IN VITRO AND *IN VIVO* ANTIBACTERIAL ACTIVITY OF FOUR NEWLY DEVELOPED QUINOLONE AGENTS AGAINST *LEGIONELLA* INFECTION

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We investigated the antibacterial activity of four newly developed quinolone agents, temafloxacin (TMFX, TA-167), sparfloxacin (SPFX, AT-4140), OPC-17116 and Y-26611 against *Legionella* was investigated both *in vitro* and *in vivo* and compared them with those of other quinolone agents and with erythromycin. *In vitro* strength of the antibacterial activity was found to be in the order of strongest to weakest, as follows: temafloxacin, ciprofloxacin, sparfloxacin, OPC-17116, Y-26611, ofloxacin, norfloxacin and erythromycin. The quinolones showed higher intracellular than extracellular concentration in human polymorphonuclear neutrophils. All quinolones and the erythromycin, at concentrations twice that of MIC, inhibited the growth of *Legionella pneumophila* in guinea pig peritoneal macrophages. In experimental *L. pneumophila* pneumonia in guinea pigs, the newly developed quinolone agents showed a significantly greater therapeutic effect than did the erythromycin.

Key words: Newly developed quinolone agents, *Legionella*, Intracellular killing activity, Experimental pneumonia.

Introduction

Legionella is a facultative intracellular bacterium that multiplies in macrophages[1~4] and may be a pathogenic factor in pneumonia[5]. Therefore *in vitro* susceptibility tests do not match the clinical therapeutic effect[6]. This is partly because of the necessity in the testing for both the high intracellular penetration of the agent and the killing activity against intracellular multiplying *Legionella*[7].

Sparfloxacin (AT-4140), temafloxacin (TA 167), OPC-17116 and Y-26611 are new synthetic oral antibacterial agents derived from fluoroquinolone. In this study, the antimicrobial activity of these agents against *Legionella* was examined with respect to the following: 1) the minimum inhibitory concentrations (MICs) against *Legionella* spp., 2) the intracellular concentration in human polymorphonuclear neutrophils, 3) the potency of these agents against intracellular *Legionella pneumophila* in guinea pig peritoneal macrophages and 4) the therapeutic efficacy of the agents against experimental *L. pneumophila* pneumonia in guinea pigs. The author then compared the total activity of the four newly developed quinolone agents with other quinolones and with erythromycin.

Materials and Methods

Antibacterial agents

Sparfloxacin (SPFX, AT-4140) was donated by Dainippon Pharmaceutical Co., Ltd. (Osaka), temafloxacin (TMFX, TA-167) by Tanabe Pharmaceutical Co., Ltd. (Osaka), OPC-17116 by Otsuka Pharmaceutical Co., Ltd. (Osaka), and Y-26611 by Yoshitomi Pharmaceutical Industries, Ltd. (Osaka)

In vitro susceptibility of the agents against *Legionella* species.

The MICs of the agents for the *Legionella* species were determined by the microdilution broth

Table 1. Strains of *Legionella* used

Source of strains	Number of strains
Standard reference strains	
Legionella pneumophila	
serogroups 1 to 13	14
Other *Legionella* spp.	27
Clinically isolated strains	
Legionella pneumophila	
serogroup 1	21
2	2
3	1
5	1
Legionella dumoffii	1
Legionella micdadei	1
Total	68 strains

method[8] using the MIC-2000 system (Dynatech Inc.). A total of 68 *Legionella* strains were tested. These are listed in detail in Table 1. This inculuded the 41 standard reference strains and the 27 clinical isolated strains, and the former included 14 strains of *L. pneumophila* and 27 strains of other *Legionella* species, and the latter included 21 strains of *L. pneumophila* serogroup 1, two strains of *L. pneumophila* serogroup 2, and one each of *L. pneumophila* serogroups 3, and 5, one of *Legionella dumoffii* serogroup 1 and one of *L. micdadei* serogroup 1.

The medium used was buffered yeast extract supplemented with α-ketoglutalate (BYEα) broth containing serial doubling dilutions of the agents, quinolones from a concentration of 1.0 to 0.00049 μg/ml and erythromycin from a concentration of 8.0 to 0.0039 μg/ml. The strains were cultured in the BYEα broth at 35°C overnight while shaking. The cultures were then adjusted with a phosphate buffered solution (pH: 6.8) to an organism concentration of 10^8 cfu/ml. The adjusted bacterial suspension was inoculated on a 96-well microplate using the MIC-2000 system inoculator. The inoculum size was 10^6 CFU of *Legionella* per well. Microplates were incubated at 35°C for 48 h.

