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Susceptibility of *Rickettsia conorii*, *R. rickettsii*, and *Coxiella burnetii* to PD 127,391, PD 131,628, Pefloxacin, Ofloxacin, and Ciprofloxacin

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Plaque formation and dye uptake assays were used to measure the MICs of PD 127,391 and PD 131,628 against *Rickettsia* species. The MICs of PD 127,391 were 0.25 μg/ml for *Rickettsia rickettsii* and 0.125 to 0.25 μg/ml for *Rickettsia conorii*. The MICs of PD 131,628 were 0.25 to 0.5 μg/ml for *R. rickettsii* and 0.5 μg/ml for *R. conorii*. As determined by the shell vial technique, 15 strains of *Coxiella burnetii* were susceptible to PD 127,391 and PD 131,628 (MIC, ≤1 μg/ml), while one strain of *C. burnetii* (MP10) was of intermediate susceptibility.

Rickettsia rickettsii and *Rickettsia conorii* are the etiological agents of Rocky Mountain spotted fever and Mediterranean spotted fever, respectively. Both rickettsiae are strict intracellular pathogens, living free in the cytoplasm of infected host cells. Despite the development of many new antibacterial agents, lethality from Rocky Mountain spotted fever remains high (2.5%) (3), as does that from Mediterranean spotted fever in several countries (7). The delay in administration of an effective antibiotic leads to enhanced mortality (17). The mainstay of treatment is tetracycline and its analogs or chloramphenicol (18). The fluoroquinolones including pefloxacin, ofloxacin, and ciprofloxacin have also been successfully used to treat patients with Mediterranean spotted fever (2, 12).

Q fever is a widespread disease caused by *Coxiella burnetii*. This unique bacterium multiplies within the phagolysosome of infected host cells, surviving the low-pH environment (pH 4.5) of this cellular compartment. Two major forms of the disease are known: acute and chronic, which includes endocarditis. The clinical form of the disease is related both to host factors (8) and strain specificity (5). Acute Q fever is a self-limiting disease, and the main goal of antibiotic therapy in patients with acute Q fever is to produce a bacteriostatic effect in order to shorten the duration of disease. Tetracycline and its analogs reduce the duration of fever (6, 19), as do the fluoroquinolones pefloxacin and ofloxacin (2). Chloramphenicol is not clinically effective (22). Clinical data corroborate experimental results obtained in ovo (19) and in cells infected with *C. burnetti* (15). Chronic Q fever is a severe, frequently lethal disease in which *C. burnetii* survives and causes relapses, despite years of treatment with conventional antibiotic regimens. The reasons for the failure of antibiotic therapy in patients with chronic Q fever have been proposed to be due to the absence of bactericidal activities of the antibiotics used to treat the disease (11) and the antibiotic resistance of some isolates. PD 127,391 and PD 131,628 are recently developed fluoroquinolones with broad spectra of antibacterial activity. In the study described here, the potential activities of these compounds compared with those of the reference quinolones pefloxacin, ofloxacin, and ciprofloxacin against rickettsial infections were tested in various cellular models.

PD 127,391 and PD 131,628 were provided by Parke-Davis, Courbevoie, France. Ten milligrams of active material was dissolved in 0.5 ml of ethyl alcohol (95%), and this solution was diluted in distilled water to a final volume of 10 ml. These stock solutions of 1 mg/ml were filter sterilized, and aliquots were kept at 4°C. Ciprofloxacin (Bayer Pharma, Sens, France), pefloxacin (Roger Bellon, Neuilly/Seine, France), and ofloxacin (Diamant, Puteaux, France) stock solutions of 1 mg/ml in distilled water were kept at 4°C.

The bacterial strains used in the study were *R. rickettsii* (Sheila Smith, ATCC VR 149) and *R. conorii* (Moroccan, ATCC VR 141) cultured on Vero cells; *C. burnetti* Nine Mile (acute Q fever type isolate), Q212 (chronic Q fever type isolate), and Priscilla (from a goat; obtained from T. Hackstadt, Rocky Mountain Laboratory, Hamilton, Mont.); and 13 new isolates (obtained from nine cardiac valves, one aortic valve, two blood samples, and one vascular prosthesis of a patient suffering from chronic Q fever; named ME1 to ME9, MP10, ME11, ME12, and ME13, respectively). They were grown in human embryonic lung fibroblasts (HEL cells) in antibiotic-free minimal essential medium supplemented with 1% glutamine–10% fetal calf serum at 37°C in a 5% CO_2 atmosphere.

