THERAPEUTIC EFFICACY OF NEW QUINOLONES, OFLOXACIN AND CIPROFLOXACIN, AGAINST *MYCOBACTERIUM KANSASII* INDUCED INFECTION IN MICE

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We examined the therapeutic efficacy of the new quinolones, ofloxacin (OFLX) and ciprofloxacin (CPFX), against *Mycobacterium kansasii* induced infection in mice, on the basis of incidence of gross lesions and changes in colony forming units (CFU) of organisms in the visceral organs. The two quinolones exhibited significant therapeutic activity against *M. kansasii* infection, when given p.o. or i.p. to mice, in a dose of 2 mg/mouse, once daily, 6 times per week. The efficacy of OFLX was dose-dependent and higher than that of CPFX. Rifampicin (RFP), as a control drug, given by gavage in a dose of 0.5 mg/mouse, exhibited significant efficacy against the infection. The two quinolones showed marked microbicidal activity against *M. kansasii* ingested by host peritoneal macrophages (Mϕs) when added at concentration of 10−20 μg/ml to an *in vitro* culture system, while RFP exhibited significant bactericidal activity even at 2 μg/ml, at which concentration the quinolones showed only a bacteriostatic effect.

Key words : New quinolones, ofloxacin, ciprofloxacin, *Mycobacterium kansasii*

Introduction

New quinolones, such as ofloxacin (OFLX)[1] and ciprofloxacin (CPFX)[2] have potent *in vitro* antimycobacterial activity, particularly against *Mycobacterium tuberculosis, M. bovis, M. xenopi, M. marinum, M. gordonae*, and *M. fortuitum*. The MIC_{90} values of the quinolones against these mycobacterial species usually range from 1—2 μg/ml[3~10] In our study[7], the MIC_{90} of the two agents against *M. kansasii* was also estimated to be 1.6 μg /ml, though other investigators have reported 2—4 μg/ml as the MIC_{90} value. These quinolones are also considerably efficacious against mycobacterial infections such as *M. tuberculosis*[11,12] and *M. fortuitum*[7,8,13] in humans and in experimental animals, even though their efficacy against tuberculosis in clinical cases is lower than that of rifampicin and in some patients the agents fail to cause negative conversion in culture[12] We have recently found that OFLX has appreciable antileprosy activity[14], and this has been confirmed by other investigators[15,16]. We now report the therapeutic efficacy of OFLX and CPFX against *M. kansasii* induced infection in mice.

Materials and Methods

Organism. *Mycobacterium kansasii* Umeda strain, originally isolated from a patient and maintained on Ogawa's egg medium[17], was cultured in Dubos Tween®-albumin (Eiken Chemical Co., Tokyo, Japan) or 7H9 medium (Difco Laboratories, Detroit, Mich. USA) at 37℃ until the optical density (540 nm) reached about 0.2, when it was used for experimental infection and macrophage (Mϕ) microbicidal testing.

Mice. Six-week old female mice of the ddY or BALB/c strain purchased from Japan SLC Co., Shizuoka, Japan, were used to study events related to experimental infection induced by *M. kansasii*. Eight to 12-week old female mice of BALB/c (SLC Co.) or CBA/JN obtained from Japan Charles River

Co., Kanagawa, Japan, were used to measure macrophage (Mϕ)-microbicidal activity.

Antimicrobial agents. OFLX and rifampicin (RFP) were obtained from Daiichi Pharmaceutical Co., Tokyo, and CPFX was provided by Bayer Pharmaceutical Co., Osaka, Japan.

In vitro antimicrobial activity. The MIC values of antimicrobial agents against various mycobacteria were measured by agar dilution method using 7H10 agar plates, as previously described[15].

Experimental infection and chemotherapy. Female mice of ddY or BALB/c strain were infected i.v. with *M. kansasii* Umeda grown in Dubos Tween-albumin or 7H9 medium. The test drugs, suspended in distilled water in a 0.1 ml volume, were given intraperitoneally (i.p.) or by gavage, once daily, 6 times per week, from day 1 for up to 8 weeks after the bacterial challenge. At various intervals, the animals were killed and observed for incidence of gross lesions in the visceral organs. The number of CFU in the organs was counted on 7H10 or 7H11 agar plates (Difco).