The MIC was defined as the lowest concentration of the agent that inhibited development of visible growth in the wells.

Intracellular concentration in human polymorphonuclear neutrophils (PMN)

An normal saline solution containing 6% dextran (High Molecular, molecular weight: 208,000, Nacalai Tesque, Inc., Tokyo, Japan) and Ficoll-Paque (Pharmatica LKB Biotechnology Inc. New Jersey, U.S.A.) were used to purify the PMNs. Three 20-ml blood samples were collected from three healthy adult volunteers. Five milliliters of a 6% dextran solution was added to the blood and mixed well in a tube. The tube was placed vertically for 30 min to allow the erythrocytes to settle to the bottom. Then the supernatant fluid was gently collected. The fluid was layered on top of 5 ml of Ficoll-Paque which was placed in a plastic tube (siliconized, Corning, Iwaki glass Co., Ltd., Japan), then centrifuged at 450×g for 10 min at room temperature. The supernatant fluid was discarded. Then the sediment was used for the source of the PMN. To lyse contaminating erythrocytes, 5 ml of 0.2% NaCl solution was added to the sediment and mixed for 15 seconds, and then an equal volume of 1.6% NaCl was added to recover the suspension from the hypotonic condition. The collected cells contained more than 95% PMNs, a percentage that was determined by using Turk solution (Wako Pure Chemical Industries Ltd. Japan). Finally, the PMNs were suspended at a concentration of from 2-5×10^7 cells/ml in 10 ml of Hanks balanced salt solution (HBSS, Nissui Seiyaku Co., Ltd. Japan) supplemented with 0.4 g/l of sodium bicarbonate.

This PMN suspension was incubated for 30 min with each agent at the final concentration of 50 μg/ml. After incubation, the PMNs were separated from the extracellular solution using the silicone oil velocity gradient centrifugalization method[9]. The 0.5 ml of PMN suspension was layered over 0.5 ml of silicone oil (Toray Silicone Co., Ltd. Tokyo) in miniature centrifuge tubes (Bio Plastic Co., Ltd. Osaka). The tubes were centrifuged at 12,000×g for 3 min, causing the PMN to pass through the water-impermeable oil to the bottom of the tube. Thus the lower layer contained PMN and the upper layer contained the extracellular solution. The upper layer and PMNs were then collected separate-

ly and the PMNs were disrupted by hypotonic treatment using distilled water and filtrated with a membrane filter (pore size: 0.22 micrometer; Millex-GV Millipore corp., Ma. U.S.A.). These samples were immediately frozen at −70°C until the assay was per formed.

The intracellular and extracellular concentrations were measured by high-performance liquid chromatography (HPLC)[10,11]. HPLC conditions were as follows: pump, TRIROTAR-V; injector, Model VL-614; detector, UVIDEC-100-V (Japan Spectroscopic Co., Ltd. Japan); column, Nucleosil-5, C 18 (Sensyu Chemical Co. Ltd.); mobilphase, mixed acetonitrile with the 0.2% tetraethylammonium phosphate buffer (pH: 1.85) (15 : 85), flow rate, 1.5 ml/min; and detected ultra violet length, 293 nm.

Activity of the agents on *L. pneumophila* grown in guinea pig peritoneal macrophages.

Peritoneal effusive cells (PEC) were collected from guinea pig peritoneal space that has been washed with cooled normal saline as described by Kitsukawa[7] PEC were washed and resuspended in RPMI MEDIUM 1640 (RPMI, GIBCO Laboratories, Life Technologies, Inc., USA.), containing 5% fetal calf serum. Then the PEC suspension was put on a glass dish and incubated for 2 h at 37°C in a 5%CO_2 humidified condition. The adherent cells were then collected and suspended at a concentration of 2×10^6 cells/ml in RPMI containing 5% fetal calf serum. This cell suspension fluid was used for the macrophage source.

