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In Vitro and In Vivo Activities of Sparfloxacin against *Mycoplasma pneumoniae*

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The in vitro and in vivo activities of sparfloxacin against *Mycoplasma pneumoniae* were compared with those of erythromycin, levofloxacin, ofloxacin, and minocycline. The MICs of sparfloxacin, erythromycin, levofloxacin, ofloxacin, and minocycline for 90% of the 43 *M. pneumoniae* strains tested were 0.063, 0.016, 0.5, 1, and 0.5 μg/ml, respectively. In the experimental pulmonary *M. pneumoniae* infection model in Syrian golden hamsters, sparfloxacin was as effective as erythromycin when orally administered at 15 mg/kg twice daily for 5 days and more effective than erythromycin when orally administered at 10 mg/kg once daily for 5 days. Sparfloxacin was more effective than levofloxacin and ofloxacin in both dosing regimens. The peak concentrations of sparfloxacin in hamster sera after administration of single oral doses of 15 mg/kg were almost the same as those in human sera after administration of single oral doses of 200 mg (the usual clinical dose), and the half-life of sparfloxacin in hamster serum was shorter than that in human serum after administration of a single oral dose of 200 mg. These results suggest that sparfloxacin may be clinically useful for the treatment of *M. pneumoniae* infections.

Mycoplasma pneumoniae is a major causative organism of pneumonia and accounts for about 20% of total pneumonia infections (6). The recommended therapeutic drug against *M. pneumoniae* infections is now erythromycin, which is efficacious in shortening the duration of symptoms (15). However, an increase in the number of mutants resistant to erythromycin has been reported (11). So, new chemotherapeutic drugs have been desired for the treatment of mycoplasmal infections. New quinolones (18) with a broad spectrum of activity against gram-positive and gram-negative bacteria have been developed during the past decade, and some of them have been reported to be active against *M. pneumoniae* (1, 2, 8, 12, 17). In particular, the in vitro activity of sparfloxacin (10) against *M. pneumoniae* has been reported to be excellent (8). In order to estimate the therapeutic potential of sparfloxacin, we compared the anti-*M. pneumoniae* activities of sparfloxacin with those of other quinolones, erythromycin, and minocycline in vitro and in vivo.

MATERIALS AND METHODS

Compounds. Sparfloxacin and erythromycin were kindly supplied by Dainippon Pharmaceutical Co., Ltd.; ofloxacin and levofloxacin were supplied by Daiichi Pharmaceutical Co., Ltd.; and minocycline was supplied by Nihon Lederle Pharmaceutical Co., Ltd. The new quinolones and minocycline were dissolved in distilled water with or without NaOH, and erythromycin was dissolved in ethanol. All the compounds were dissolved or suspended in 0.2% carboxymethyl cellulose for oral administration.

Organisms. Forty clinical strains of *M. pneumoniae* were isolated and identified at Nagasaki University Hospital, and three standard strains, FH, Mac, and M129, which had been obtained from M. F. Barile (Food and Drug Administration, Bethesda, Md.), were used for the in vitro test; the virulent strain M129 was used for experimental mycoplasmal pneumonia in hamsters.

MIC determination. MICs were determined by a modified broth microdilution method (17) with modified Chanock broth medium (4) consisting of 7 parts PPLO broth without crystal violet (Difco Laboratories, Detroit, Mich.), 2 parts uninactivated horse serum, 1 part 25% fresh yeast extract, 1% glucose, and 0.002% phenol red and adjusted to pH 7.8 and inocula of 10^5 CFU/ml. The plates, sealed with plate sealers, were then incubated at 37°C in air. In each case, when the color of the medium of the drug-free control changed from red to yellow, the minimal concentration of drug preventing the color change was defined as the MIC (17).

Assessment of in vivo activities. The in vivo activities of drugs were assessed in a pulmonary *M. pneumoniae* infection model using Syrian golden hamsters (1, 3, 16). Each group consisted of five 6-week-old male Syrian golden hamsters weighing about 100 g each (Japan SLC, Shizuoka, Japan). Animals were anesthetized by intraperitoneal injection of ketamine (Sankyo Co., Ltd.) and infected by the intratracheal intubation method described previously (3) with modification. The animals were mounted upside down at an angle of 45 degrees from vertical. A bent, blunted 2-inch 20-gauge Angiocath (Becton Dickinson Vascular Access) was inserted under the glottis into the trachea and the inner needle was drawn. A 1-ml tuberculin syringe was attached to the outer catheter and moved as far forward as possible. A 0.15-ml sample of the suspension of *M. pneumoniae* M129 strain (5.6×10^7 CFU/ml) was inoculated directly into the trachea. Control intratracheal inoculation with India ink verified the limited distribution of the inoculum into the right lung, especially into the lower right lobe. For a negative control, medium not containing *M. pneumoniae* was also inoculated, and no lung lesions were observed. Drugs were administered orally at a dose of 15 mg/kg twice daily or 10 mg/kg once daily, starting on day 5 after inoculation with *M. pneumoniae* and continuing for 5 days. Animals were sacrificed by cutting the abdominal aorta under ketamine anesthesia 24 h after the final drug administration.

