56	0.39	1.56
	Ofloxacin	0.2–1.56	0.39	0.78
Staphylococcus epidermidis (38)	AT-4140	0.0125–1.56	0.1	0.2
	Ciprofloxacin	0.025–12.5	0.2	1.56
	Ofloxacin	0.1–3.13	0.39	1.56
Enterococcus faecalis (58)	AT-4140	0.05–0.78	0.39	0.78
	Ciprofloxacin	0.05–1.56	0.78	1.56
	Ofloxacin	0.2–3.13	1.56	3.13
Streptococcus pyogenes (21)	AT-4140	0.1–1.56	0.39	0.78
	Ciprofloxacin	0.1–1.56	0.39	1.56
	Ofloxacin	0.39–1.56	0.78	1.56
Streptococcus pneumoniae (24)	AT-4140	0.1–0.78	0.2	0.78
	Ciprofloxacin	0.39–1.56	0.78	1.56
	Ofloxacin	0.39–3.13	3.13	3.13

[a] 50% and 90%, MIC for 50 and 90% of the strains tested, respectively.
[b] MRSA, Methicillin-resistant *S. aureus* (MIC of methicillin, >12.5 μg/ml).

TABLE 3. Antibacterial activities of AT-4140 and related compounds against anaerobes

Organism	MIC (μg/ml)[a]				
	AT-4140	CPFX	OFLX	ENX	NFLX
Streptococcus intermedius 17408	1.56	3.13	6.25	25	25
Streptococcus parvulus VPI 0546	0.39	0.78	1.56	12.5	12.5
Peptostreptococcus asaccharolyticus GAI 0290	0.2	3.13	12.5	6.25	1.56
Staphylococcus saccharolyticus ATCC 13953	0.39	0.39	0.78	1.56	3.13
Clostridium perfringens ATCC 13123	0.2	0.39	0.78	3.13	1.56
Eubacterium limosum ATCC 8486	0.78	1.56	3.13	12.5	6.25
Eubacterium aerofaciens ATCC 25986	0.39	1.56	1.56	12.5	6.25
Propionibacterium acnes ATCC 11827	0.39	1.56	1.56	12.5	6.25
Propionibacterium granulosum ATCC 25564	0.2	0.78	0.39	6.25	3.13
Bacteroides fragilis ATCC 25285	0.78	6.25	1.56	12.5	25
Bacteroides vulgatus ATCC 29327	0.78	25	3.13	25	100
Bacteroides thetaiotaomicron WAL 3304	1.56	12.5	6.25	25	>100
Bacteroides distasonis GM 7007	3.13	25	12.5	25	>100
Fusobacterium varium ATCC 8501	6.25	12.5	12.5	50	100
Fusobacterium mortiferum ATCC 9817	12.5	12.5	12.5	50	50
Fusobacterium nucleatum GAI 0476	1.56	3.13	3.13	12.5	50

[a] See Table 1, footnote *a*.

epidermidis, *E. faecalis*, *S. pyogenes*, and *S. pneumoniae*, the MICs of AT-4140 for 90% of the strains tested were uniformly lower than those of ciprofloxacin and ofloxacin. Methicillin-resistant *S. aureus* was also highly susceptible to AT-4140.

Activities against anaerobes. The antibacterial activities of AT-4140 and related compounds against anaerobes are shown in Table 3. Pathogenic anaerobes, such as *Clostridium perfringens* and *Bacteroides fragilis*, were inhibited by AT-4140 at concentrations of 0.2 and 0.78 μg/ml, respectively. AT-4140 was generally more potent than the related compounds compared in activity against anaerobes.