The plaque assay was used to determine the susceptibilities of *R. rickettsii* and *R. conorii* to the test agents. The plaque assay procedure used in this study was essentially the same as that described earlier (13). The endpoint was defined as that concentration of antibiotic which produced greater than a 1,000-fold reduction in plaque formation compared with that of the control inoculum diluted 10^{-3} in a petri dish.

A previously described (14) dye uptake assay was also used to determine the susceptibilities of *R. rickettsii* and *R. conorii*. The inoculum of *R. rickettsii* or *R. conorii* was added at a final volume of 50 μl per well, so that 2,000 PFU of rickettsiae was added to each well of the second row, 200 PFU was added to each well of the third row, and 20 PFU was added to each well of the fourth row. The first row was used as a control, and so no rickettsiae were added. Two thousand PFU of rickettsiae was added to each well of the next seven rows for the antibiotic assay (PD 127,391 and PD 131,628). A concentration of antibiotic was estimated to be active if the mean optical density was found to be between

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those of the inoculum containing 20 PFU and the cell control line.

Another model was used to test the susceptibility of *C. burnetii* to antibiotics during chronic cell infection. L929 cells persistently infected with phase I *C. burnetii* (Nine Mile or Q212) were maintained as described previously (1). The ratio of infected to uninfected cells was 0.95. Antibiotic was added to a final concentration of 1 μg/ml. Cells were maintained for 11 days at 37°C in a 5% CO_2 environment, and the culture medium containing the antibiotic was changed twice a week. The ratio of cell infection in control and antibiotic-treated cell cultures was monitored daily by observation of Gimenez-stained smears (4). Smears were prepared onto glass slides with a centrifugal slide maker (Cytospin II; Shandon, Cheshire, Great Britain).

In a second model, the multiplication of L929 cells persistently infected with *C. burnetti* was blocked with cycloheximide at 1 μg/ml, as described previously (11). Antibiotics were added to final concentrations of 1 μg/ml. Medium without antibiotic was added to control cultures. All cultures were incubated at 25°C and were maintained by changing the medium every 72 h, at which time appropriate amounts of antibiotic and cycloheximide were added. The percentage of infected cells was determined as described above.

The antibiotic challenge in a shell vial assay was used to determine the bacteriostatic effect of the antibiotics on the different *Coxiella* isolates (15, 16). As described previously (15), for each *C. burnetii* isolate, a series of centrifugation shell vials was inoculated with the dilution of infected cell suspension that resulted in infection of 30 to 50% of the cells after 6 days in the inoculum titration test. After centrifugation, the supernatant was discarded and 100 μl of the antibiotic solution and 900 μl of the cell culture medium were added to each shell vial. For each isolate, a reference vial of infected HEL cells without antibiotic was prepared as a positive control. After 6 days at 37°C in a 5% CO_2 environment, the number of HEL cells infected with *C. burnetti* was determined by indirect immunofluorescence (15).

PD 127,391, PD 131,628, ciprofloxacin, ofloxacin, and pefloxacin were shown by the dye uptake and shell-vial assays to be nontoxic for Vero and HEL cells, respectively, at concentrations of up to 64 μg/ml. This result proved that the cellular toxicities of the different antibiotics did not interfere with our susceptibility tests, which were run at concentrations of 0.0256 to 8 μg/ml. As determined by the dye uptake assay, the MICs of PD 127,391 and PD 131,628 were 0.25 μg/ml for *R. rickettsii* and 0.25 and 0.5 μg/ml, respectively, for *R. conorii*. As determined by the plaque assay, the MICs of PD 127,391 and PD 131,628 were 0.25 and 0.5 μg/ml, respectively, for *R. rickettsii* and 0.125 and 0.5 μg/ml, respectively, for *R. conorii*. As determined by the plaque assay, the MICs of pefloxacin, ciprofloxacin, and ofloxacin were 1 μg/ml, as observed previously (14). The results obtained by the plaque assay were consistent with those obtained by the dye uptake assay.