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TABLE 1. Evaluation of the macroscopic severity of lung lesions for *M. pneumoniae* pneumonia in hamsters

Extent of macroscopic lung lesion	Ratio
Less than 1/8 lobe	0
More than 1/8 and less than 1/4 lobe	1/8
More than 1/4 and less than 1/2 lobe	1/4
More than 1/2 and less than 3/4 lobe	1/2
More than 3/4 and less than 1 lobe	3/4
More than 1 lobe	1

Lungs were removed aseptically, and the viable *M. pneumoniae* cells per lung were counted by the method described previously (14). Some colonies were used for identification of *M. pneumoniae* by a growth-inhibition test with anti-*M. pneumoniae* rabbit serum and by their hemadsorption properties (5).

For an untreated control, five hamsters similarly received the vehicle 0.2% carboxymethyl cellulose only twice daily for 5 days, and the viable-cell counts in hamster lungs and the estimates of macroscopic severity were performed at 24 h after the final administration. Before treatment (on day 5 after infection), five hamsters were sacrificed and the viable-cell counts in hamster lungs and the estimates of macroscopic severity were also done. In advance of this in vivo estimation, in order to estimate the possibility of the lung lesions being caused by other microorganisms, some homogenized lung lesions were cultured on chocolate agar, BCYE-α agar, and Sabouraud agar but no microorganisms were recovered.

Assessment of macroscopic pulmonary lesions. Lesions in the lungs were limited to the lower right lobe, and the severity of the pulmonary lesions was assessed macroscopically by measuring the extent of the macroscopic pulmonary lesion area according to the criteria shown in Table 1. This assessment was performed by the investigator who was blinded to the nature of the treatment regimen.

Lung and serum levels of sparfloxacin and ofloxacin. The concentrations of sparfloxacin and ofloxacin in the lungs and sera of male Syrian golden hamsters weighing about 100 g each and given each drug at a single oral dose of 15 mg/kg were determined. Samples were taken 0.5, 1, 2, 4, 6, 12, and 24 h following administration. Three hamsters were sacrificed at each time point to take the lungs and sera. Lungs were weighed and homogenized in 1/15 M phosphate buffer (pH 7.4). After centrifugation, the supernatants were obtained. The sera were appropriately diluted in normal hamster serum. The lung extracts and sera were assayed by the agar well diffusion method with *Escherichia coli* Kp as a test organism by the method described previously (9).

Statistical analysis. Differences in the CFUs in lungs and the extent of macroscopic lung lesions between the treated and untreated groups were analyzed by the Mann-Whitney U test for nonparametric analysis. All analyses were conducted with Version II Statview SE software (Abacus Concepts, Inc.).

RESULTS

In vitro activity. The antimycoplasmal activities of sparfloxacin, erythromycin, levofloxacin, ofloxacin, and minocycline against 43 strains of *M. pneumoniae*, including 3 standard strains, is shown in Table 2.

The ranges of the MICs of sparfloxacin, erythromycin, levofloxacin, ofloxacin, and minocycline were 0.031 to 0.063, 0.0039 to 0.031, 0.25 to 0.5, 0.5 to 1, and 0.25 to 1 μg/ml, respectively.

TABLE 2. Susceptibilities of 43 clinically isolated *M. pneumoniae* strains, including three standard strains, M129, Mac, and FH, to sparfloxacin, erythromycin, levofloxacin, ofloxacin, and minocycline

Drug	MIC (μg/ml)[a]		
	Range	50%	90%
Sparfloxacin	0.031–0.063	0.031	0.063
Erythromycin	0.0039–0.031	0.0078	0.016
Levofloxacin	0.25–0.5	0.25	0.5
Ofloxacin	0.5–1	0.5	1
Minocycline	0.25–1	0.5	0.5

[a] 50% and 90%, MICs for 50 and 90% of strains tested, respectively.

The MICs at which 90% of the strains tested were inhibited by sparfloxacin, erythromycin, levofloxacin, ofloxacin, and minocycline were 0.063, 0.016, 0.5, 1, and 0.5 μg/ml, respectively. The activities of levofloxacin and ofloxacin were less potent than that of erythromycin but similar to that of minocycline, and sparfloxacin showed the highest activity among new quinolones. The MICs of sparfloxacin, erythromycin, levofloxacin, and ofloxacin against the strain M129, which was used for the in vivo test, were 0.063, 0.016, 0.25, and 0.5 μg/ml, respectively.

