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In Vitro and In Vivo Activities of AM-1155, a New Fluoroquinolone, against *Chlamydia* spp.

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The in vitro and in vivo activities of AM-1155, a new quinolone, against *Chlamydia* spp. were investigated. The MIC of AM-1155 for 10 standard strains of different *Chlamydia* spp. and 25 wild-type strains of *Chlamydia pneumoniae* isolated in Japan, which were morphologically different from clinical isolates from the United States, ranged from 0.063 to 0.125 μg/ml. Its activity was almost the same as those of sparfloxacin and tosufloxacin and was 4 and 16 times superior to those of levofloxacin and ciprofloxacin, respectively, but lower than those of clarithromycin and minocycline (range for each, 0.016 to 0.031 μg/ml). The minimal chlamydiacidal concentration of AM-1155 ranged from 0.063 to 0.125 μg/ml, while those of clarithromycin and minocycline ranged from 0.016 to 0.031 μg/ml and 0.016 to 0.063 μg/ml, respectively. The therapeutic effect of a 7-day course of AM-1155 at doses of 5 and 10 mg/kg of body weight administered orally twice daily to mice with experimental *Chlamydia psittaci* pneumonia was excellent, with a 100% survival rate at 21 days after infection. The efficacy was equal to those of clarithromycin and minocycline and higher than those of ciprofloxacin and ofloxacin.

AM-1155 is a new 8-methoxy quinolone with the formula (±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate. AM-1155 possesses potent in vitro and in vivo activities not only against gram-positive and -negative bacteria but also against mycoplasmas and mycobacteria (9–11, 21, 22). This study was designed to determine the susceptibilities of three *Chlamydia* species, *Chlamydia pneumoniae*, *Chlamydia psittaci*, and *Chlamydia trachomatis*, to AM-1155 and compared them with those to other conventional antimicrobial agents. 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. The antimicrobial agents tested were AM-1155 (obtained from Kyorin Pharmaceutical Co.), sparfloxacin (obtained from Dainippon Pharmaceutical Co.), tosufloxacin (obtained from Toyama Chemical Co.), levofloxacin and ofloxacin (obtained from Daiichi Pharmaceutical Co.), ciprofloxacin (obtained from Bayer Yakuhin Co.), erythromycin (obtained from Shionogi & Co.), azithromycin and doxycycline (obtained from Pfizer Pharmaceutical Co.), roxithromycin (obtained from Nippon Roussel Co.), clarithromycin (obtained from Taisho Pharmaceutical Co.), and minocycline (obtained from Lederle Japan Co.). The new quinolones and tetracyclines were dissolved in sterile distilled water with or without 0.1 N NaOH, and macrolides were dissolved in ethanol.

Chlamydial strains. The standard strains of chlamydia tested were *C. pneumoniae* TW-183, AR-39, and AR-388 (purchased from the Washington Research Foundation, Seattle), IOL-207, and Kajaani-6 (supplied by P. Saikku, University of Helsinki, Helsinki, Finland); *C. psittaci* Budgerigar-1 (supplied by the National

TABLE 1. MICs of AM-1155 and other antimicrobial agents for standard strains of *Chlamydia* species

Agent	MIC (μg/ml)									
	C. pneumoniae					*C. psittaci*[a]		*C. trachomatis*[b]		
	IOL-207	Kajaani-6	TW-183	AR-39	AR-388	Bud	Cal 10	D	E	L_2
AM-1155	0.063	0.125	0.063	0.063	0.125	0.063	0.063	0.063	0.063	0.125
Sparfloxacin	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.125
Tosufloxacin	0.063	0.125	0.125	0.063	0.125	0.125	0.125	0.063	0.125	0.125
Levofloxacin	0.5	0.25	0.5	0.25	0.5	0.25	0.25	0.5	0.25	0.5
Ofloxacin	1.0	0.5	1.0	0.5	1.0	0.5	0.5	0.5	0.5	1.0
Ciprofloxacin	1.0	2.0	1.0	1.0	2.0	1.0	2.0	1.0	1.0	1.0
Erythromycin	0.125	0.25	0.125	0.125	0.25	0.125	0.25	0.125	0.125	0.125
Azithromycin	0.125	0.125	0.125	0.25	0.125	0.125	0.125	0.125	0.125	0.125
Roxithromycin	0.063	0.063	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.063
Clarithromycin	0.016	0.031	0.016	0.016	0.016	0.016	0.016	0.016	0.031	0.031
Doxycycline	0.031	0.063	0.031	0.031	0.031	0.031	0.063	0.031	0.063	0.031
Minocycline	0.016	0.016	0.016	0.031	0.031	0.031	0.016	0.016	0.016	0.031