Activities against the genera *Legionella*, *Mycoplasma*, and *Chlamydia*. Table 4 shows the comparative antibacterial activities of AT-4140, related compounds, and some reference antibiotics against *Legionella* spp., *Mycoplasma* spp., and *Chlamydia* spp. *Legionella* spp. were inhibited by AT-4140, ciprofloxacin, and ofloxacin at MICs of 0.0125 to 0.05 μg/ml, which were lower than those of enoxacin, norfloxacin, and erythromycin but higher than those of rifampin. *Mycoplasma pneumoniae* strains were highly susceptible to AT-4140 (MIC for 50% of the strains studied, 0.1 μg/ml), which was more potent than the related compounds and minocycline but less potent than erythromycin. Other *Mycoplasma* spp., such as *M. buccale*, *M. fermentans*, *M. hominis*, *M. orale*, and *M. salivarium*, which were quite resistant to erythromycin were also highly susceptible to AT-4140 (MICs, 0.0125 to 0.2 μg/ml). *C. trachomatis* and *C. psittaci* were inhibited by AT-4140 at MICs of 0.031 to 0.063

TABLE 4. Antibacterial activities of AT-4140 and reference compounds against members of the genera *Legionella*, *Mycoplasma*, and *Chlamydia*

Organism (no. of strains)	MIC (μg/ml) for 50% of strains tested[a]							
	AT-4140	CPFX	OFLX	ENX	NFLX	EM	RFP	MINO
Legionella pneumophila (7)	0.05	0.05	0.05	0.2	0.1	1.56	≦0.0031	
Legionella bozemanii ATCC 33127	0.025	0.0125	0.0125	0.2	0.05	0.78	≦0.0031	
Legionella micdadei ATCC 33218	0.0125	0.0125	0.05	0.2	0.05	3.13	≦0.0031	
Legionella dumoffii ATCC 33279	0.05	0.025	0.05	0.2	0.1	0.78	≦0.0031	
Mycoplasma pneumoniae (11)	0.1	0.78	0.78	6.25	6.25	0.0063		1.56
Mycoplasma buccale CH-20247	0.0125	0.39	0.39	1.56	1.56	>100		0.05
Mycoplasma fermentans PG-18	0.0125	0.1	0.1	0.78	0.39	25		0.05
Mycoplasma hominis PG-21	0.05	1.56	0.78	12.5	12.5	>100		0.1
Mycoplasma orale CH-19299	0.2	1.56	1.56	6.25	12.5	>100		0.1
Mycoplasma salivarium PG-20	0.1	3.13	6.25	25	25	>100		0.1
Chlamydia trachomatis (8)	0.063	1.0	1.0	8.0	16	0.13		0.031
Chlamydia psittaci MP	0.031	1.0	0.5	8.0		0.13		0.031

[a] See Table 1, footnote *a*, for abbreviations of quinolones; EM, erythromycin; RFP, rifampin; MINO, minocycline.

μg/ml, which were comparable to those of minocycline and much lower than those of ciprofloxacin, ofloxacin, enoxacin, and erythromycin.

Activities against *Mycobacterium* spp. AT-4140 inhibited *M. tuberculosis* at an MIC of 0.1 μg/ml, which was 1/3 of that of ciprofloxacin, 1/10 of that of ofloxacin, and 1/30 of that of enoxacin and norfloxacin (Table 5). The antitubercular activity of AT-4140 was comparable to those of isoniazid, *p*-aminosalicylic acid, and rifampin. AT-4140 also inhibited *M. tuberculosis* organisms resistant to isoniazid, *p*-aminosalicylic acid, and streptomycin at the same MIC of 0.1 μg/ml, indicating that it was not cross-resistant with such drugs. AT-4140 inhibited *M. intracellulare* and *M. fortuitum*, which were highly resistant to most antitubercular drugs. The MICs of AT-4140 for both of the *Mycobacterium* spp. were 0.1 to 0.3 μg/ml, which were equal to those of ciprofloxacin and generally lower than those of ofloxacin, enoxacin, and norfloxacin.

