ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1992, p. 188–190
0066-4804/92/010188-03$02.00/0

Vol. 36, No. 1

NOTES

In Vitro and In Vivo Activities of Sparfloxacin, Other Quinolones, and Tetracyclines against *Chlamydia trachomatis*

KATSUHISA NAKATA,* HIROSHI MAEDA, AKIRA FUJII, SOICHI ARAKAWA, KEIICHI UMEZU, AND SADAO KAMIDONO

Department of Urology, School of Medicine, Kobe University, Chuo-ku, Kobe 650, Japan

Received 24 June 1991/Accepted 26 October 1991

Sparfloxacin was more potent than other quinolones (tosufloxacin, lomefloxacin, ciprofloxacin, ofloxacin, fleroxacin, enoxacin, and norfloxacin) and as potent as minocycline and doxycycline in activity against *Chlamydia trachomatis* in vitro and in vivo. Sparfloxacin was more bactericidal than minocycline against *C. trachomatis* D/UW-3/Cx.

Chlamydia trachomatis is an important pathogen causing various urogenital and ophthalmic infections (14). Tetracyclines have been used frequently for chlamydial infections for many years, although they have some undesirable side effects. Recently, some new quinolones have been reported to have antichlamydial activity in vitro (1, 7, 9, 16), and some of them have successfully been applied to chlamydial infections in humans (2, 3). Sparfloxacin has been reported to have potent antichlamydial activity (10), but its position in antichlamydial chemotherapy remains uncertain because of the relatively few comparative data available. In the current study, we tested antichlamydial activities of sparfloxacin in vitro and in vivo in comparison with other quinolones and tetracyclines.

(Parts of this study were presented at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, 1989 [11].)

Sparfloxacin and enoxacin were provided by Dainippon Pharmaceutical Co., tosufloxacin was provided by Toyama Chemical Co., lomefloxacin was provided by Hokuriku Seiyaku Co., ciprofloxacin was provided by Bayer Yakuhin Co., ofloxacin was provided by Daiichi Seiyaku Co., norfloxacin and fleroxacin were provided by Kyorin Pharmaceutical Co., and minocycline and doxycycline were provided by Lederle Japan Co. All the drugs were dissolved in sterile distilled water with or without NaOH for in vitro studies and dissolved or suspended in 0.2% carboxymethyl cellulose for in vivo ones.

Twenty-five clinical strains were isolated from patients who attended our clinic. *C. trachomatis* D/UW-3/Cx was supplied by National Institute of Health, Tokyo, Japan. *C. trachomatis* KN (serovar E) was a clinical isolate from a patient suffering from nongonococal urethritis (11). The MIC was determined by the inhibition of inclusion body formation in McCoy cells, as described previously (7), except with 5% fetal bovine serum instead of 10%. The MBC was determined by inability to produce inclusion bodies in the drug-free medium. The McCoy cells cultured with drugs at or above their MICs were washed twice with the drug-free growth medium and suspended in 0.2 ml of the same medium. Fresh 24-h-old McCoy cell monolayers were inoculated with 0.1 ml of the cell suspensions, cultured in the drug-free test medium, fixed and stained. The MBC was defined as the lowest drug concentration at which no inclusion bodies were detected at all throughout five serial passages in the drug-free medium. In vivo activities of drugs were assessed in a urogenital infection model in leukopenic mice (11). Female 6- to 8-week-old Jcl-ICR mice (CLEA Japan Inc.) weighing 23 to 25 g were intraperitoneally injected with cyclophosphamide (Shionogi & Co.) at a dose of 300 mg/kg of body weight 48 h before chlamydial inoculation. Groups of five to six cyclophosphamide-induced leukopenic mice were intravenously anesthetized with sodium pentobarbital (Dainippon Pharmaceutical Co.) at a dose of 25 mg/kg, forced to void urine from the bladder by compressing gently the abdominal wall, and transurethrally inoculated into the bladder with 0.1 ml of a *C. trachomatis* KN suspension (2×10^5 inclusion-forming units per mouse) by using a tuberculin syringe with a blunt-end 23-gauge needle. The external urethral meatus of the mice was clamped with a clip for 5 h after inoculation, and they were refrained from drinking water for 24 h. Drugs were orally administered to them six times: 3, 8, 24, 30, 48, and 54 h after inoculation. On day 5 postinoculation, the mice were sacrificed under ether anesthesia. Their urinary bladders were exposed through a small suprapubic incision, opened, and rubbed with cotton swabs. The bladder mucous membrane in the swabs was suspended in 0.5 ml of a cold sucrose-phosphate-glutamate medium (sucrouse, 75 g; KH_2PO_4, 0.52 g; Na_2HPO_4, 1.22 g; L-glutamic acid, 0.72 g; and distilled water, 1,000 ml) containing gentamicin (10 μg/ml) and amphotericin B (2 μg/ml) and stored at −70°C until cultivation. Fresh 24-h-old McCoy cell monolayers were inoculated with 0.25 ml of the thawed membrane suspensions and cultured in the drug-free medium as described in the MBC determination. No finding of inclusion bodies in the primary culture was regarded as being protected from chlamydial infection. The 50% effective dose (ED_{50}) was calculated by probit analysis (8) and a 95% confidence limit by the method of Litchfield and Wilcoxon (6).