The strain of *Legionella* used in this study was *L. pneumophila* serogroup 1 (# 80-045), which was isolated from the lung tissue of the first case of Legionnaires' disease in Japan[12]. *L. pneumophila* was suspended at a concentration of $2\text{-}4\times10^6$ CFU/ml in RPMI containing 5% fetal calf serum.

Macrophage suspension was mixed with an equal volume of *L. pneumophila* serogroup 1 (# 80-045) suspension and incubated at 37°C on a rotary shaker for 16 h. After the incubation, extracellular organisms were washed out with fresh RPMI. The macrophage suspension was then incubated with twice the MIC of each agent.

At 0, 12, 24, and 36 h after mixed culture, extracellular organisms were washed out with fresh RPMI by centrifuge. Macrophages were suspended in 1 ml of fresh RPMI and disrupted by adding glass beads and shaken in a vortex mixer. The number of viable organisms was then counted on the buffered charcoal yeast extract supplemented with a α-ketoglutalate (BCYEα) agar plate.

Therapeutic effect of the agents on experimental *Legionella* pneumonia in guinea pigs.

The study was performed dividing two different periods. Male Hartley strain guinea pigs weighing 300—370 g were used. *L. pneumophila* serogroup 1 (# 80-045) was used as the challenge strain. The bacterial suspension was prepared at 8×10^7 CFU/ml in normal saline in the first study and 2.5×10^8 CFU/ml in the second. The method used to infect the animals are those described in Edelstein *et. al.*[13].

The animals were anesthetized with an intraperitoneal injection mixture of 80 mg/kg ketamine hydrochloride (Sankyo Pharmaceutical Inc. Japan) and 5 mg/kg of xylazine (Bayer AG Japan). For local anesthesia, a lidocaine hydrochloride (1% solution, Fujisawa Pharmaceutical Co., Ltd. Japan) was injected into neck subcutaneous tissue. The trachea was then surgically exposed with an aseptic technique, and 0.2 ml of bacterial suspension injected into trachea. After the injection, the animal was shaken by hand in an upright position for about 30 seconds in order to force all of the bacterial suspension into the lung. The incision was then closed with a steel clip.

The animals were observed and weighed twice daily for 14 days. Treatment with antimicrobial agents was started at 24 h after inoculation. The quinolone agents were suspended at a concentration of 5 mg/ml in a 0.5% tragacanth gum solution and the erythromycin was suspended at a concentration of 10 mg/ml in a 0.5% tracaganth gum solution, which was administrated through an orogastric tube twice a day for 7 days. The daily dosage of the six quinolones was 10 mg/kg (1.0 ml/kg) and that of the erythromycin was 20 mg/kg (1.0 ml/kg).

The therapeutic effect of each antimicrobial agent was followed for 14 days and evaluated by survival rate and mean survival dates after inocula-

Table 2. Antibacterial activity of the eight agents against 41 standard strains of *Legionella* species

