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In Vitro Susceptibility of *Coxiella burnetii* to Trovafloxacin in Comparison with Susceptibilities to Pefloxacin, Ciprofloxacin, Ofloxacin, Doxycycline, and Clarithromycin

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The antibiotic susceptibilities of eight Greek isolates of *Coxiella burnetii* to trovafloxacin were determined by the shell vial assay. MICs of trovafloxacin and ofloxacin ranged from 1 to 2 μg/ml, those of pefloxacin ranged from 1 to 4 μg/ml, those of ciprofloxacin ranged from 4 to 8 μg/ml, those of doxycycline ranged from 1 to 2 μg/ml, and those of clarithromycin ranged from 2 to 4 μg/ml. Trovafloxacin exhibited no activity against *C. burnetii* at 4 μg/ml.

Coxiella burnetii is the etiologic agent of Q fever. Two major forms of the disease are known: acute and chronic (1). The acute form of the disease is usually a self-limiting acute febrile illness, during which pneumonia or hepatitis may occur. However, the chronic form is a severe disease in which endocarditis predominates (8). Whereas acute *C. burnetii* infections respond to antibiotic therapy, chronic infections are hard to cure (3, 12).

The efficacies of quinolones against these types of infection have yet to be established. In this paper we studied the activity of a new quinolone, trovafloxacin, against *C. burnetii*, using Vero cell tissue cultures. We also compared its bacteriostatic effect with those of five other antibiotic compounds on eight Greek isolates derived from human samples.

Stock solutions of trovafloxacin (20 mg/ml; kindly provided by Pfizer Inc., New York, N.Y.), pefloxacin (400 mg/5 ml; Rhone Poulenc S.A., Paris, France), ciprofloxacin (100 mg/50 ml; Bayer AG, Leverkuzen, Germany), and ofloxacin (220 mg/100 ml; Hoechst AG, Frankfurt am Main, Germany) were prepared. For doxycycline (Pfizer Inc.) and clarithromycin (Abbott Laboratories, Chicago, Ill.), stock solutions at 6 mg/ml were prepared by using methanol.

All strains were grown in Vero cells as previously described (10). The bacteriostatic and bactericidal effects on *C. burnetii* were tested by the shell vial assay. For this purpose an acute-infection model with the Nine-Mile strain and a chronic-infection model with the Q212 strain were employed (1, 10). In addition, the concentration of the inoculum that was previously determined to infect 30 to 50% of Vero cells was used to infect the shell vials for the antibiotic challenge (10). Bacterial growth was evaluated after 6 days of incubation by indirect immunofluorescence, and the results were scored as follows: R for resistance, with growth comparable to that of the control; I for intermediate susceptibility, with less than 10% infected cells; and S for susceptibility, with the absence of infected cells (10).

The bactericidal activity of trovafloxacin was assessed by the quantitative method described by Maurin and Raoult (6). Trovafloxacin was added to the culture medium at 4 μg/ml. Viable organisms were identified after 6 days in the culture, and bactericidal activity corresponded to a significant reduction in bacterial titer (at least 2 to 3 dilutions) after antibiotic exposure, compared with that in the primary inoculum dose (5, 6).

Trovafloxacin showed bacteriostatic activity toward *C. burnetii*. Complete bacterial growth inhibition was obtained with 1 μg per ml in tests with the Nine-Mile isolate and with seven of eight Greek isolates. Inhibition was also obtained with 2 μg/ml in tests with the Q212 strain and the last Greek isolate (Table 1). These concentrations are below those achieved in human serum with the recommended single oral dose of 200 mg of trovafloxacin (2.2 μg/ml).

Vero cells were persistently infected with the Nine-Mile and Q212 *C. burnetii* strains for 5 months before being tested. Only these two strains were tested in bactericidal experiments with trovafloxacin. No bactericidal activity was demonstrated with 4 μg of trovafloxacin per ml when these cells were examined.

All MICs recorded were compared with each other and with the peak concentration of each antibiotic in serum. The MICs of all the antibiotics tested are summarized in Table 2.

At the concentrations tested, trovafloxacin, ofloxacin, and doxycycline showed improved bacteriostatic activities in vitro

TABLE 1. Susceptibilities of *C. burnetii* strains to trovafloxacin determined by the shell vial technique

Trovafloxacin concn (μg/ml)	Susceptibility[a] of indicated strain									
	Nine Mile	Q212	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8
0.25	R	R	R	R	R	R	R	R	R	R
0.5	I	R	R	I	I	I	I	I	I	I
1.0	S	I	I	S	S	S	S	S	S	S
2.0	S	S	S	S	S	S	S	S	S	S
4.0	S	S	S	S	S	S	S	S	S	S
8.0	S	S	S	S	S	S	S	S	S	S
16.0	S	S	S	S	S	S	S	S	S	S

[a] See the text.

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TABLE 2. Antibiotic susceptibilities of *C. burnetii* isolates

Isolate	MIC (μg/ml) of:					
	Pefloxacin at PCS[a] 3.8	Ofloxacin at PCS 3	Ciprofloxacin at PCS 1.6	Trovafloxacin at PCS 2.2	Clarithromycin at PCS 4	Doxycycline at PCS 4.4
Nine Mile	1	1	4	1	2	1
Q212	4	2	8	2	4	2
CP1	4	2	8	2	4	2
CP2	1	1	4	1	2	1
CP3	1	1	4	1	2	1
CP4	2	1	4	1	2	2
CP5	1	1	4	1	2	1
CP6	1	1	4	1	2	1
CP7	1	1	4	1	2	1
CP8	1	1	4	1	2	1

[a] PCS, peak concentration in serum (in micrograms per milliliter) for a single dose of antibiotic.

in tests with the Greek *C. burnetii* isolates as well as the tested reference strains. However, the same isolates were less susceptible to pefloxacin and clarithromycin. Ciprofloxacin presented higher MICs for all tested strains.