Antimicrobial activity of drugs against organisms phagocytosed by Mϕs. To prepare the Mϕ monolayer culture, resident peritoneal cells or zymosan A (injected i.p. at 1 mg/mouse 4 days before harvest)-induced peritoneal exudate cells suspended in RPMI 1640 medium supplemented with 10 % fetal bovine serum (FBS) (MAB Bioproduct Co., Walkersville, Md., U.S.A.) were cultured on 23- or 34 mm culture wells (Corning Glass works, Corning, New York, U.S.A.) for 2—3 h at 37 ℃ in a CO_2 incubator (5 % CO_2: 95 % humidified air), then the wells were vigorously vibrated for 30 seconds followed by a thorough rinsing with Hanks' balanced salt solution (HBSS) (Nissui Pharmaceutical Co., Tokyo). The resultant Mϕ monolayer culture was incubated in a medium containing 4×10^6 or 1×10^5/ml of *M. kansasii* for 1 or 2 h. The Mϕ monolayer was thoroughly washed with HBSS and incubated in the FBS-RPMI 1640 medium at 37℃ in a CO_2 incubator for up to 6 days. At intervals, the Mϕ monolayer was rinsed with HBSS and subjected to CFU-counting of organisms in Mϕ cells on 7H11 agar plates after distilled water-treatment and sonication to lyse Mϕs. In some experiments, the number of acid-fast bacilli in Mϕ cells was counted microscopically.

Results

In vitro antimycobacterial activity. As shown in Table 1, the *in vitro* anti-*M. kansasii* activity of the two quinolones, OFLX and CPFX, was similar to that of RFP, although the quinolones were less active against the *M. avium - intracellulare -*

Table 1. *In vitro* antimycobacterial activity of ofloxacin, ciprofloxacin and rifampicin

Species	Number of strains	MIC_{90} (μg/ml)		
		OFLX	CPFX	RFP
M. tuberculosis	25	3.13	1.6	100[a]
M. kansasii	19	6.25	3.13	3.13
M. marinum	10	25	12.5	0.4
M. avium complex	64[b]	100	50	50
M. scrofulaceum	19	50	25	6.25
M. fortuitum	20	3.13	0.8	100
M. chelonae subsp. *abscessus*	15[c]	>100	100	>100
M. chelonae subsp. *chelonae*	20	50	12.5	>100

[a] MICs for majority of test strains (19 strains) were lower than 0.2 μg/ml.
[b] For rifampicin, 52 strains were tested.
[c] For rifampicin, 20 strains were tested.
[d] Abbreviations used are: OFLX, ofloxacin; CPFX, ciprofloxacin; RFP, rifampicin.

scrofulaceum complex and *M. marinum* than was RFP. Although a large part of *M. tuberculosis* strains (19 of 25 strains) tested were more susceptible to RFP than to the quinolones, the remaining 6 strains were highly resistant to RFP. In the case of the quinolones, there was no *M. tuberculosis* strain showing such a high resistance. The MIC values of the two quinolones and RFP against *M. kansasii* strain Umeda were as follows: 1.56, 1.56 and 0.78 μg/ml (in 7H10 agar); 3.13, 1.56 and 0.78 μg/ml (in 7H11 agar) for OFLX, CPFX, and RFP, respectively.

Therapeutic activity. The two quinolones were compared for *in vitro* activity against *M. kansasii* infection. When the agents were given to mice infected with organisms by gavage in a dose of 2 mg/mouse, once daily, six times per week, from day 1 for up to 8 weeks after infection, OFLX produced a considerable decrease in the severity of gross lesions in the lungs but in the kidneys (Table 2). CPFX, however, did not have this effect. There was no significant difference in the organ weights of the infected animals, given or not given either quinolone (data not shown). Table 3 shows the efficacy of OFLX and CPFX given by gavage against murine experimental *M. kansasii* infection, on the basis of CFU recovered from visceral organs. OFLX exhibited significant therapeutic activity. CPFX had no such significant efficacy, but it did enhance the elimination of organisms from the spleen (8 weeks) in a manner comparable to that seen with OFLX.