Name	Sero-group	ATCC	MICs (μg/ml)							
			TMFX	SPFX	OPC-17116	Y-26611	OFLX	CPFX	NFLX	EM
L. pneumophila	1	33152	0.0078	0.0039	0.0078	0.0625	0.0625	0.0312	0.25	0.25
L. pneumophila	1	33153	0.0078	0.0039	0.0078	0.0156	0.0625	0.0312	0.125	0.125
L. pneumophila	2	33154	0.0039	0.0039	0.0078	0.0312	0.0312	0.0312	0.25	0.25
L. pneumophila	3	33155	0.0039	0.0039	0.0078	0.0312	0.0312	0.0156	0.125	0.25
L. pneumophila	4	33156	0.0156	0.0312	0.0312	0.0625	0.0625	0.0312	0.25	0.25
L. pneumophila	5	33216	0.0312	0.0625	0.0625	0.0312	0.0625	0.0312	0.125	0.25
L. pneumophila	6	33215	0.0078	0.0039	0.0156	0.0312	0.0625	0.0625	0.25	0.5
L. pneumophila	7	33823	0.0039	0.0039	0.0078	0.0312	0.0625	0.0312	0.125	0.25
L. pneumophila	8	35096	0.0039	0.0039	0.0078	0.0312	0.0625	0.0312	0.125	0.0625
L. pneumophila	9	35289	0.0039	0.0039	0.0078	0.0156	0.0312	0.0078	0.0312	1.0
L. pneumophila	10	43283	0.0078	0.0078	0.0156	0.0625	0.0625	0.0312	0.25	0.125
L. pneumophila	11	43130	0.0039	0.0039	0.0078	0.0625	0.0625	0.0312	0.25	0.25
L. pneumophila	12	43290	0.0078	0.0078	0.0156	0.0625	0.125	0.0625	0.5	0.125
L. pneumophila	13	43736	0.0078	0.0039	0.0156	0.125	0.0625	0.0312	0.25	0.125
L. bozemanii	1	33217	0.0156	0.0312	0.0312	0.0312	0.0312	0.0156	0.0625	0.5
L. bozemanii	2	35453	0.0078	0.0625	0.0625	0.125	0.0625	0.0312	0.125	0.25
L. dumoffii	1	33279	0.0312	0.0625	0.0625	0.0625	0.0625	0.0156	0.0625	0.25
L. gormanii	1	33297	0.0156	0.0312	0.0625	0.0625	0.0625	0.0156	0.0625	0.25
L. micdadei	1	33218	0.0078	0.0019	0.0039	0.0156	0.0312	0.0078	0.0625	1.0
L. longbeachae	1	33469	0.0156	0.0312	0.0625	0.0625	0.0625	0.0312	0.0312	0.125
L. longbeachae	2	33484	0.0078	0.0039	0.0156	0.0312	0.0312	0.0156	0.125	0.5
L. jordanis	1	33623	0.0312	0.0156	0.0312	0.0312	0.125	0.0156	0.0625	1.0
L. oakridgensis	1	33761	0.0039	0.0078	0.0156	0.0312	0.0625	0.0156	0.25	0.5
L. wadsworthii	1	33877	0.0156	0.0312	0.0312	0.0078	0.0312	0.0156	0.0625	0.5
L. feeleii	1	35072	0.0078	0.0039	0.0078	0.0156	0.0625	0.0156	0.0625	0.5
L. feeleii	2	35849	0.0078	0.0039	0.0078	0.0625	0.125	0.0312	0.25	0.5
L. sainthelensis	1	35248	0.0312	0.0312	0.0312	0.25	0.0625	0.0625	0.25	0.125
L. anisa	1	35292	0.0312	0.0312	0.125	0.0312	0.0625	0.0312	0.0625	0.5
L. santicrucis	1	35301	0.0078	0.0078	0.0156	0.0625	0.0312	0.0078	0.25	0.25
L. steigerwaltii	1	35302	0.0312	0.0625	0.125	0.0625	0.0625	0.0156	0.0625	0.25
L. parisiensis	1	35299	0.0156	0.0156	0.0312	0.0312	0.0625	0.0156	0.0625	0.5
L. spiritensis	1	35249	0.0078	0.0039	0.0078	0.0312	0.0312	0.0078	0.0625	1.0
L. hackeliae	1	35250	0.0625	0.0625	0.125	0.5	0.125	0.0625	0.5	0.5
L. hackeliae	2	35999	0.0625	0.0625	0.125	0.25	0.125	0.0625	0.25	2.0
L. maceachernii	1	35300	0.0039	0.0019	0.0039	0.0078	0.0312	0.0078	0.0625	0.5
L. jamestowniensis	1	35298	0.0078	0.0078	0.0156	0.0312	0.0625	0.0156	0.0625	1.0
L. cherrii	1	35252	0.0156	0.0312	0.0312	0.0312	0.0625	0.0156	0.0625	0.25
L. rubrilucens	1	35304	0.0078	0.0078	0.0156	0.0312	0.0312	0.0078	0.0625	2.0
L. erythra	1	35303	0.0078	0.0078	0.0156	0.0156	0.0312	0.0078	0.0312	1.0
L. israelensis	1	43119	0.0078	0.0078	0.0156	0.0312	0.0312	0.0078	0.25	0.125
L. birminghamesis	1	43702	0.0078	0.0039	0.0078	0.0312	0.0625	0.0625	0.0625	0.5

TMFX, temafloxacin; SPFX, sparfloxacin; OFLX, ofloxacin; CPFX, ciprofloxacin; NFLX, norfloxacin; EM, erythromycin.

tion. Dead animals were autopsied, and pathological and bacteriological examinations were then performed.

Statistical analysis.