In vivo activity. The in vivo activity of sparfloxacin was compared with those of erythromycin, levofloxacin, and ofloxacin in experimentally induced *M. pneumoniae* pneumonia in golden hamsters (Fig. 1 and 2).

Sparfloxacin, erythromycin, and levofloxacin at 15 mg/kg twice daily for 5 days and 10 mg/kg once daily for 5 days and ofloxacin at 15 mg/kg twice daily for 5 days decreased the numbers of viable *M. pneumoniae* cells significantly ($P < 0.05$) compared with that of the untreated control. Sparfloxacin at 10 mg/kg once daily for 5 days was significantly more effective than levofloxacin, ofloxacin, and erythromycin at 10 mg/kg once daily for 5 days ($P < 0.01$). Sparfloxacin showed the same potency irrespective of the dosing regimens employed. With

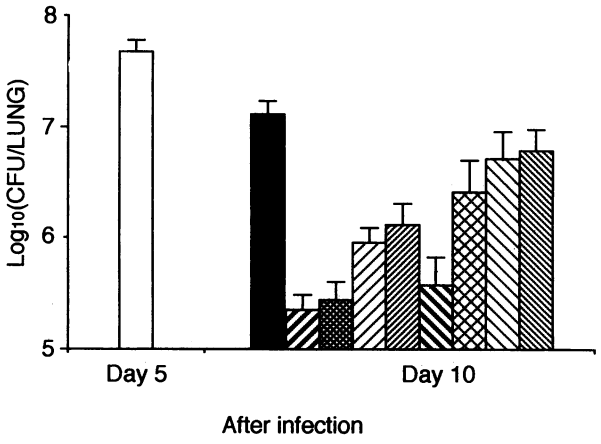

FIG. 1. Efficacy of drugs on viable mycoplasma cells in lungs after oral administration. Data, including those of the early and untreated controls, are the means of the cell counts from five hamsters per group with standard deviations. Symbols: □, early control; ■, untreated control; ▨, sparfloxacin at 15 mg/kg twice daily for 5 days; ▩, erythromycin at 15 mg/kg twice daily for 5 days; ▨, levofloxacin at 15 mg/kg twice daily for 5 days; ▨, ofloxacin at 15 mg/kg twice daily for 5 days; ▨, sparfloxacin at 10 mg/kg once daily for 5 days; ▩, erythromycin at 10 mg/kg once daily for 5 days; ▨, levofloxacin at 10 mg/kg once daily for 5 days; ▨, ofloxacin at 10 mg/kg once daily for 5 days.

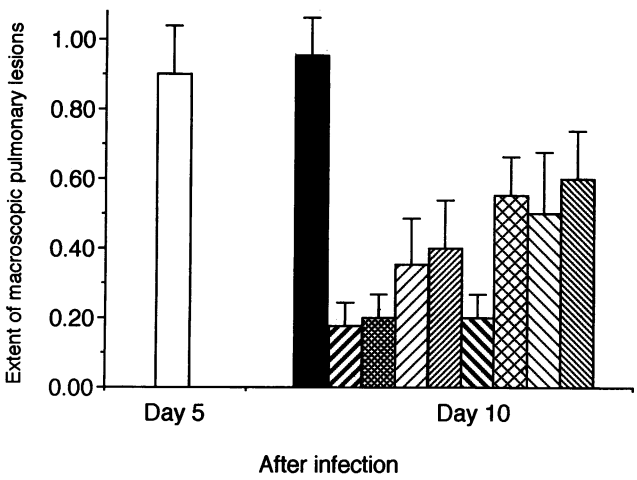

FIG. 2. Efficacy of drugs on macroscopic pulmonary lesions after oral administration. Data, including those of the early and untreated controls, are the means of the assessments for five hamsters per group with standard deviations. Symbols: □, early control; ■, untreated control; ▨, sparfloxacin at 15 mg/kg twice daily for 5 days; ▩, erythromycin at 15 mg/kg twice daily for 5 days; ▨, levofloxacin at 15 mg/kg twice daily for 5 days; ▨, ofloxacin at 15 mg/kg twice daily for 5 days; ▧, sparfloxacin at 10 mg/kg once daily for 5 days; ▩, erythromycin at 10 mg/kg once daily for 5 days; ▧, levofloxacin at 10 mg/kg once daily for 5 days; ▧, ofloxacin at 10 mg/kg once daily for 5 days.

respect to macroscopic pulmonary lesions, all the drugs had the potency to reduce macroscopic pulmonary lesions in both dosing regimens ($P < 0.05$), and sparfloxacin and erythromycin at 15 mg/kg twice daily for 5 days were generally more effective than the other drugs. The results indicated that sparfloxacin at 10 mg/kg once daily for 5 days had the same potency as sparfloxacin and erythromycin at 15 mg/kg twice daily for 5 days.