Fig. 1 shows the therapeutic activity of OFLX given i.p. against *M. kansasii* infection. In this case, OFLX exhibited considerably higher therapeutic efficacy than that seen with oral administration (Table 3). Moreover, it was found that OFLX showed nearly dose-dependent efficacy.

RFP, a representative antituberculous drug, significantly enhanced the elimination of organisms from visceral organs when given to mice by gavage, using the protocol described in Table 2, even at a dose of 0.5 mg/mouse. On the other hand, OFLX given in the same dose had no significant therapeutic efficacy. The values of Log (CFU/organ) for control and RFP-treated mice at week 8 after infection (challenge dose; 1.6×10^6/mouse) were as follows: control mice ($n=8$): 6.44 ± 0.10, 7.49 ± 0.10, 5.92 ± 0.13 and 7.36 ± 0.08 for lungs, liver, kidneys and spleen, respectively; RFP-treated mice ($n=8$): 1.68 ± 0.26, 5.22 ± 0.07, 0.52 ± 0.34 and 3.74 ± 0.14 for lungs, liver, kidneys and spleen, respectively ($P<0.005$ in all cases; Student's t-test).

Pharmacokinetics. Serum concentrations of OFLX, CPFX and RFP in mice administered orally

Table 2. Effect of ofloxacin and ciprofloxacin treatments on incidence of gross lesions in the lungs and kidneys of infected mice[a]

Administration	No. of mice	Gross lesions							
		Lungs[b]				Kidneys[c]			
		0	1+	2+	3+	0	1+	2+	3+
None	6	0	2	1	3	0	6	0	0
OFLX	6	0	4	2	0	0	6	0	0
CPFX	6	0	2	1	3	0	6	0	0

[a] Female ddY mice were infected i.v. with 1.4×10^6 of *M. kansasii* Umeda and given 0.2 ml of the indicated drug suspended in distilled water, in a dose of 2 mg/mouse, as described in the text.

[b] The degree of gross lesions was scored by the following criteria: 0, no lesion; 1+, a few small lesions (<20); 2+, many small lesions (≧20); 3+, many small lesions with some large lesions (φ≧2 mm).

[c] The degree of gross lesions was scored by the following criteria: 0, no lesion; 1+, lesions covering <25% surface; 2+, confluent lesions covering 25~50% surface; 3+, confluent lesions covering ≧50% surface.

[d] Abbreviations used are: OFLX, ofloxacin; CPFX, ciprofloxacin.

Table 3. Therapeutic efficacy of ofloxacin and ciprofloxacin against *M. kansasii* infection in mice[a]

Treatment with	Time after infection	Log CFU / organ			
		Lungs	Liver	Spleen	Kidneys
—	1 day	5.58 ± 0.04[b]	6.86 ± 0.06	6.36 ± 0.03	4.72 ± 0.05
Solute control	4 weeks	6.93 ± 0.06	6.04 ± 0.03	6.24 ± 0.09	6.03 ± 0.18
	8 weeks	6.19 ± 0.26	5.69 ± 0.09	5.85 ± 0.04	6.14 ± 0.28
OFLX	4 weeks	6.48 ± 0.09[c,e]	5.69 ± 0.09[d,f]	5.94 ± 0.04[c,f]	5.55 ± 0.18
	8 weeks	5.85 ± 0.08[f]	5.31 ± 0.09[d]	5.40 ± 0.14[d]	5.49 ± 0.17
CPFX	4 weeks	6.92 ± 0.06	5.95 ± 0.05	6.20 ± 0.08	6.36 ± 0.03
	8 weeks	6.24 ± 0.08	5.64 ± 0.17	5.41 ± 0.41	5.68 ± 0.22

[a] Details as in Table 1.
[b] The mean ± SEM (n=6).
[c] Difference from the value of control mice was statistically (t-test) significant ($P<0.01$).
[d] Ibid. ($P<0.05$).
[e] Difference from the value of ciprofloxacin-treated mice was statistically significant ($P<0.01$, t-test).
[f] Ibid. ($P<0.05$).
[g] Abbreviations used are: OFLX, ofloxacin; CPFX, ciprofloxacin.