Susceptibility of 25 clinical isolates of *C. trachomatis* to quinolones and tetracyclines is shown in Table 1. The MICs for 90% of the strains tested (MIC_{90}s) of sparfloxacin,

* Corresponding author.

TABLE 1. In vitro antichlamydial activity of sparfloxacin, other quinolones, and tetracyclines against 25 clinically isolated strains of *C. trachomatis*

Drug	MIC (μg/ml)		
	Range	50%	90%
Sparfloxacin	0.025–0.05	0.05	0.05
Tosufloxacin	0.05–0.2	0.1	0.1
Lomefloxacin	1.56–6.25	3.13	6.25
Ciprofloxacin	0.78–3.13	1.56	1.56
Ofloxacin	0.78–1.56	0.78	1.56
Fleroxacin	3.13–6.25	3.13	6.25
Enoxacin	3.13–6.25	6.25	6.25
Norfloxacin	12.5–25	12.5	12.5
Minocycline	0.025–0.05	0.025	0.05
Doxycycline	0.025–0.05	0.025	0.05

minocycline, and doxycycline were 0.05 μg/ml each, and that of tosufloxacin was 0.1 μg/ml. The MIC_{90}s of ciprofloxacin and ofloxacin were both 1.56 μg/ml, about 1 order of magnitude higher than those of the above four drugs, and the MIC_{90}s of lomefloxacin, fleroxacin, enoxacin, and norfloxacin were 6.25 to 12.5 μg/ml, about 2 orders of magnitude higher than those of the four drugs. The MIC_{90}s of sparfloxacin, tosufloxacin, ciprofloxacin, and ofloxacin would be attained in plasma at their usual clinical doses (4, 18), but those of lomefloxacin, fleroxacin, enoxacin, and norfloxacin seemed to be above their usual plasma levels in humans (13, 15, 17, 18). No quinolone- or tetracycline-resistant strains were detected at all in the clinical isolates of *C. trachomatis* tested, showing that a drug-resistance problem has not occurred as yet in our clinical population. The MBCs of sparfloxacin, ofloxacin, and minocycline against *C. trachomatis* D/UW-3/Cx were 0.1, 1.56, and 0.2 μg/ml, respectively, or two, two, and eight times higher than their MICs. Nagayama et al. have reported that the MBCs of ofloxacin, ciprofloxacin, lomefloxacin, fleroxacin, and norfloxacin are only 1 or 2 times higher than their MICs but those of minocycline and doxycycline are 4 to 16 times higher than their MICs (9). Therefore, quinolones seem to inhibit the multiplication of chlamydial strains completely in a single growth cycle at concentrations relatively close to their MICs, while tetracyclines do so at concentrations about 1 order of magnitude higher than their MICs. This fact suggests an advantage of sparfloxacin over minocycline and doxycycline.