Results are expressed as the mean ± standard deviation. The statical comparisons of the mean was made by t-test. Mean survival dates were calculated by the Kaplan-Meir method. Statistical comparisons of survival rate were made by the Cox -Mantel test.

Results

***In vitro* susceptibility of *Legionella* species.**

Table 2 shows the MICs of 41 standard reference strains. The MIC of temafloxacin ranged from 0.0039 to 0.0625 μg/ml, OPC-17116 from 0.0039 to 0.125 μg/ml, sparfloxacin from 0.0019 to 0.625 μg/ml, Y-26611 from 0.0078 to 0.5 μg/ml, ofloxacin from 0.0313 to 0.125 μg/ml, ciprofloxacin from 0.0078 to 0.625 μg/ml, norfloxacin from 0.0312 to 0.5 μg/ml and erythromycin from 0.0625 to 2.0 μg/ml.

Table 3 shows MIC_{50} and MIC_{90} and the range of MICs of clinical isolates. The MIC of temafloxacin ranged from 0.0039 to 0.0312 μg/ml, OPC-17116 from 0.0078 to 0.0625 μg/ml, sparfloxacin from 0.00195 to 0.0625 μg/ml, Y-26611 from 0.0156 to 0.125 μg/ml, ofloxacin from 0.0312 to 0.0625 μg/ml, ciprofloxacin from 0.0039 to 0.0625 μg/ml, norfloxacin from 0.0312 to 0.5 μg/ml and erythromycin from 0.125 to 1.0 μg/ml.

Table 3. MIC of the 8 agents against 27 clinically isolated strains of *Legionella*

Agents	MIC (μg/ml)		
	Range	50%	90%
TMFX	0.0039 −0.0312	0.0078	0.0078
OPC*	0.0078 −0.0625	0.0078	0.0312
SPFX	0.00195−0.0625	0.0039	0.0312
Y**	0.0156 −0.125	0.0625	0.0625
OFLX	0.0312 −0.0625	0.0625	0.0625
CPFX	0.0039 −0.0625	0.0312	0.0625
NFLX	0.0312 −0.5	0.25	0.25
EM	0.125 −1.0	0.125	0.5

*OPC-17116, **Y-26611

TMFX, temafloxacin; SPFX, sparfloxacin; OFLX, ofloxacin; CPFX, ciprofloxacin; NFLX, norfloxacin; EM, erythromycin.

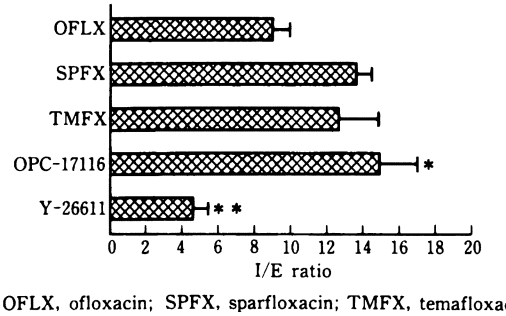
The MIC of temafloxacin against *L. pneumophila* serogroup 1 (clinicaly isolated #80-045) was 0.0078 μg/ml, that of sparfloxacin was 0.0078 μg/ml, OPC-17116 was 0.0156 μg/ml, Y-26611 was 0.625 μg/ml, ofloxacin was 0.0625 μg/ml, ciprofloxacin was 0.0313 μg/ml norfloxacin was 0.25 μg/ml and erythromycin was 0.125 μg/ml.

Intracellular concentration in the human PMNs.

The intracellular to extracellular concentration ratio (I/E ratio) of the agents in shown in Fig. 1. From highest to lowest the I/E ratios were as follows: OPC -17116, 14.99 ± 0.72; sparfloxacin, 13.70 ± 2.20; temafloxacin, 12.69 ± 2.06; ofloxacin, 9.07 ± 0.76; Y-26611; 4.68 ± 0.10. OPC -17116 showed the highest I/E ratio which was significantly higher than that of ofloxacin ($p<0.005$, t-test), and Y-26611 showed a significantly lower ratio than that of ofloxacin ($p<0.005$, t-test).

Antimicrobial activity of the agents on *L. pneumophila* grown in guinea pigs' peritoneal macrophages.