[a] Bud, Budgerigar-1; Cal 10, California 10.
[b] D, D/UW-3/Cx; E, E/UW-5/Cx; L_2, L_2/434/Bu.

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TABLE 2. MCCs of AM-1155 and other antimicrobial agents for standard strains of *Chlamydia* species[a]

Agent	MCC (μg/ml)									
	C. pneumoniae					*C. psittaci*		*C. trachomatis*		
	IOL-207	Kajaani-6	TW-183	AR-39	AR-388	Bud	Cal 10	D	E	L_2
AM-1155	0.063	0.125	0.063	0.063	0.125	0.063	0.063	0.063	0.063	0.125
Sparfloxacin	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.125
Tosufloxacin	0.063	0.125	0.125	0.125	0.125	0.125	0.125	0.063	0.125	0.125
Levofloxacin	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.5	0.25	0.5
Ofloxacin	1.0	0.5	1.0	0.5	1.0	0.5	1.0	0.5	0.5	1.0
Ciprofloxacin	1.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0	1.0	1.0
Erythromycin	0.125	0.25	0.25	0.125	0.25	0.125	0.25	0.125	0.125	0.25
Azithromycin	0.125	0.125	0.25	0.25	0.125	0.125	0.125	0.25	0.125	0.25
Roxithromycin	0.063	0.063	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.063
Clarithromycin	0.016	0.031	0.016	0.016	0.016	0.016	0.016	0.016	0.031	0.031
Doxycycline	0.031	0.063	0.063	0.063	0.063	0.031	0.063	0.031	0.063	0.063
Minocycline	0.016	0.016	0.031	0.031	0.031	0.031	0.031	0.031	0.016	0.031

[a] See footnotes to Table 1 for strain abbreviations.

Institute for Health, Tokyo, Japan) and California 10 (supplied by A. Matsumoto, Department of Microbiology, Kawasaki Medical School, Kurashiki, Japan); and *C. trachomatis* D/UW-3/Cx, E/UW-5/Cx, and L_2/434/Bu (supplied by the National Institute for Health, Japan).

In the past 4 years, we successively isolated 25 *C. pneumoniae* strains from nasopharyngeal swab specimens of patients with respiratory tract infections in Japan (24 isolates, designated KKpn-1 to KKpn-24, from the Kawasaki Medical School Hospital and 1 isolate, designated YK-41, from Hiroshima Prefectural Hiroshima Hospital [12, 16, 17]). We reported that some of the above-mentioned clinical isolates, strains KKpn-1, KKpn-15, KKpn-16, and YK-41, were morphologically different from TW and AR strains (TW-83, AR-39, and AR-388) from the United States (16–19). The elementary bodies (EBs) of these four clinical isolates from Japan were round, whereas those of the TW and AR strains were pear shaped. We also confirmed that the EBs of the other isolates (KKpn-2 to KKpn-14 and KKpn-17 to KKpn-24) were morphologically identical to those of strains KKpn-1, KKpn-15, KKpn-16, and YK-41 (data not published). These 25 clinical isolates of *C. pneumoniae* were also used.