TABLE 2. Oral effects of sparfloxacin, other quinolones, and tetracyclines on the *C. trachomatis* KN infection in leukopenic mice

Drug	ED_{50} (range [mg/kg/dose])	MIC (μg/ml)
Sparfloxacin	3.4 (1.9–8.3)[a]	0.05
Tosufloxacin	13.6 (3.4–54)	0.1
Lomefloxacin	58.7 (18.4–187)	3.13
Ciprofloxacin	57.5 (17.9–185)	1.56
Ofloxacin	19.9 (10.8–37)	0.78
Fleroxacin	69.9 (21.2–230)	6.25
Enoxacin	95.9 (40.4–228)	6.25
Norfloxacin	>100	12.5
Minocycline	4.2 (1.2–15.0)	0.025
Doxycycline	3.7 (1.3–11.0)	0.025

[a] 95% confidence limit.

In vivo antichlamydial activities of quinolones and tetracyclines were examined in the *C. trachomatis* KN infection in leukopenic mice (Table 2). In unmedicated control mice infected with *C. trachomatis* KN, marked inflammatory reactions occurred with remarkable infiltration of neutrophils and membraneous exfoliation in the bladder membrane on days 2 to 9 after infection, and *C. trachomatis* was recovered from the bladder membrane at relatively high titers throughout the period (11). Administration of effective drugs to the mice results in the relatively less severe inflammatory responses and disappearance of *C. trachomatis*. The ED_{50} of sparfloxacin (3.4 mg/kg) was the lowest and was followed by that of doxycycline (3.7 mg/kg), minocycline (4.2 mg/kg), tosufloxacin (13.6 mg/kg), ofloxacin (19.9 mg/kg), ciprofloxacin (57.5 mg/kg), lomefloxacin (58.7 mg/kg), fleroxacin (69.9 mg/kg), enoxacin (95.9 mg/kg), and norfloxacin (>100 mg/kg), showing that sparfloxacin, doxycycline, and minocycline were the most effective in vivo and were followed by tosufloxacin and ofloxacin, but the other quinolones were relatively less effective or were ineffective.

Clinical studies with quinolones have disclosed that ofloxacin (2) and ciprofloxacin (3) are effective against chlamydial infections, although their effective daily doses (600 to 800 mg) are usually higher than those (200 mg) of minocycline (5) and doxycycline (12). On the other hand, it has been reported that sparfloxacin is effective on chlamydial infections at the same daily dose as the tetracyclines (16a), so the experimental results obtained here are consistent with the reported clinical results.

The present study indicates that sparfloxacin has the most potent antichlamydial activity in quinolones and is comparable to or better than tetracyclines in vitro and in vivo. Sparfloxacin may occupy an important position in antichlamydial chemotherapy, as have tetracyclines.

This study was supported by a grant from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan.