Two major problems are associated with the evaluation of the antibiotic treatment of acute Q fever. First, it is usually a self-limited disease and mostly retrospectively diagnosed and in the chronic form, an evaluation of the success of therapy requires prolonged follow-up due to late relapses (3–5, 7–9). Second, the experimental evaluation of antibiotic therapy is problematic because *C. burnetii* is a strictly intracellular pathogen and no successful animal model of chronic Q fever has been described so far (9). In cases of acute Q fever, a bacteriostatic effect is sufficient for enabling recovery, whereas in cases of chronic Q fever, a bacteriostatic regimen is not curative (9).

Tetracycline has been the mainstay therapy for endocarditis (8, 9). However, recovery of viable *C. burnetii* from valve tissue after 4 years of therapy with doxycycline has been reported (8). The use of cotrimoxazole alone has failed to cure Q fever endocarditis (8, 9). Combinations of rifampin with either doxycycline or cotrimoxazole have been used in treating Q fever endocarditis, with apparent success (9). Clinical data on the efficacies of macrolides are lacking (4).

More recently, a combination of doxycycline with the lysosomotropic agent chloroquine displayed bactericidal activity in vitro, but no definitive clinical data were presented (4). Quinolones exhibit in vitro activities against rickettsiae and have produced encouraging clinical results in Q fever patients (2, 3). In our study, trovafloxacin showed bacteriostatic activities (MIC, 1 to 2 μg/ml) in tests with *C. burnetii* strains that were better than those obtained with pefloxacin, clarithromycin, and ciprofloxacin. The bacteriostatic activities of doxycycline and ofloxacin were comparable to those of trovafloxacin.

A comparison between its MIC and peak concentration in serum cannot be used as evidence of trovafloxacin's clinical efficacy, since *C. burnetii* is an intracellular pathogen. It is, however, interesting that the intracellular concentrations of ciprofloxacin for all the tested strains and those of pefloxacin for two strains (Q212 and CP1) needed to be higher than their peak concentrations in serum in order to achieve bacteriostatic levels. This was not true for the other quinolones tested. The same type of comparison produced favorable results for clarithromycin and doxycycline (Table 2). However, the determination of the activities of trovafloxacin against *C. burnetii* strains showed that at a concentration of 4 μg/ml, it was not bactericidal to either tested strain.

In conclusion, our results indicate that trovafloxacin possesses promising in vitro activities against bacteria of the *C. burnetii* species. Careful clinical studies are now required to evaluate it for the treatment of Q fever infection.

REFERENCES

1. **Jabarit-Aldighieri, N., H. Torres, and D. Raoult.** 1992. Susceptibility of *Rickettsia conori*, *R. rickettsii*, and *Coxiella burnetii* to PD 127,391, PD 131,628, pefloxacin, ofloxacin, and ciprofloxacin. Antimicrob. Agents Chemother. **36:** 2529–2532.
2. **Keren, G., A. Keysary, R. Goldwasser, and E. Rubinstein.** 1994. The inhibitory effect of fluoroquinolones on *Coxiella burnetii* growth in in-vitro systems. J. Antimicrob. Chemother. **33:**1253–1255.
3. **Levy, P. Y., M. Drancourt, J. Etienne, J. C. Auvergnat, J. Beytout, J. M. Sainty, F. Goldstein, and D. Raoult.** 1991. Comparison of different antibiotic regimens for therapy of 32 cases of Q fever endocarditis. Antimicrob. Agents Chemother. **35:**533–537.
4. **Maurin, M., and D. Raoult.** 1996. Optimum treatment of intracellular infection. Drugs **52:**45–49.
5. **Maurin, M., A. M. Benoliel, P. Bongrand, and D. Raoult.** 1992. Phagolysosomal alkalinization and the bactericidal effect of antibiotics: the *Coxiella burnetii* paradigm. J. Infect. Dis. **166:**1097–1102.
6. **Maurin, M., and D. Raoult.** 1993. In vitro susceptibilities of spotted fever group rickettsiae and *Coxiella burnetii* to clarithromycin. Antimicrob. Agents Chemother. **37:**2633–2637.
7. **Raoult, D.** 1991. Antibiotic treatment of rickettsiosis, recent advances and current concepts. Eur. J. Epidemiol. **7:**276–281.
8. **Raoult, D.** 1993. Treatment of Q fever. Antimicrob. Agents Chemother. **37:** 1733–1736.
9. **Raoult, D., and T. Marrie.** 1995. Q fever. Clin. Infect. Dis. **20:**489–496.
10. **Raoult, D., H. Torres, and M. Drancourt.** 1991. Shell-vial assay: evaluation of a new technique for determining antibiotic susceptibility, tested in 13 isolates of *Coxiella burnetii*. Antimicrob. Agents Chemother. **35:**2070–2077.
11. **Torres, H., and D. Raoult.** 1993. In vitro activities of ceftriaxone and fusidic acid against 13 isolates of *Coxiella burnetii*, determined using the shell vial assay. Antimicrob. Agents Chemother. **37:**491–494.
12. **Tselentis, Y., A. Gikas, D. Kodteridis, E. Kyriakakis, N. Lydataki, D. Bouros, and N. Tsaparas.** 1995. Q fever in the Greek island of Crete: epidemiological, clinical, and therapeutic data from 98 cases. Clin. Infect. Dis. **20:**1311–1316.
13. **Yeaman, M. R., L. A. Mitscher, and O. G. Baca.** 1987. In vitro susceptibility of *Coxiella burnetii* to antibiotics, including several quinolones. Antimicrob. Agents Chemother. **31:**1079–1084.