For *C. burnetii*, only one concentration of antibiotic (1 μg/ml) was tested in the chronic infection and shell vial assays. Thus, the MIC could not be determined; however, both test antibiotics prevented persistent infection of L929 and HEL cells at this concentration. In the chronic Q fever model, PD 127,391 reduced the ratio of L929 cells infected with the Q212 isolate from 95 down to 13% within 11 days (Fig. 1). The reduction of the percentage of infected cells was slightly quicker with the Nine Mile type isolate (90 down to 10% within 7 days) than with the Q212 type isolate (Fig. 2). PD 131,628 reduced the persistent infection to only 50%

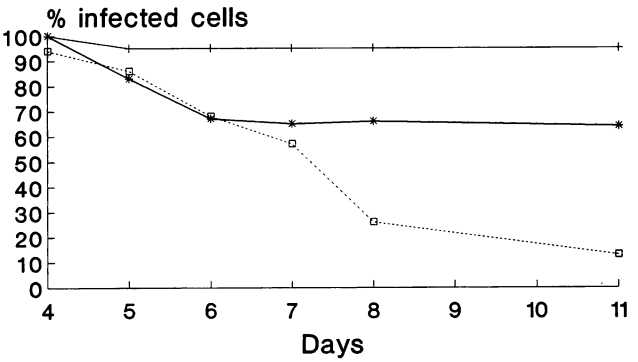

FIG. 1. Ratio of infected to uninfected L929 cells in which growth was not blocked with cycloheximide. Cells were infected with *C. burnetii* Q212 and were not treated (control) (+) or were treated with PD 127,391 (□) or PD 131,628 (*).

in 11 days. During this time, 95% of control cells remained infected.

When cells persistently infected with *C. burnetii* Nine Mile were blocked with cycloheximide, a ratio of 20% infected cells was found after 11 days of treatment with PD 127,331 (Fig. 3), whereas 50% infection was found with the Nine Mile type isolate treated with PD 131,628 or with the Q212 type isolate treated with either PD 127,391 or PD 131,628; 95% of control cells remained infected (Fig. 4).

Both the plaque and dye uptake assays can be used to test the antibiotic susceptibilities of *Rickettsia* isolates. The dye uptake assay studies the viabilities of cells infected with a high inoculum and is an indirect measure of antibiotic activity, because it measures the number of living host cells. It also shows the decrease in cell multiplication. The plaque assay, on the other hand, studies the generation of plaque from each *Rickettsia* isolate. In the present study, the efficacies of the new fluoroquinolones PD 127,391 and PD 131,628 were tested on *R. rickettsii* and *R. conorii* by using these techniques. PD 127,391 was found to be active, with MICs of 0.25 μg/ml for *R. rickettsii* and 0.125 to 0.25 μg/ml for *R. conorii*. PD 131,628 was found to be highly active also, with MICs of 0.25 to 0.5 μg/ml for *R. rickettsii* and 0.5 μg/ml for *R. conorii*. The results obtained with PD 127,391 were slightly better than those obtained with the other fluoroquinolones tested, while for PD 131,628 the results were equiv-

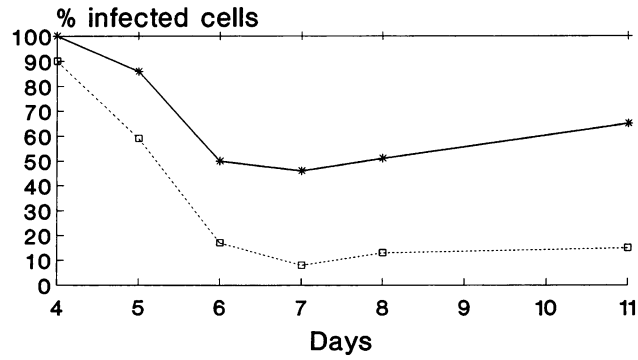

FIG. 2. Ratio of infected to uninfected L929 cells in which growth was not blocked with cycloheximide. Cells were infected with *C. burnetii* Nine Mile and were not treated (control) (+) or were treated with PD 127,391 (□) or PD 131,628 (*).