Measurement of MICs and MCCs. MICs and minimal chlamydiacidal concentrations (MCCs) were determined by the standard method of the Japan Society of Chemotherapy (13, 14). 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 one of the chlamydial species per well. The IFU were determined by the following method. A serially diluted suspension containing chlamydiae was inoculated onto confluent monolayers of HeLa 229 cells. After inoculation, the number of inclusion bodies was counted. The number of IFU per milliliter was calculated on the basis of the number of inclusion bodies, inoculum size, and fold dilution of the suspension. After the inoculation, 1 ml of a preparation of culture medium consisting of Eagle's minimal essential medium (Nissui Pharmaceuticals Co.), 10% heat-inactivated fetal calf serum (GIBCO Laboratories Inc.), and cycloheximide (Nakarai Tesque Inc.) at a final concentration of 1 μg/ml and containing one concentration of each test agent was applied. Then, the plates were incubated in 5% CO_2 at 35°C for 72 h for *C. pneumoniae*, at 37°C for 48 h for *C. psittaci*, or at 37°C for 72 h for *C. trachomatis*. Following incubations, Cultureset (Ortho Diagnostics Systems Inc.), a genus-specific fluorescein isothiocyanate-conjugated monoclonal antibody, was used to stain inclusions. Inclusion bodies formed in cells were observed with a Nikon epifluorescence microscope at ×200 or ×400 magnification. The MIC was defined as the lowest concentration at which complete inhibition of inclusion formation was observed. The MCC was determined by aspirating antibiotic-containing medium, washing wells twice with phosphate-buffered saline, and adding antibiotic-free medium. Cultures were frozen at −70°C, thawed, passed onto new cells, reincubated under the conditions described above, and then fixed and stained as described above. The MCC was the lowest antibiotic concentration which resulted in no inclusions after passage.

Therapeutic effect in mice with *C. psittaci* pneumonia. Cells infected with *C. psittaci* California 10 were micronized by ultrasonic treatment and were diluted with sucrose-phosphate-glutamic acid medium to an appropriate titer. Then, 5-week-old male MCH mice (CLEA Japan Inc.) were infected with the cell solution (10^5 IFU per animal) by nasal instillation. Five compounds (AM-1155, ofloxacin, ciprofloxacin, clarithromycin, and minocycline) were used for treatment. All agents were suspended in 5% gum arabic, and two doses, 5 and 10 mg per kg of body weight, were prepared. The animals were divided into groups of 10 each. Each animal received 0.2 ml of the antibiotic suspension orally every 12 h for 7 days. Control mice received only 5% gum arabic. The treatment was begun 24 h after infection. The animals were monitored every day for 21 days to determine the survival rates and to compare the therapeutic effects of each drug.

A two-tailed Fisher exact probability test was used for statistical analysis of the survival/death ratio on day 21, and a P of <0.05 was considered to be significant.

RESULTS

MICs and MCCs. The MICs and MCCs of AM-1155 and other antimicrobial agents for standard strains of three different species of chlamydiae and 25 clinical isolates of *C. pneumoniae* are shown in Tables 1, 2, and 3, respectively. All chlamydial strains showed almost identical drug susceptibility. Clarithromycin and minocycline showed the lowest MICs (range for each, 0.016 to 0.031 μg/ml), and the MIC at which

TABLE 3. MICs and MCCs of AM-1155 and other antimicrobial agents for 25 clinically isolated strains of *C. pneumoniae*

Agent	MIC (μg/ml) range	MIC_{50}	MIC_{90}	MCC (μg/ml) range	MCC_{50}	MCC_{90}
AM-1155	0.063–0.125	0.063	0.125	0.063–0.125	0.063	0.125
Sparfloxacin	0.031–0.125	0.063	0.063	0.031–0.125	0.063	0.063
Tosufloxacin	0.063–0.25	0.125	0.125	0.063–0.25	0.125	0.125
Levofloracin	0.25–1.0	0.25	0.5	0.25–1.0	0.25	0.5
Ofloxacin	0.5–1.0	0.5	1.0	0.5–1.0	0.5	1.0
Ciprofloxacin	1.0–2.0	1.0	2.0	1.0–4.0	1.0	4.0
Erythromycin	0.063–0.25	0.125	0.25	0.063–0.25	0.125	0.25
Azithromycin	0.125–0.25	0.125	0.25	0.125–0.5	0.125	0.5
Roxithromycin	0.063–0.25	0.125	0.125	0.063–0.25	0.125	0.125
Clarithromycin	0.016–0.031	0.016	0.031	0.016–0.031	0.016	0.031
Doxycycline	0.031–0.063	0.031	0.063	0.031–0.125	0.063	0.125
Minocycline	0.016–0.031	0.016	0.031	0.016–0.063	0.031	0.063