REFERENCES

1. **Azmar, M. J., M. C. Caballero, M. C. Lazano, C. de Miguel, J. C. Palomares, and E. J. Perea.** 1985. Activity of new quinolone derivatives against gential pathogens. Antimicrob. Agents Chemother. **27**:76–84.
2. **Biscoff, W.** 1986. Ofloxacin: therapeutic results in *Chlamydia trachomatis* urethritis. Infection **14**(Suppl. 4):S316–S317.
3. **Ignatius, W. F., W. Linton, M. Simbul, R. Thorup, B. Mclanghlin, V. Rohm, and P. A. Quinn.** 1987. Treatment of nongonococcal urethritis with ciprofloxacin. Am. J. Med. **82**(Suppl. 4A):311–316.
4. **Kanamaru, M., M. Nakashima, T. Uematsu, and Y. Takikuchi.** 1988. Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother, abstr. 1490.
5. **Kovacs, G. T., M. Westcott, J. Rusden, V. Asche, H. King, S. E. Haynes, E. K. Moore, and B. E. Hall.** 1989. A prospective single-blind trial of minocycline and doxycycline in the treatment of genital *Chlamydia trachomatis* infection in women. Med. J. Aust. **150**:483–485.
6. **Litchfield, J. T., and F. Wilcoxon.** 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. **96**:99–113.
7. **Maeda, H., A. Fujii, K. Nakata, S. Arakawa, and S. Kamidono.** 1988. In vitro activity of T-3262, NY-198, fleroxacin (AM-833; RO23-6240), and other new quinolone agents against clinically isolated *Chlamydia trachomatis* strains. Antimicrob. Agents Chemother. **32**:1080–1081.
8. **Miller, L. C., and M. L. Tainter.** 1944. Estimation of the ED_{50} and its error by means of logarithmic-probit graph. Proc. Soc. Exp. Biol. Med. **57**:261–264.
9. **Nagayama, A., T. Nakano, and H. Taen.** 1988. In vitro activities

of ofloxacin and four other new quinolone-carboxylic acids against *Chlamydia trachomatis*. Antimicrob. Agents Chemother. **32**:1735–1737.

10. **Nakamura, S., A. Minami, K. Nakata, N. Kurobe, K. Kouno, Y. Sakaguchi, S. Kashimoto, H. Yoshida, T. Kojima, T. Ohue, K. Fujimoto, M. Nakamura, M. Hashimoto, and M. Shimizu.** 1989. In vitro and in vivo antibacterial activities of AT-4140, a new broad-spectrum quinolone. Antimicrob. Agents Chemother. **33**: 1167–1173.
11. **Nakata, K., H. Maeda, A. Fujii, S. Arakawa, K. Umezu, and S. Kamidono.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr 1200.
12. **Noguera, X., M. Ferrer, E. Ortora, and L. Lopez-Marin.** 1986. Evaluation of doxycycline in the treatment of urethritis and cervicitis caused by *Chlamydia trachomatis*. Clin. Ther. **9**(Suppl. A):33–37.
13. **Ogawa, N., H. Uchida, S. Murayama, K. Hirai, Y. Oomori, Y. Abe, and T. Irikura.** 1981. Phase 1 study on AM-715. Chemotherapy (Tokyo) **29**(Suppl. 4):136–145.
14. **Schachter, J.** 1978. Chlamydial infections. N. Engl. J. Med. **298**:428–435, 490–495, 540–549.
15. **Siba, K., A. Saito, J. Shimada, S. Hori, M. Kaji, T. Miyahara, H. Kusajima, S. Kaneko, S. Saito, T. Ooie, and H. Ueda.** 1990. Renal handling of fleroxacin in rabbits, dogs, and humans. Antimicrob. Agents Chemother. **34**:58–64.
16. **Sloney, L., H. Chubb, A. Ronald, and R. Brunham.** 1990. In vitro activity of azithromycin, erythromycin, ciprofloxacin and norfloxacin against *Neisseria gonorrhoeae*, *Haemophilus ducreyi*, and *Chlamydia trachomatis*. J. Antimicrob. Chemother. **25**(Suppl. A):1–5.

16a. **Takagi, S., S. Arakawa, O. Matsumoto, and S. Kamidono.** 1990. Fundamental and clinical studies on sparfloxacin in the field of urology, abstr. 077. Abstr. 38th Gen. Meet. West Branch Jap. Soc. Chemother.

17. **Wise, R., Lockley, J. Dent, and M. Webberly.** 1984. Pharmacokinetics and tissue penetration of enoxacin. Antimicrob. Agents Chemother. **26**:17–19.
18. **Yamasaku, H., and Y. Suzuki.** 1988. A comparative study of the pharmacokinetics of ofloxacin, ciprofloxacin, NY-198 and T-3262 in the same volunteers. Chemotherapy (Tokyo) **36**(Suppl. 9):195–200.