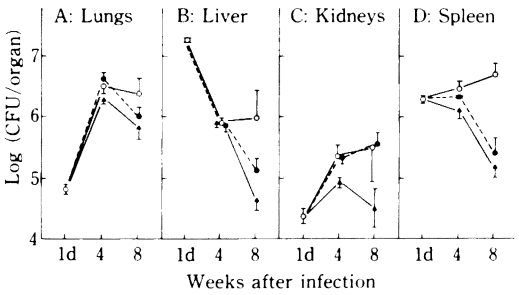

Fig. 1. Theraputic efficacy of ofloxacin against *M. kansasii* infection in mice given i.p. Mice infected i.v. with the organisms (7.2×10^6/mouse) were given the drug in a dose of 1 (●) or 2 (▲) mg/mouse, once daily, 6 times per week, from day 1 for up to 8 weeks after the bacterial challenge. Solute control (○). Each symbol indicates the mean ± SEM (n=5)

(by gavage) or i.p. at a dose of 2 mg/mouse were measured. Sample sera were obtained from mice 30 min after administration, because their serum concentrations peaked about this time[18,19] (our preliminary experiments). In this experiment, concentrations of the agents in sample sera were measured by disc method using tryptosoy agar medium overlaid with agar medium containing spores of *Bacillus subtilis* (ca. 10^6 CFU/ml), as previously described[20]. When mice (n=2) were given the agents by gavage, the serum concentrations were 19.3, 9.0 and 21.5 μg/ml (mean values; the 95 % confidence limit was 11 % of the original volume) for OFLX, CPFX and RFP, respectively. When the agents were injected i.p. to mice, the same values were 12.0, 3.5 and 26.0 μg/ml for OFLX, CPFX and RFP, respectively. It is noteworthy that the serum concentration of OFLX was considerably higher than that of CPFX regardless of the administration route.

Antimicrobial activity of quinolones against intracellular *M. kansasii*. OFLX, CPFX and RFP were examined for antimicrobial activity against *M. kansasii* phagocytosed in resident peritoneal Mφs, by measuring the change in CFU of organisms per Mφ during a 3-day incubation after bacterial phagocytosis (Table 4). The quinolones, as well as RFP, inhibited growth of the organisms at concentrations below the serum level achieved by oral administration. The antimicrobial activity of OFLX was somewhat higher than that of CPFX and a

Table 4. Antimicrobial activity of ofloxacin, ciprofloxacin and rifampicin against *M. kansasii* phagocytosed in resident murine peritoneal Mϕs

Addition		Chase incubation (days)	CFU / Mϕ × 10^2	Bacilli / Mϕ* × 10^2
Agent	μg / ml			
None	–	0	1.09 ± 0.02[b]	3.72 ± 1.26[b]
None	–	3	57.1 ± 9.9	29.0 ± 9.6
OFLX	2	3	11.1 ± 0.1	8.77 ± 0.52[c]
OFLX	10	3	1.25 ± 0.15[d]	7.34 ± 0.67
CPFX	2	3	11.9 ± 0.1	23.9 ± 0.7
CPFX	10	3	6.28 ± 0.71	7.98 ± 1.65
RFP	2	3	1.15 ± 0.05[e]	3.28 ± 0.65[f]
RFP	10	3	0.52 ± 0.06	3.84 ± 1.51

* The number of acid fast bacilli in Mϕ was counted microscopically.
[b] The mean ± SEM (n=2).
[c] Difference from the value for ciprofloxacin was statistically significant ($P<0.01$, t-test).
[d] Ibid. ($P<0.05$).
[e] Difference from the values for quinolones was statistically (t-test) significant ($P<0.01$).
[f] Ibid. ($P<0.05$).
[g] Abbreviations: OFLX, ofloxacin; CPFX, ciprofloxacin; RFP, rifampicin.

statistically significant difference was seen in some cases ($P < 0.05$; t-test). The quinolones showed no microbicidal activity against the organisms, even when added at a concentration of 10 μg/ml, while RFP did show bacterial killing ability at the same concentration. Similar results were obtained when the number of acid-fast bacilli in Mϕ cells was enumerated microscopically, although this method did not allow for evaluation of the microbicidal activity (Table. 4).