Activities against systemic infections. The in vivo activities of AT-4140 were compared with those of ciprofloxacin, ofloxacin, enoxacin, and norfloxacin in systemic infection models in mice (Table 6). The ED_{50}s of AT-4140 for infections with the gram-positive organisms *S. aureus*, *S. pyogenes*, and *S. pneumoniae* were 0.828, 3.36, and 6.31 mg/kg, respectively, which were 1/5 to 1/10 of those of ciprofloxacin, 1/3 to 1/7 of those of ofloxacin, 1/12 to 1/39 of those of enoxacin, and 1/33 to 1/56 of those of norfloxacin. The ED_{50}s of AT-4140 for infections with the gram-negative organisms *E. coli* and *P. aeruginosa* were 0.478 and 1.57 mg/kg, respectively, which ranged from unity to 1/2 of those of ciprofloxacin, from 1/2 to 1/4 of those of ofloxacin and from 1/10 to 1/11 of those of norfloxacin and were 1/5 of those of enoxacin. The in vivo activities of AT-4140 were thus generally superior to those of ciprofloxacin, ofloxacin, enoxacin, and norfloxacin. It was noted that the in vivo activity of AT-4140 was higher than that of ciprofloxacin in infections

TABLE 5. Antibacterial activities of AT-4140 and reference compounds against *Mycobacterium* species

Compound	MIC(s) (μg/ml)[a] for:			
	M. tuberculosis (2)[b]	*M. tuberculosis* INH^r PAS^r SM^r (1)[b,c]	*M. intracellulare* (3)[b]	*M. fortuitum* (2)[b]
AT-4140	0.1_2	0.1	0.1_1, 0.3_2	0.3_2
Ciprofloxacin	0.3_2	0.3	0.1_1, 0.3_2	0.3_1, 1_1
Ofloxacin	1_2	1	0.3_1, 1_1, 3_1	0.3_2
Enoxacin	3_2	3	1_1, 3_2	1_1, 3_1
Norfloxacin	3_2	3	1_2, 3_1	1_1, 10_1
Isoniazid	0.03_2	>30	10_2, 100	30_1, 100
p-Aminosalicylic acid	0.1_2	100	100_2, >100	$>100_2$
Ethambutol	3_2	3	1_1, 10_2	30_1, >100
Streptomycin	3_2	>100	0.3_1, 3_1, 10_1	30_2
Kanamycin	0.3_1, 1_1	1	3_1, 10_2	100_2
Rifampin	0.1_2	0.1	<0.01, 0.1_2	30_1, 100_1

[a] The inferior number is the number of isolates with the MIC indicated.
[b] Number of strains.
[c] Isoniazid, *p*-aminosalicylic acid, and streptomycin resistant.

TABLE 6. Comparative oral activities of AT-4140 and related compounds against systemic infections in mice

Infecting organism (challenge dose [CFU/mouse])	Compound	MIC (μg/ml)	ED_{50} (95% confidence limits) (mg/kg)
S. aureus 50774 (5×10^8)	AT-4140	0.05	0.828 (0.618–1.11)
	Ciprofloxacin	0.2	8.24 (5.41–12.6)
	Ofloxacin	0.2	5.18 (4.32–6.21)
	Enoxacin	0.39	9.89 (7.31–13.4)
	Norfloxacin	0.78	27.0 (19.3–37.8)
S. pyogenes A65 (3×10^7)	AT-4140	0.39	3.36 (2.39–4.43)
	Ciprofloxacin	0.2	20.3 (13.8–29.7)
	Ofloxacin	0.39	10.8 (7.82–14.9)
	Enoxacin	1.56	86.4 (76.7–97.3)
	Norfloxacin	1.56	188 (152–233)
S. pneumoniae I Neufeld (3×10^3)	AT-4140	0.39	6.31 (3.83–10.4)
	Ciprofloxacin	0.78	31.3 (15.6–62.7)
	Ofloxacin	1.56	41.7 (23.4–74.2)
	Enoxacin	6.25	247 (196–313)
	Norfloxacin	3.13	340 (175–659)
E. coli P-5101 (9×10^6)	AT-4140	0.0125	0.478 (0.390–0.585)
	Ciprofloxacin	0.0063	0.468 (0.367–0.597)
	Ofloxacin	0.05	0.749 (0.603–0.931)
	Enoxacin	0.05	2.15 (1.79–2.59)
	Norfloxacin	0.05	4.84 (3.57–6.57)
P. aeruginosa 12 (4×10^3)	AT-4140	0.39	1.57 (1.25–1.98)
	Ciprofloxacin	0.1	2.78 (2.17–3.56)
	Ofloxacin	0.78	6.62 (5.85–7.48)
	Enoxacin	0.78	8.41 (7.61–9.29)
	Norfloxacin	0.78	17.4 (14.7–20.7)