Figs. 2 and 3 show the chronological changes of intracellular viable organisms incubated at twice the MIC of each agent. *L. pneumophila* did not multiply in guinea pig peritoneal macrophages incubated with each agent. With the passage of incubation time, the numbers of intracellular viable



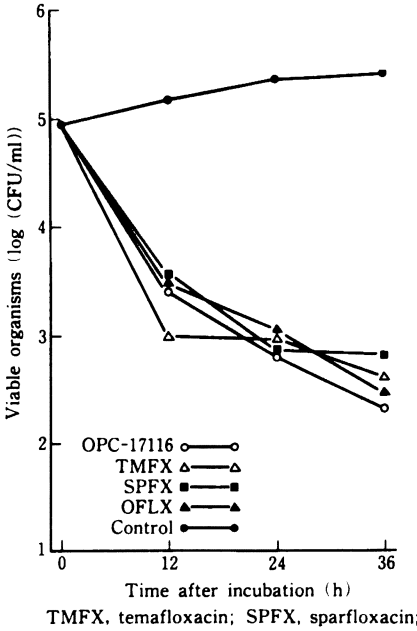
OFLX, ofloxacin; SPFX, sparfloxacin; TMFX, temafloxacin.

Fig. 1. The intracellular to extracellular concentration ratio (I/E ratio) of the agents. (*significantliy higher than ofloxacin, $p<0.005$ with t-test, **significantly lower than ofloxacin, $p<0.005$ with t-test)

organisms decreased, but there was no significant differences for the agents.

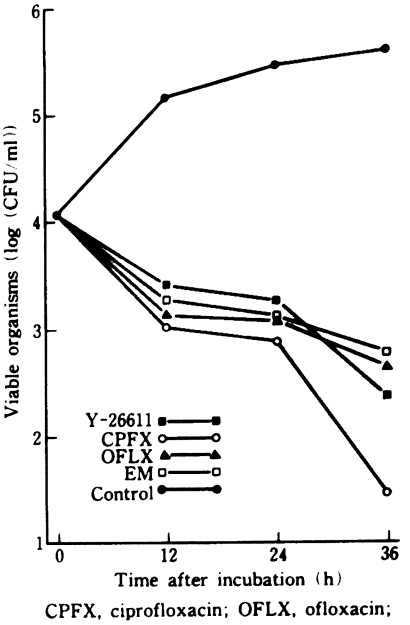
Therapeutic effect of the agents on experimental *Legionella* pneumonia in guinea pigs.

The results of the first experiment are shows in Fig. 4. The survival rates up to 14 days after infection were 90%, 80%, 70%, 10%, and 0% respectively in the groups treated with temafloxacin,



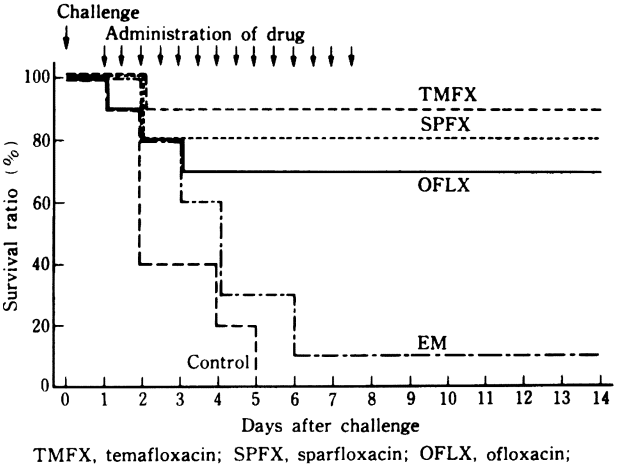
TMFX, temafloxacin; SPFX, sparfloxacin; OFLX, ofloxacin.

Fig. 2. Effect of the agents on *L. pneumophila* grown in guinea pig peritoneal macrophages.