Drug levels in lungs and sera. The peak concentration of sparfloxacin in lungs after the administration of a single oral dose of 15 mg/kg was 3.20 ± 1.75 μg/g (Table 3). This value was about 1.3-fold higher than that of ofloxacin at the same dose. The half-life of sparfloxacin in lungs was 3.4 h, which was about 4-fold longer than that of ofloxacin.

The mean concentration of sparfloxacin in lungs at 24 h after the administration of a single oral dose of 15 mg/kg was 0.10 μg/g, which was higher than the MIC for the M129 strain. The peak concentrations and half-lives of sparfloxacin and ofloxacin in sera after the administration of single oral doses of 15 mg/kg were 0.54 ± 0.16 μg/ml and 3.5 h and 2.40 ± 0.31 μg/ml and 0.6 h, respectively.

These results show that the peak concentrations of sparfloxacin and ofloxacin in hamster sera are similar to those of sparfloxacin and ofloxacin in human sera after clinical dosing and that the half-lives of sparfloxacin and ofloxacin in hamster sera are shorter than those of sparfloxacin and ofloxacin in human sera (7, 13).

TABLE 3. Pharmacokinetic parameters of sparfloxacin and ofloxacin in lungs and sera after single oral doses of 15 mg/kg

Sample source and drug	C_{max} (μg/g)[a]	T_{max} (h)[b]	$t_{1/2}$ (h)[c]
Lung			
Sparfloxacin	3.20 ± 1.75	1	3.4
Ofloxacin	2.47 ± 0.37	0.5	0.8
Serum			
Sparfloxacin	0.54 ± 0.16	1	3.5
Ofloxacin	2.40 ± 0.31	0.5	0.6

[a] C_{max}, maximum concentration of drug. Values are the means ± standard deviations ($n = 3$).
[b] T_{max}, time to maximum concentration of drug.
[c] $t_{1/2}$, half-life. Values are calculated from the data of mean concentrations.

DISCUSSION

M. pneumoniae is a major causative organism of pneumonia, and the recommended therapeutic drug for mycoplasmal pneumonia is considered to be erythromycin. But Niitu et al. reported that *M. pneumoniae* strains resistant to erythromycin and other macrolide antibiotics were isolated after erythromycin therapy (11).

New quinolone antibacterial agents have broad and potent antibacterial activities and have been applied to various kinds of infections in humans. It has been reported that new quinolones are active against *M. pneumoniae* and that their MICs are comparable to those of tetracyclines but inferior to those of macrolides (8, 12, 17). Sparfloxacin is a new quinolone, which has been reported to show much superior activity against gram-positive bacteria (10). In the in vitro study, we confirmed that sparfloxacin demonstrated much more activity against clinical isolates of *M. pneumoniae* than levofloxacin and ofloxacin, although it was less active than erythromycin. Sparfloxacin seems to have the greatest antimycoplasmal potency among the new quinolones tested in this in vitro study.

In the in vivo study, all the drugs tested were able to decrease the numbers of viable *M. pneumoniae* cells in lungs, but sparfloxacin and erythromycin were more potent than levofloxacin and ofloxacin. Sparfloxacin was the most effective among the three new quinolones which were tested in vivo. The pharmacokinetic study demonstrated that sparfloxacin showed high levels in lungs, with long half-lives. Such pharmacokinetic properties and the high antimycoplasmal potency of sparfloxacin may be the reasons why sparfloxacin showed greater efficacy than the other drugs. Arai et al. have already reported on the effects of quinolones against experimental *M. pneumoniae* pneumonia in hamsters, but the doses used were 100 and 200 mg/kg/day, which are far higher than the clinical doses for humans (1), though they did not make any estimate for sparfloxacin. In the present study, we used doses of 10 and 30 mg/kg/day.

The peak concentrations of sparfloxacin in hamster sera after the administration of single oral doses of 15 mg/kg were almost the same as those in human sera after the administration of single oral doses of 200 mg (the usual clinical dose), and the half-lives of sparfloxacin in hamster sera were shorter than those in human sera (13). As the maximum concentration of sparfloxacin in lungs was higher than that in hamster serum, it is likely that this is the case in humans. These results suggest that sparfloxacin may be clinically useful for the treatment of *M. pneumoniae* infection. The excellent clinical efficacy of sparfloxacin against respiratory infection, including mycoplasmal pneumonia, has been reported for clinical studies carried out in Japan (19).

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