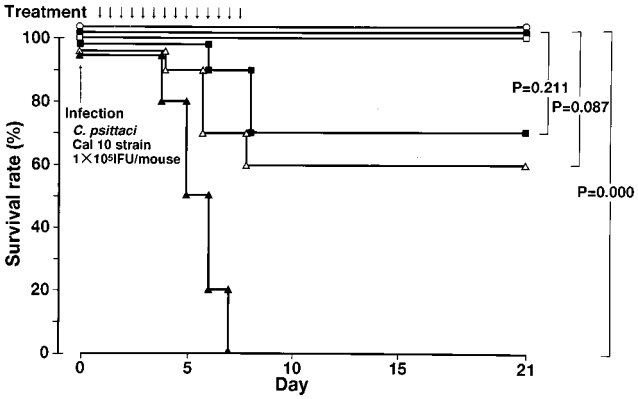

FIG. 1. Therapeutic effects of 10-mg/kg doses of AM-1155 in mice with *C. psittaci* California 10 (Cal 10) pneumonia. Treatment with 10 mg of AM-1155 (○), clarithromycin (●), minocycline (□), ofloxacin (■), or ciprofloxacin (△) per kg (twice daily for 7 days) was started 24 h after infection. Control mice received 5% gum arabic (▲). A two-tailed Fisher exact probability test was used for statistical analysis of the survival/death ratio on day 21, and a P of <0.05 was considered to be significant (n = 10).

90% of the clinical isolates are inhibited (MIC_{90}) was 0.031 μg/ml for each of these agents. Among the fluoroquinolones tested, AM-1155, sparfloxacin, and tosufloxacin showed the most potent activities. The MIC_{90} of AM-1155 was 0.125 μg/ml, and its activity was slightly lower than those of clarithromycin and minocycline. The MIC_{90}s of levofloxacin and ciprofloxacin (0.5 and 2.0 μg/ml, respectively) were 4 and 16 times higher, respectively, than that of AM-1155. The MCC of AM-1155 ranged from 0.063 to 0.125 μg/ml, while those of clarithromycin and minocycline ranged from 0.016 to 0.031 μg/ml and 0.016 to 0.063 μg/ml, respectively. Quinolone-, tetracycline-, or macrolide-resistant *C. pneumoniae* strains were not detected, indicating that a drug resistance problem has not yet occurred in our clinical population.

Therapeutic effects in mice with *C. psittaci* pneumonia. As shown in Fig. 1, the survival rate on day 21 for mice treated with 10-mg/kg doses of AM-1155, clarithromycin, or minocycline was 100%, while all control animals died within 7 days of the initiation of treatment. The survival rates of mice treated with 5-mg/kg doses of these drugs are shown in Fig. 2. The survival rate with AM-1155 treatment was significantly superior to that obtained with ofloxacin ($P < 0.005$) or ciprofloxacin ($P < 0.001$) and equal to those obtained with clarithromycin and minocycline.