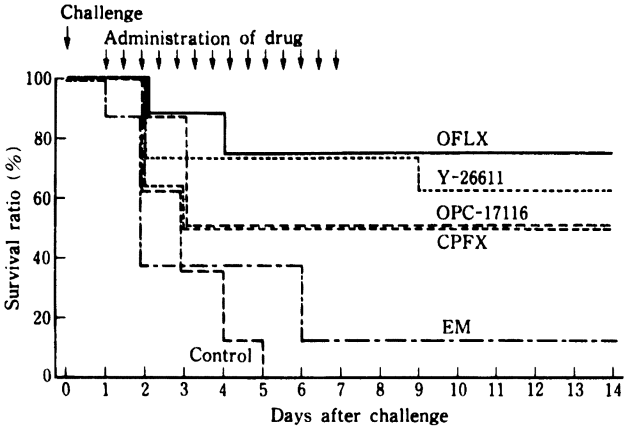
CPFX, ciprofloxacin; OFLX, ofloxacin; EM, erythromycin.

Fig. 3. Effect of the agents on *L. pneumophila* grown in guinea pig peritoneal macrophages.



TMFX, temafloxacin; SPFX, sparfloxacin; OFLX, ofloxacin; EM, erythromycin.

Fig. 4. The therapeutic efficacy of the agents on experimental *L. pneumophila* pneumonia in guinea pigs. (n=10, each group)



OFLX, ofloxacin; CPFX, ciprofloxacin; EM, erythromycin.

Fig. 5. The therapeutic efficacy of the agents on experimental *L. pneumophila* pneumonia in guinea pigs. (n=8, each group)

sparfloxacin, ofloxacin, erythromycin, and without treatment. The mean survival time was 12.9 ± 3.6 days for the temafloxacin-treated group, 11.6 ± 5.0 days for the sparfloxacin group, 10.5 ± 5.4 days for the ofloxacin group, 4.8 ± 3.5 days for the erythromycin group and 2.9 ± 1.4 days without treatment. The control group and erythromycin treated group differed significantly in survival rate ($p<0.05$, Cox-Mantel test) from the quinolone-treated group.

The results of the second experiment are shows in Fig. 5. The survival rates up to 14 days after infection were 62.5%, 50%, 75.0%, 50%, 12.5% and 0% respectively in the groups treated with Y-26611, OPC-17116, ofloxacin, ciprofloxacin erythromycin, and without treatment. The mean survival time was 10.4 ± 5.1 days for the Y-26611 treated group, 8.4 ± 5.7 days for the OPC-17116 group, 11.3 ± 4.8 days for the ofloxacin group, 8.1 ± 5.9 days for the ciprofloxacin group, 4.4 ± 4.1 days for the erythromycin group and 3.1 ± 1.1 days for the group without treatment. The control group and erythromycin-treated group differed significantly in survival rate ($p<0.05$ Cox-Mantel test) from the quinolone treated group.

Discussion

The four newly developed oral quinolone agents, sparfloxacin, temafloxacin, OPC-17116 and Y-26611, showed excellent antibacterial activity against *Legionella in vitro* and *in vivo*.

Thier MICs against *Legionella* species were better than that of either norfloxacin or erythromycin.

The four agents penetrated well in human PMN, the intracellular concentration being 4 to 13 times higher than the extracellular concentration. When these four agents were compared with ofloxacin, sparfloxacin, temafloxacin and OPC-17116 showed higher I/E ratios than ofloxacin but Y-26611 showed a lower ratio. The I/E ratio of ciprofloxacin has been reported as 3.49[11] and that of erythromycin as 6.01[14]. The I/E ratio of other antimicrobial agents have been also reported. The pipercilin ratio was less than 0.01, ceftizoxime was 0.09, ceftazizime was 0.56, rifampicin was 8.23[11], roxithromycin was 19.81[14] and gentamicin was 0.73[9]. When the I/E ratio of these agents was compared with that of the quinolone agents, the penicillines, the cephems and the aminoglicocides were found to be much lower than quinolones, but the ratio of rifampicin and macrolides was equal or higher to that of the quinolones.

The results of in this study showed that all agents tested were effective against intracellular *Legionella*. The intracellular killing activity of other agents has been reported by Kitsukawa *et. al.*[7]. In their report, rifampicin and roxithromycin shown a good

intracellular killing effect against *L. pneumophila* but less killing effect was shown for piperacillin, ceftizoxime, ceftazizime and gentamycin.

When comparing those resuts of the I/E ratios with the intracellular killing effects of the agents, a relationship can been seen between the I/E ratio and the intracellular killing effect.

The four newly developed quinolone agents exhibited significantly greater *in vivo* activity against *Legionella* pneumonia in guinea pigs than did erythromycin.