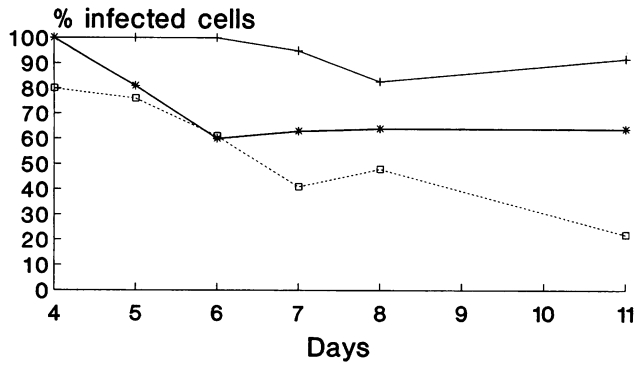

FIG. 3. Ratio of infected to uninfected L929 cells in which growth was blocked with cycloheximide. Cells were infected with *C. burnetii* Nine Mile and were not treated (control) (+) or were treated with PD 127,391 (□) or PD 131,628 (*).

alent to those obtained with the other fluoroquinolones tested. The MICs of ciprofloxacin are 0.25 μg/ml for *R. conorii* and 1 μg/ml for *R. rickettsii*, the MICs of pefloxacin are 0.5 μg/ml for *R. conorii* and 1 μg/ml for *R. rickettsii*, the MICs for ofloxacin are 1 μg/ml for both rickettsiae, and the MICs of sparfloxacin are 0.5 μg/ml for *R. conorii* and 0.25 μg/ml for *R. rickettsii* (9). Ciprofloxacin (12) as well as pefloxacin and ofloxacin (2) have been successfully used to treat Mediterranean spotted fever (10). However, the potential toxicities of the fluoroquinolones limit their use during pregnancy and childhood.

The management of patients with chronic Q fever remains problematic. Viable *C. burnetii* organisms have been isolated from excised cardiac valves after years of tetracycline therapy (20). This fact prompted Yeaman et al. (21) to use a cell line persistently infected with *C. burnetii* to evaluate antibiotic efficacies. They demonstrated that tetracycline, doxycycline, chloramphenicol, and trimethoprim were not bactericidal and that after 10 days of exposure to physiologically relevant levels of antibiotics, more than 50% of the cells remained infected. PD 131,628 gave a similar result, with 50% of the cells remaining infected after 11 days. However, as shown previously with rifampin; the fluoroquinolones difloxacin, ciprofloxacin, oxolinic acid, sparfloxacin; and in this study, PD 127,391, a higher degree of inhibition was obtained. As with other compounds, a difference in susceptibilities to PD 127,391 was found between the Nine Mile and the Q212 type isolates, with the Nine Mile being more susceptible. In that model, the observed bactericidal effect may have resulted from dilution of nondividing rickettsiae within multiplying cells (10). Therefore, we tested these antibiotics on the new cellular model of chronic Q fever infection in which persistently infected cells were blocked from dividing by treatment with cycloheximide. As with previously tested antibiotics (11), when cellular multiplication was blocked in this way, neither of the two new fluoroquinolones could eliminate the cellular infection, although with PD 127,391, less than 50% of the cells were infected with the Q212 type isolate. In the present study, PD 127,391 and PD 131,628, at concentrations of 1 μg/ml, were found to inhibit the multiplication of 15 of the 16 *C. burnetti* type isolates in the shell vial assay. The remaining type isolate (MP10) was not inhibited at this concentration. PD 127,391 was the most active and PD 131,628 had activity equal to that of ofloxacin in the model described here. However, as has been reported previously for other fluoroquinolones (11), neither PD 127,391 nor PD 131,628 cured persistently infected cells blocked with cycloheximide.

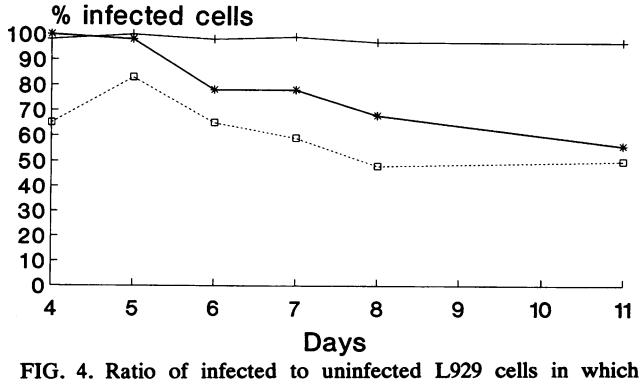

FIG. 4. Ratio of infected to uninfected L929 cells in which growth was blocked with cycloheximide. Cells were infected with *C. burnetii* Q212 and were not treated (control) (+) or were treated with PD 127,391 (□) or PD 131,628 (*).