Fig. 2 shows the dose-dependent antimicrobial activity of OFLX, CPFX and RFP against *M. kansasii* ingested in zymosan A-stimulated peritoneal Mϕs. All the test agents killed the Mϕ-associated organisms during the course of chase incubation after phagocytosis, when added at a concentration of 20 μg/ml. Both quinolones showed bacteriostatic but not bactericidal activity when added at a concentration of 2 μg/ml, whereas RFP at the same dose exhibited significant microbicidal activity against Mϕ-associated organisms.

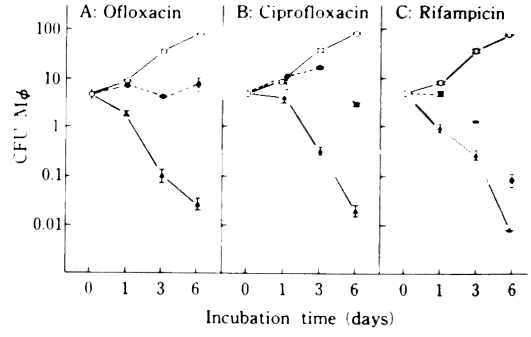

Fig. 2. Antimicrobial effects of ofloxacin (A), ciprofloxacin (B), and rifampicin (C), against *M. kansasii* phagocytosed in zymosan A-induced murine peritoneal Mϕs. None added (○); + agent at 2 μg/ml (●); + agent at 20 μg/ml (▲). Each symbol indicates the mean ± SEM (n=2)

Discussion

Our study showed the therapeutic efficacy of OFLX and CPFX against *M. kansasii* infection induced in mice, based either on growth inhibition or on elimination of the organisms from visceral organs. The efficacy was considerably higher in OFLX than in CPFX, but the activity of both agents

was significantly lower than that of RFP. Since the MIC values of the two quinolones against *M. kansasii* Umeda were at the same level, the difference in efficacy between them would seem to originate primarily from their pharmacokinetics. It is known that OFLX is better absorbed than CPFX after oral administration[6] and maximum levels achieved in serum are 3- to 5-fold higher in OFLX[21,22]. We also observed much higher (2—3-fold higher) serum concentrations of OFLX, given either orally (by gavage) or i.p. to mice, than those of CPFX. This may explain the considerably lower therapeutic efficacy of CPFX, compared to OFLX.

As shown in Table 4, the antimicrobial activity of the drugs tested against *M. kansasii* phagocytosed in host Mϕ cells was in the order of; RFP≫OFLX≧CPFX. Since the MICs of these agents against *M. kansasii* were at a similar level, the observed difference in bactericidal efficacy in the phagocytic cells may be attributed either to disparity in the mode of penetration into and accumulation in Mϕ cells[23] or to differences in stability against enzymatic degradation in phagolysosomes of Mϕs.

In any case, the *in vivo* efficacy of OFLX and CPFX against experimental *M. kansasii* infection was appreciable, although lower than that of RFP. Therefore, these drugs may be used for combination therapy in patients receiving other antituberculous drugs, including RFP.

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新キノロン剤，オフロキサシンならびにシプロフロキサシンの実験的マウス *Mycobacterium kansasii* 感染症に対する治療効果

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新キノロン剤，オフロキサシン（OFLX）ならびにシプロフロキサシン（CPFX）の実験的マウス *Mycobacterium kansasii* 感染症に対する治療効果について，内臓での肉眼病変，還元生菌数を指標として検討した。両キノロンを連日週に6回，2 mg 宛で経口あるいは腹腔内投与した場合有意な治療効果がみられた。その薬効はOFLX＞CPFXであったが，対照として用いたリファンピシン（RFP）にくらべてはかなり低いものであった。両キノロンは宿主の腹腔マクロファージ内に貪食された *M. kansasii* に対して強い殺菌能を示したが，RFPはそれらよりもさらに優れた殺菌活性を示した。

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