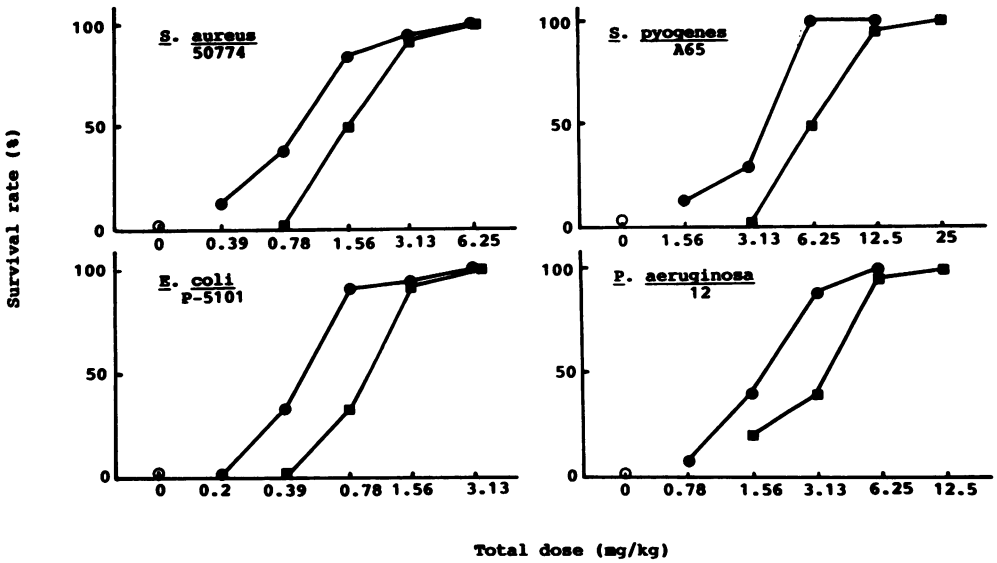

FIG. 2. Influence of medication times on the activity of orally administered AT-4140 against systemic infections in mice. ○, No medication; ●, single-dose medication (immediately after challenge); ■, equivalent two-dose medication (immediately after challenge and 6 h later).

with *S. pyogenes* and *P. aeruginosa*, which were less susceptible to AT-4140 than to ciprofloxacin in vitro, and higher than that of ofloxacin in infections with *S. pyogenes*, against which AT-4140 and ofloxacin showed identical in vitro activities.

The influence of medication times on the activity of orally administered AT-4140 was examined in systemic infections in mice (Fig. 2). Single-dose medication showed a consistently better effect than equivalent two-dose medication, although the efficacies were the same at higher doses.

Activities against pulmonary, dermal, and urinary tract infections. Comparisons of the in vivo activities of AT-4140 and its analogs in pulmonary, dermal, and urinary tract infections in mice were also made (Table 7). The ED_{50}s of AT-4140 were 1.61 and 2.86 mg/kg for pulmonary *K. pneumoniae* and *P. aeruginosa* infections, respectively, which were 1/2 to 1/3 of those of ciprofloxacin, 1/2 of those of ofloxacin, 1/3 to 1/9 of those of enoxacin, and 1/5 to 1/81 of those of norfloxacin. In dermal infections with *P. aeruginosa* in mice, AT-4140 showed an ED_{50} of 1.06 mg/kg, which was 1/5, 1/7, 1/11, and 1/31 of those of ciprofloxacin, ofloxacin, enoxacin, and norfloxacin, respectively. The in vivo antipseudomonal activity of AT-4140 was superior to that of ciprofloxacin, irrespective of its relatively inferior in vitro activity. In urinary tract infections with *S. aureus*, *E. coli*, *P. aeruginosa*, and *Serratia marcescens* in mice, AT-4140 exhibited ED_{50}s of 17.8, 0.782, 1.12, and 5.77 mg/kg, respectively, which ranged from unity to 1/5 of those of ciprofloxacin and from unity to 1/3 of those of enoxacin.

