In Vitro and In Vivo Antichlamydial Activities of HSR-903, a New Fluoroquinolone Antibiotic

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The in vitro and in vivo antichlamydial activities of HSR-903 were investigated. The MICs of HSR-903 for different species of chlamydia were 0.016 to 0.063 $\mu\text{g/ml}$, which were superior to those of conventional fluoroquinolones. The therapeutic effect of HSR-903 in experimental mouse *Chlamydia psittaci* pneumonia was also excellent and almost equal to that of minocycline and superior to that of ofloxacin. These results indicate that HSR-903 may be useful in the treatment of respiratory infections caused by chlamydiae.

HSR-903 is a new oral fluoroquinolone antimicrobial agent developed by Hokuriku Seiyaku Co., Ltd. (Katsuyama, Japan), which has been reported to have broader and more potent activity against gram-positive and -negative bacteria than those of conventional quinolones, such as ofloxacin, ciprofloxacin, and sparfloxacin (8). In this study, the in vitro and in vivo activities of HSR-903 against chlamydiae were investigated and compared with those of other antibiotics.

In vitro activities (MICs) were measured by the standard methods of the Japan Society of Chemotherapy (3). 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 various chlamydia strains per well.

The IFU were counted by the following method. A serially diluted suspension containing chlamydiae was inoculated onto confluent monolayers of HeLa 229 cells. After incubation, 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. The standard strains of *Chlamydia* tested were *C. psittaci* Budgerigar (obtained from Japan National Institute of Health, Tokyo, Japan) and California 10 (obtained from A. Matsumoto, Department of Microbiology, Kawasaki Medical School), *C. trachomatis* D/UW-3/CX (obtained from Washington Research Foundation, Seattle), and *C. pneumoniae* TW-183 (obtained from Washington Research Foundation). The MICs for three strains of *C. psittaci*, three strains of *C. trachomatis*, and four strains of *C. pneumoniae* isolated from patients with chlamydial respiratory and urinary tract infections were also examined. The antimicrobial agents tested were HSR-903 (a gift from Hokuriku Seiyaku Co., Ltd.), ofloxacin, sparfloxacin, erythromycin, clarithromycin, and minocycline.

After inoculation, dilutions of the test compound were added to the culture medium (Eagle minimal essential medium supplemented with 10% fetal bovine serum, 1 μg of cycloheximide per ml, and antibiotics), and the plates were incubated at 37°C in 5% CO_2 for 48 h (*C. psittaci*), 37°C in 5% CO_2 for 72 h (*C. trachomatis*), and 35°C in 5% CO_2 for 72 h (*C. pneumoniae*) prior to staining of the inclusions.

Cultureset (Ortho Diagnostic Systems Co., Ltd., Tokyo, Ja-

pan), a genus-specific fluorescein isothiocyanate-conjugated monoclonal antibody to chlamydial lipopolysaccharide, was used to stain the inclusions.

The MICs of HSR-903 for standard strains and clinical isolates of three different species of chlamydia were 0.016 to 0.063 $\mu\text{g/ml}$. Clarithromycin and minocycline showed the strongest activity, with a MIC of 0.016 $\mu\text{g/ml}$. The MIC ranges of erythromycin, sparfloxacin, and ofloxacin were 0.25 to 0.5, 0.063 to 0.125, and 0.5 to 1.0 $\mu\text{g/ml}$, respectively. There were no significant differences in the MICs of each drug for the different chlamydial species. MICs of HSR-903 and other drugs for 10 clinical isolates are shown in Table 1.

The therapeutic effect of HSR-903 in mouse experimental chlamydial pneumonia was also evaluated. HeLa 229 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 ICR mice were infected with the cell solution (10^5 IFU per animal) by nasal instillation.

The animals were divided into nine groups (eight animals in each treatment group and five animals in the control group), and at 24 h after the infection, oral administration of antibacterial agents was started. These agents were HSR-903, sparfloxacin, ofloxacin, and minocycline. The three fluoroquinolones were suspended in 5% gum arabic and were administered at doses of 5 and 10 mg/kg of body weight twice daily for 5 days. Minocycline was also suspended in 5% gum arabic and was administered at a dose of 5 mg/kg twice daily for 5 days. Control mice received only 5% gum arabic. Animals were monitored every day for 14 days to determine the survival rates and compare the therapeutic effects of the drugs. Statistical analyses were done by the log rank test for comparison of the survival times and Fisher's exact test for the survival rates at day 14.

