Susceptibility of Coxiella burnetii to Pefloxacin and Ofloxacin In Ovo and in Persistently Infected L929 Cells

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Received 28 September 1988/Accepted 31 January 1989

The relative efficacy of the antibiotic treatment of chronic Q fever endocarditis justifies the further evaluation of the susceptibility of Coxiella burnetii to the modern quinolone antibiotics. We evaluated the efficacies of pefloxacin and ofloxacin in controlling the Nine Mile isolate of C. burnetii by using an embryonated egg assay and persistently infected L929 cells in culture. Pefloxacin was effective in controlling the intracellular parasite at a concentration of 50 μg per egg and 1 μg/ml in cultures of infected cells. Ofloxacin was effective at a concentration of 25 μg per egg and 0.5 μg/ml in infected-cell cultures. In light of the fact that the concentrations of antibiotics used fall within physiological ranges used in humans, ofloxacin and pefloxacin may be useful in the clinical management of chronic Q fever, for which, to date, results have been poor.

Q fever is a widely distributed zoonosis which also affects humans. The agent of the disease is the obligate intracellular procaryotic rickettsial organism Coxiella burnetii (1, 2). The disease manifestations may include pneumonia, hepatitis, and chronic endocarditis (4, 7, 8). Conventional antibiotic regimens prescribed for chronic Q fever are not very effective and may involve years of administration (8, 12, 13). Recently, it was demonstrated that the quinolone antibiotics ciprofloxacin, ofloxacin, and oxolinic acid are effective in controlling the Nine Mile isolate of C. burnetii persistently infecting L929 cells (15). In the present study, the efficacies of two new quinolone antibiotics, pefloxacin and ofloxacin, in controlling the Nine Mile isolate of C. burnetii were evaluated by using embryonated eggs and persistently infected L929 cells.

MATERIALS AND METHODS

Antibiotic preparation. Freshly prepared antibiotic solutions were used for all experiments. Ofloxacin (Roussel Uclaf, Paris, France) and pefloxacin (Roger Bellon, Paris, France) were dissolved in 47.5% ethanol adjusted to pH 8.0 with NaOH to obtain a stock solution of 1.0 mg/ml. The solutions were filter sterilized (pore size, 0.22 μm), and aliquots were kept at 4°C.

Embryonated-egg assay. The phase II Nine Mile isolate of C. burnetii was grown in L929 cells for amplification. After 4 days of incubation, the L929 cells were harvested and disrupted in a 0.5% solution of trypsin. The material was centrifuged for 10 min at 163 × g, the supernatant was collected, and the rickettsiae were pelleted at 10,000 × g for 10 min. The organisms were further purified by centrifugation through an aqueous solution of 7% Renografin (E. R. Squibb & Sons, Princeton, N.J.) and 30% sucrose (14). Purified rickettsiae were suspended in phosphate-buffered saline and adjusted to a concentration of 4 × 106 cells per ml by the method of Silverman and Fiset (10). Embryonated eggs (antibiotic free) were obtained from the National Institute of Agronomy Research, Marseilles, France. Sets of 30 eggs were used in separate tests for each antibiotic concentration tested (25, 50, 100, and 200 μg per egg). The antibiotics were injected in 0.25-ml volumes into the yolk sacs of 5- to 7-day-old embryonated eggs, which were then inoculated with 1 × 108 C. burnetii organisms (0.25 ml of purified C. burnetii suspension). Control eggs received equal volumes of phosphate-buffered saline (0.25 ml) and antibiotic solvent (0.25 ml) containing no antibiotics. In calculating the mean survival time by the method of Spicer et al. (11), we used the assumption that all embryos alive at the end of the experiment had died on day 13 of the experiment period.

Cell cultures. Mouse L929 fibroblast cells had been persistently infected with the phase 1 Nine Mile isolate of C. burnetii and were maintained as described previously (2). At the time of this study, the L929 cells had been persistently infected for 1,323 days. The viability of the cells was determined by the dye exclusion technique with erythrosine B stain (6). Smears were prepared on glass slides with the aid of a centrifugal slide maker (Cytospin II; Shandon, Cheshire, England) and subsequently stained by the method of Gimenez (3); the percentage of infected cells was then enumerated. Antibiotics were added to yield final concentrations (in 10 ml of cell culture) of 0.5, 1, and 5 μg/ml for ofloxacin and 1, 5, and 10 μg/ml for pefloxacin. Solvent containing no antibiotic was added to control cultures. All cultures were incubated at 35°C in a shaker incubator at 100 rpm. Cultures were maintained via passage every 48 h, at which time appropriate amounts of antibiotic were added.