DISCUSSION

Recently developed quinolone antibacterial agents, such as ciprofloxacin, ofloxacin, enoxacin, and norfloxacin, have broad and potent antibacterial activities and have been applied to various kinds of infections in humans. However, their antibacterial activities are not sufficient against some important pathogens, e.g., gram-positive cocci, *Mycoplasma* spp., *Chlamydia* spp., *Mycobacterium* spp., etc., in contrast with their potent activities against gram-negative bacilli. As shown in Results, the antibacterial activity of AT-4140 against such pathogens was generally equivalent to its potent activity against gram-negative organisms.

Respiratory tract infections are caused by various kinds of organisms, and proper therapeutic drugs for the infections depend on the causative organisms. Infections due to *S. pneumoniae* can be successfully treated with β-lactams, but those due to *M. pneumoniae* would need treatment with macrolides. Chronic infections due to gram-negative organisms may require treatment with β-lactams, aminoglycosides, or quinolones. Tuberculosis should be treated with antitubercular drugs. However, identification of causative organisms is not always easy, and inadequate treatment may bring about critical conditions. If a drug has a broad antibacterial spectrum covering most respiratory pathogens, it would be useful for treatment of infections in which the causative organisms are not easily identified. AT-4140 seems to have potential as a drug useful for such situations.

Methicillin-resistant *S. aureus* is said to have been frequently isolated recently as an organism causing nosocomial infections, and treatment of such infections is usually difficult because of the resistance of the organism to most available antibacterial agents. Therefore, it is noteworthy that AT-4140 is highly active against methicillin-resistant *S. aureus*, as well as methicillin-susceptible *S. aureus*, and is different from currently used quinolones.

C. psittaci induces psittacosis, and *C. trachomatis* is a causative organism of sexually transmitted diseases, such as urethritis and cervicitis, for which tetracyclines have been used. However, tetracyclines have some untoward effects which restrict their clinical use. It has been reported that some quinolones are clinically effective on *C. trachomatis* infections (abstr. 2nd Int. Symp. New Quinolones, Geneva, Switzerland, 25 to 27 August 1988, p. 243–257). The potent antichlamydial activity of AT-4140, comparable to that of minocycline, encourages us to examine its potential usefulness for chlamydial infections.

Nontuberculous *Mycobacterium* spp., such as *M. intracellulare* and *M. fortuitum*, are usually resistant to available antibacterial agents, including antitubercular drugs, and there are few effective drugs against them. Therefore, it was noted that AT-4140 showed potent antibacterial activity

TABLE 7. Comparative oral activities of AT-4140 and related compounds against pulmonary, dermal, and urinary tract infections in mice