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REFERENCES

1. **Baca, O. G., T. O. Scott, E. T. Akporiaye, R. DeBlassie, and H. A. Crissman.** 1985. Cell cycle distribution patterns and generation times of L929 fibroblast cells persistently infected with *Coxiella burnetii*. Infect. Immun. **47**:366–369.
2. **Bertrand, A., F. Janbon, O. Jonquet, and J. Reynes.** 1988. Infections par les rickettsiales et fluoroquinolones. Pathol. Biol. (Paris) **36**:493–495.
3. **Fishbein, D. B., M. G. Frontini, R. Giles, and L. L. Vernon.** 1990. Fatal cases of Rocky Mountain spotted fever in the United States, 1981–1988. Ann. N.Y. Acad. Sci. **590**:246–247.
4. **Gimenez, D. F.** 1964. Staining rickettsiae in yolk-sac cultures. Stain Technol. **39**:135–140.
5. **Hackstadt, T.** 1990. The role of lipopolysaccharides in the virulence of Coxiella burnetii. Ann. N.Y. Acad. Sci. **590**:27–32.
6. **Powell, O. W., K. P. Kennedy, M. McIver, and H. Silverstone.** 1962. Tetracycline in the treatment of Q fever. Aust. Ann. Med. **11**:184–188.
7. **Ramsey, P. G., and O. W. Press.** 1984. Successful treatment of Rocky Mountain 'spotless' fever. West. J. Med. **140**:94–96.
8. **Raoult, D.** 1990. Host factors in the severity of Q fever. Ann. N.Y. Acad. Sci. **590**:33–38.
9. **Raoult, D., P. Bres, M. Drancourt, and G. Vestris.** 1991. In vitro susceptibilities of *Coxiella burnetii*, *Rickettsia rickettsii*, and *Rickettsia conorii* to the fluoroquinolone sparfloxacin. Antimicrob. Agents Chemother. **35**:88–91.
10. **Raoult, D., and M. Drancourt.** 1991. Antimicrobial therapy of rickettsial diseases. Antimicrob. Agents Chemother. **35**:2457–2462.
11. **Raoult, D., M. Drancourt, and G. Vestris.** 1990. Bactericidal effect of doxycycline associated with lysosomotropic agents on *Coxiella burnetii* in P388D1 cells. Antimicrob. Agents Chemother. **34**:1512–1514.
12. **Raoult, D., H. Gallais, P. De Micco, and P. Casanova.** 1986. Ciprofloxacin therapy for Mediterranean spotted fever. Antimicrob. Agents Chemother. **30**:606–607.
13. **Raoult, D., P. Roussellier, V. Galicher, R. Perez, and J. Tamalet.** 1986. In vitro susceptibility of *Rickettsia conorii* to ciprofloxacin as determined by suppressing lethality in chicken embryos and by plaque assay. Antimicrob. Agents Chemother. **29**:424–425.
14. **Raoult, D., P. Roussellier, G. Vestris, and J. Tamalet.** 1987. In vitro antibiotic susceptibility of Rickettsia rickettsii and Rickettsia conorii: plaque assay and microplaque colorimetric assay. J. Infect. Dis. **155**:1059–1062.

15. **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.
16. **Raoult, D., G. Vestris, and M. Enea.** 1990. Isolation of 16 strains of *Coxiella burnetii* from patients by using a sensitive centrifugation cell culture system and establishment of strains in HEL cells. J. Clin. Microbiol. **28:**2482–2484.
17. **Raoult, D., P. Zuchelli, P. J. Weiller, C. Charrel, J. L. San Marco, H. Gallais, and P. Casanova.** 1986. Incidence, clinical observations and risk factors in the severe form of Mediterranean spotted fever among patients admitted to hospital in Marseilles 1983–1984. J. Infect. **12:**111–116.
18. **Smadel, J. E., E. B. Jackson, and A. B. Cruise.** 1949. Chloromycetin in experimental rickettsial infections. J. Immunol. **62:**49–65.
19. **Spelman, D. W.** 1982. Q fever: a study of 111 consecutive cases. Med. J. Aust. **1:**547–548, 551, 553.
20. **Turck, W. P., G. Howitt, L. A. Turnberg, H. Fox, M. Longson, M. B. Matthews, and R. Das Gupta.** 1976. Chronic Q fever. Q. J. Med. **45:**193–217.
21. **Yeaman, M. R., J. R. Miriam, and O. G. Baca.** 1989. Antibiotic susceptibilities of two *Coxiella burnetii* isolates implicated in distinct clinical syndromes. Antimicrob. Agents Chemother. **33:**1052–1057.
22. **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.