When OPC-17116 and ciprofloxacin were compared with ofloxacin, there was a discrepancy between *in vitro* studies (MIC, I/E ratio and intracellular killing activity) and the therapeutic effect of experimental pneumonia. In the case of ciprofloxacin, the cause is suspected to be due to a lower serum and tissue concentration in the guinea pig. The serum and lung concentration of ciprofloxacin in guinea pigs has been reported[15]. When ciprofloxacin was administrated intraperitonealy in a dosage of 30 mg/kg, the serum or lung tissue concentration at 30 min was 0.2 μg/ml or 0.3 μg/g and at 90 min, 2.7 μg/ml or 2.7 μg/g. When ofloxacin was administrated in one oral dosage of 20 mg/kg, the serum and lung tissue concentration was as follows: at 30 min, 15 μg/ml and 15 μg/g; at 1 h 17 μg/ml and 20 μg/g; at 2 h, 12 μg/ml and 15 μg/g; at 4 h 3 μg/ml and 2 μg/ml[16]. The data on OPC-17116 with one oral dosage of 40 mg/kg, the plasma and lung tissue concentrations of OPC-17116 were as follows: 1.68 μg/ml and 27.07 μg/g at 30 minutes after; 2.20 μg/ml and 48.10 μg/g at 1 h; 1.15 μg/ml and 31.36 μg/g at 2 h and 0.32 μg/ml and 10.94 μg/g at 4 h. (Unpublished data, results received from Tokushima research institute of Otsuka pharmaceutical Co., Ltd. by personal communication.) So the cause of the discrepancy is thought to be a lower plasma concentration and high metabolic speed or some other factor specific to guinea pigs.

But as these two agents have excellent therapeutic activity compared with erythromycin, they still have an acceptable potency level for experimental *Legionell pneumophila* pneumonia in guinea pigs.

Previous studies of quinolone agents have shown them to be active *in vitro* and in animal models of *L. pneumophila* infection[18~17].

However, number of clinical cases of legionellosis that have been treated with quinolone agents was not enough for evaluating these drugs; some cases were successfully treated with pefloxacin but some treated with ciprofloxacin were unsuccessful[18,19].

As the results of this study clearly show that the four newly developed quinolone agents have excellent antibacterial activity against *Legionella* both *in vitro* and *in vivo*, they seem to have a definite potential as useful clinical therapeutic drugs for *Legionella* infection in humans.

But for clinical use, we must also be consider the data concerning pharmacokinetics and the side effects. The author suggest that following additional data of side effecst and that of pharmacological obtaining in humans, the next step would be to carry out in clinical trials.

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新しく開発されたキノロン4剤のレジオネラ感染症に対する *in vitro* および *in vivo* 抗菌力に関する検討

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新しく開発されたキノロン剤であるtemafloxacin (TMFX, TA-167), sparfloxacin (SPFX, AT-4140), OPC-17116 および Y-26611 の4薬剤の *Legionella* 感染症に対する抗菌力を *in vitro* および *in vivo* において他のニューキノロン剤を含む対照薬剤と比較検討した。1) *Legionella* 標準株41株および臨床分離株27株に対する上記4薬剤および ofloxacin, norfloxacin, ciprofloxacin, erythromycin の計8薬剤の MIC を求めた。*Legionella* 属に対する MIC は, temafloxacin≧ciprofloxacin≧sparfloxacin＞OPC-71776＞Y-26611≧ofloxacin＞norfloxacin＞erythromycin の順であった。2) *Legionella* 感染症の治療の際に重要となる抗菌剤の細胞内移行について検討し, 4薬剤はヒト好中球において細胞内濃度が細胞外濃度より高い値を示し良好な細胞球内移行を示した。3) モルモット腹腔マクロファージ内に貪食された *Legionella pneumophila* serogroup 1 に対し各薬剤とも2 MIC の濃度において増殖抑制作用を示した。4) *L. pneumophila* serogroup 1 (#80-045: 臨床分離株) の気管内投与によるモルモットの実験肺炎モデルに対する経口投与による治療効果は4薬剤とも erythromycin 投与群との比較で生存率において有意差をもって良好な治療成績を示した。以上の検討の結果より, いずれの薬剤とも各項目において優れた結果を示し上記4薬剤は *Legionella* 感染症に対して有効であるものと考えられた。

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