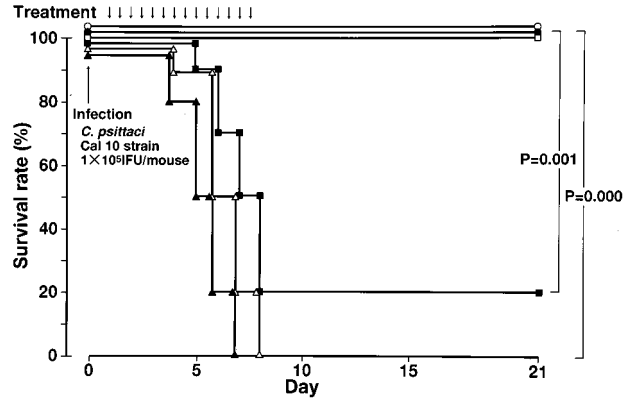

FIG. 2. Therapeutic effects of 5-mg/kg doses of AM-1155 in mice with *C. psittaci* California 10 (Cal 10) pneumonia. Treatment with 5 mg of AM-1155 (○), clarithromycin (●), minocycline (□), ofloxacin (■), or ciprofloxacin (△) per kg (twice daily for 7 days) was started 24 h after infection. Control mice received 5% gum arabic (▲). A two-tailed Fisher exact probability test was used for statistical analysis of the survival/death ratio on day 21, and a P of <0.05 was considered to be significant (n = 10).

DISCUSSION

Chlamydia species are well-known respiratory pathogens that cause upper and lower respiratory tract infections and pneumonia. *C. trachomatis* causes pneumonia in newborns, *C. psittaci* is known as a causative organism of psittacosis, and *C. pneumoniae* is recognized as an important pathogen of acute respiratory infections worldwide (3–5). Tetracyclines and macrolides have been used frequently for treatment of chlamydial infections. Recently, some fluoroquinolones as well as macrolides and tetracyclines have been demonstrated to be active in vitro against chlamydial species (6, 7, 16, 17). Clinical studies on erythromycin, clarithromycin, and azithromycin in which cultures were performed showed that these macrolides are effective drugs for treatment of genital infection with *C. trachomatis* as well as respiratory infection with *C. pneumoniae* (1, 2, 8). However, there are no studies of treatment of *C. pneumoniae* infection with any quinolone in which cultures were done.

C. pneumoniae is now well established as a major respiratory pathogen (3). This organism commonly causes pneumonia, bronchitis, sinusitis, and pharyngitis, as well as being responsible for up to 10% of the cases of community-acquired pneumonia (4, 5). However, data on the in vitro susceptibility of *C. pneumoniae* are limited because the number of clinical isolates available for testing is small and all isolates have been obtained from a limited geographic area (6, 7, 15). In this study, five *C. pneumoniae* strains isolated in several countries (IOL-207, Iran; Kajaani-6, Finland; TW-183, Taiwan; and AR-39 and -388, United States) (17) showed drug susceptibilities almost identical to those of the 25 *C. pneumoniae* clinical isolates tested in this study (Table 1, 2, and 3). We have previously reported that EBs of some of these 25 *C. pneumoniae* strains, KKpn-1, KKpn-15, KKpn-16, and YK-41, were round whereas those of TWAR were pear shaped (16–19). And we also confirmed that the EBs of the other isolates (KKpn-2 to KKpn-14 and KKpn-17 to KKpn-24) were morphologically identical to those of strains KKpn-1, KKpn-15, KKpn-16, and YK-41 (data not published). These facts indicate that the antichlamydial activity of drugs, including AM-1155, against *C. pneumoniae* might not be affected by morphological difference.

In our study, AM-1155 showed in vitro antichlamydial activities that were equal to or much higher than those of sparfloxacin, tosufloxacin, ofloxacin, ciprofloxacin, and roxithromycin, and in experiments with animals, we found that the therapeutic effect of AM-1155 against *C. psittaci* pneumonia was excellent and was equal to those of clarithromycin and minocycline. Now, the phase III clinical studies of AM-1155 are under way in Japan. According to the strong in vitro and in vivo antichlamydial activities of AM-1155 and to its pharmacokinetic profile in humans (for an oral 200-mg dose of AM-1155, the maximum concentration in serum is 1.71 μg/ml and the half-life is 7.1 h [20]), AM-1155 is expected to have clinical potential against chlamydial infections, and clinical study such as a prospective treatment study utilizing cultures is therefore recommended.

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