As shown in Fig. 1, the survival rates of mice treated daily with 10 and 20 mg of HSR-903 and minocycline per kg for 5 days were 100%, while all control animals and animals treated daily with 10 mg of ofloxacin per kg died within 7 and 8 days after the infection, respectively. The survival rate of the group treated daily with 20 mg of ofloxacin per kg was 12.5%, and 87.5% of animal treated with 10 and 20 mg of sparfloxacin per kg survived at day 14.

The survival rates or times of mice treated with HSR-903 were significantly higher or longer than those of ofloxacin-treated groups or controls ($P < 0.001$ for both tests). However,

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TABLE 1. MICs of HSR-903 and other antimicrobial agents for 10 clinical isolates of *Chlamydia* spp.

Organism and strain	MIC (μ g/ml) of:				
	HSR-903	Sparfloxacin	Ofloxacin	Erythromycin	Minocycline
<i>C. psittaci</i>					
OZAKI-1	0.031	0.031	0.5	0.25	0.016
OZAKI-2	0.031	0.063	0.5	0.25	0.016
IZAWA-1	0.031	0.063	0.5	0.5	0.016
<i>C. trachomatis</i>					
1043	0.031	0.063	1.0	0.5	0.016
1936	0.016	0.063	1.0	0.5	0.016
1921	0.031	0.063	0.5	0.25	0.016
<i>C. pneumoniae</i>					
KKpn-1	0.031	0.063	0.5	0.25	0.016
KKpn-2	0.031	0.063	1.0	0.5	0.016
KKpn-15	0.016	0.063	1.0	0.25	0.016
KKpn-16	0.031	0.063	0.5	0.25	0.016

no significant difference was observed between HSR-903-, sparfloxacin-, and minocycline-treated groups.

Chlamydial species are now well-known respiratory pathogens that cause upper and lower respiratory tract infections and pneumonia. *C. psittaci* is known as a causative organism of psittacosis, and *C. pneumoniae* is a newly recognized chlamydial species which has been shown to be an important pathogen of acute respiratory infections worldwide (1, 4).

Some of the recently developed antibiotics, such as new quinolones and new macrolides, showed excellent antichlamydial activities in vitro, in vivo, and also in clinical studies (2, 5-7, 9, 10). According to epidemiological studies of acute respiratory infections, good activity against chlamydiae is an important criterion to consider when choosing an oral antibiotic for the treatment of respiratory infections, since such infections are commonly caused by chlamydiae.

We previously reported the experimental and clinical effectiveness of sparfloxacin against acute chlamydial respiratory infections (5, 6, 10). An animal experiment using the same model of *C. psittaci* pneumonia in mice indicated that sparfloxacin was one promising agents in the treatment of chlamydial respiratory infections. Sparfloxacin showed an 88.9% efficacy rate in the treatment of 18 proven chlamydial infections in a clinical study. It was also effective in 66 nonchlamydial infections registered for the study, at an efficacy rate of 78.8%. Therefore, the overall success rate of sparfloxacin as the first-choice drug for acute respiratory infections was sufficiently high at 81.0%.

In our study, HSR-903 showed the strongest in vitro activity for three different chlamydial species, an activity which was equal or 2 times superior to that of sparfloxacin and 4 to 32 times superior to that of ofloxacin. And in the animal experi-

FIG. 1. Therapeutic effects of HSR-903 and related antibiotics against mouse *C. psittaci* pneumonia. *, $P < 0.001$ (both tests); 5 $\times$ 2 and 10 $\times$ 2, 5 and 10 mg/kg twice daily, respectively; N.S., not significant. Arrows indicate times of drug administration.

ment, animals treated with 10 mg of HSR-903 per kg per day had 100% survival while sparfloxacin showed some treatment failure even at a dosage of 20 mg/kg/day.

According to the above and former reports of potent and broad antibacterial activity of HSR-903, we can conclude that HSR-903 may be a useful oral agent for the acute treatment of respiratory tract infections.

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