Type of infection and organism (challenge dose [CFU/mouse])	Compound	MIC (μg/ml)	ED_{50} (95% confidence limits) (mg/kg)
Pulmonary			
K. pneumoniae 1775b (2×10^5)	AT-4140	0.025	1.61 (0.764–3.39)
	Ciprofloxacin	0.025	4.03 (1.87–8.65)
	Ofloxacin	0.1	3.39 (2.07–5.54)
	Enoxacin	0.2	14.9 (10.2–21.8)
	Norfloxacin	0.1	130 (67.7–251)
P. aeruginosa 12 (2×10^7)	AT-4140	0.39	2.86 (1.81–4.52)
	Ciprofloxacin	0.1	5.39 (3.41–8.52)
	Ofloxacin	0.78	5.84 (3.28–10.4)
	Enoxacin	0.78	9.28 (5.89–14.6)
	Norfloxacin	0.78	13.9 (6.59–29.4)
Dermal; *P. aeruginosa* 12 (9×10^6)	AT-4140	0.39	1.06 (0.555–2.03)
	Ciprofloxacin	0.1	5.32 (4.02–7.03)
	Ofloxacin	0.78	7.49 (5.08–11.1)
	Enoxacin	0.78	11.3 (7.40–17.2)
	Norfloxacin	0.78	32.6 (20.4–52.0)
Urinary tract			
S. aureus 50774 (2×10^5)	AT-4140	0.05	17.8 (12.5–25.2)
	Ciprofloxacin	0.2	54.4 (17.6–168)
	Enoxacin	0.39	24.2 (6.13–95.4)
E. coli P-5101 (2×10^7)	AT-4140	0.0125	0.782 (0.304–2.01)
	Ciprofloxacin	0.0063	0.939 (0.658–1.34)
	Enoxacin	0.05	1.48 (0.773–2.85)
P. aeruginosa 12 (2×10^4)	AT-4140	0.39	1.12 (0.344–3.65)
	Ciprofloxacin	0.1	5.88 (1.88–18.4)
	Enoxacin	0.78	3.38 (1.46–7.79)
S. marcescens S-9 (3×10^7)	AT-4140	0.2	5.77 (3.95–8.45)
	Ciprofloxacin	0.05	11.7 (5.72–24.1)
	Enoxacin	0.2	11.9 (2.81–50.1)

against such organisms, although many studies should be done before its clinical application.

The in vivo antibacterial activities of AT-4140 were generally higher than those of ciprofloxacin, ofloxacin, enoxacin, and norfloxacin. As the in vitro activity of AT-4140 against gram-positive organisms was generally higher than that of the reference quinolones, it can partly account for its good in vivo activity against infections due to gram-positive organisms. However, AT-4140 was also more active than the reference quinolones against infections with gram-negative organisms, some of which were less susceptible to AT-4140 than to ciprofloxacin in vitro. This suggests that factors other than in vitro antibacterial activity are responsible for the good in vivo activity of AT-4140.

Pharmacokinetic studies revealed that AT-4140 was well absorbed orally, had a relatively long half-life in plasma, and showed good tissue distribution (Y. Sekine, Y. Matsunaga, H. Miyazaki, T. Yamaguchi, Y. Mizuki, T. Itoh, N. Kurobe, S. Nakamura, M. Hashimoto, and M. Shimizu, 28th ICAAC, abstr. no. 1489, 1988). When compared with ciprofloxacin, ofloxacin, enoxacin, and norfloxacin, AT-4140 has a significantly longer half-life in plasma in mice (unpublished data). Therefore, such pharmacokinetic properties of AT-4140 may favorably affect its in vivo activity.

When the influence of medication times on the activity of AT-4140 was examined in systemic infection models, single-dose medication was better than equivalent two-dose medication, suggesting that the duration of the compound may be sufficient and its level in plasma may be important for efficacy.

The phase 1 study indicated that the peak levels of AT-4140 in plasma were 0.44 to 1.39 μg/ml in human volunteers given a single oral dose of 100 to 400 mg and its half-life in plasma was about 16 h (M. Kanamaru, M. Nakashima, T. Uematsu, and Y. Takikuchi, 28th ICAAC, abstr. no. 1490, 1988), significantly longer than the half-lives of ciprofloxacin (3.3 to 4.9 h), ofloxacin (7 h), enoxacin (6.2 h), and norfloxacin (3 to 4 h) (3). The long half-life of AT-4140 in plasma seems to support once-a-day treatment, although the existing quinolones have been clinically administered twice or three times a day.

Toxicological studies revealed that AT-4140 was low enough in toxicity to be applied clinically (M. Hashimoto, A. Minami, K. Nakata, Y. Sakaguchi, T. Kojima, K. Fujimoto, H. Yoshida, S. Nakamura, K. Ohnishi, and M. Shimizu, 28th ICAAC, abstr. no. 1488, 1988). Clinical trials are in progress in Japan.

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