RESULTS

Embryonated-egg assay. The average time to death was 7.5 days in C. burnetii-inoculated eggs without antibiotics, and the increase in mean survival time (IMST) was 5 days for negative controls. With ofloxacin, the IMST was 1.7 days at a concentration of 25 μg per egg, 3.1 days at 50 μg per egg, and an IMST equivalent to that of non-C. burnetii-inoculated eggs at 100 and 200 μg per egg. With pefloxacin, the IMST was 0 at 25 μg per egg, 2.4 days at 50 μg per egg, 3.5 days at 100 μg per egg, and 4 days at 200 μg per egg.

Persistently infected cell cultures. The efficacies of the quinolone antibiotics ofloxacin and pefloxacin in reducing the percentage of L929 cells persistently infected with C. burnetii were determined by using microscopic assay methods in the evaluation of Gimenez-stained smears of control...
FIG. 1. Efficacy of ofloxacin in reducing persistent infection of L929 mouse fibroblast cells infected with the phase I Nine Mile isolate of C. burnetii. Untreated (solvent-only) control (○) and ofloxacin concentrations of 0.5 μg/ml (●), 1.0 μg/ml (□), and 5.0 μg/ml (■) were tested in concurrent, duplicate experiments. The L929 cells had been persistently infected for 1,323 days before the experiment was started.

(antibiotic solvent only) and antibiotic-treated, persistently infected cell cultures (Fig. 1 and 2). Pefloxacin and ofloxacin were tested at concentrations of 1.5, 10 μg/ml and 0.5, 1, and 5 μg/ml, respectively, in L929 cell cultures treated for 10 days. MICs for 50% and 90% of strains (MIC50 and MIC90, respectively) were determined by using standard dose-response graphic analyses as previously described (15). The MIC50 and MIC90 of pefloxacin were 0.6 and 1.4 μg/ml, respectively, and the MIC50 and MIC90 of ofloxacin were 0.1 and 0.3 μg/ml, respectively.

The in vitro results obtained with the embryonated-egg and persistently infected cell culture assays were in agreement. They indicate that the two new quinolone antibiotics pefloxacin and ofloxacin are very effective in controlling the Nine Mile isolate of C. burnetii, with ofloxacin being the more effective of the two.

FIG. 2. Efficacy of pefloxacin in reducing persistent infection of L929 mouse fibroblast cells infected with the phase I Nine Mile isolate of C. burnetii. Untreated (solvent-only) control (○) and pefloxacin concentrations of 1.0 μg/ml (●), 5.0 μg/ml (□), and 10.0 μg/ml (■) were tested in concurrent, duplicate experiments. The L929 cells had been persistently infected for 1,323 days before the experiment was started.

DISCUSSION

Previous investigators evaluated a number of antibiotics for their efficacies against C. burnetii (5, 11). These included oxytetracycline, aureomycin, terramycin, doxycycline, rifampin, chloramphenicol, trimethoprim, and erythromycin. Chloramphenicol was not very effective, since 500 μg per egg (approximately 10 μg/g of egg) was necessary to obtain an IMST of 2.4 days (5). Tetracyclines, rifampin, and trimethoprim were reported to be somewhat effective; however, inconsistent results were obtained (5, 11). Spicer et al. (11) reported that one isolate of C. burnetii (CB-CY PI) was relatively resistant to tetracycline. Despite the apparently high in vitro efficacies of some of these drugs, C. burnetii could still be isolated from cardiac valves of patients suffering from chronic Q fever endocarditis even after years of tetracycline therapy (12, 13).

In this first work reporting the susceptibility of C. burnetii to the quinolones pefloxacin and ofloxacin, we found that these drugs were more effective than rifampin, doxycycline, or trimethoprim in ovo. Previously, the poor clinical success in the management of chronic Q fever endocarditis prompted Yeaman et al. (15) to use a cell line model persistently infected with C. burnetii for evaluating antibiotic efficacies. This work demonstrated that tetracycline, chloramphenicol, trimethoprim, and doxycycline were not bactericidal and that after 10 days of exposure to physiologically relevant levels, of antibiotic, more than 50% of the cells remained infected. Only rifampin and the quinolones difloxacin, ciprofloxacin, and oxolinic acid were apparently bactericidal. In the work described in this report, two new quinolones, pefloxacin and ofloxacin, were also shown to be effective. A comparison of previous (15) and present MICs of quinolones shows that ofloxacin is apparently the most effective quinolone tested to date in vitro. Because rifampin could be difficult to use in patients with prosthetic valves, owing to concomitant treatment with oral anticoagulants, we believe that these quinolone compounds may have particular value in patients with chronic Q fever endocarditis. Other aspects of quinolone treatment remain to be investigated both in vivo and in vitro; the potency of quinolones and their possible association with doxycycline or other antibiotics in potentially synergistic combinations; reference treatment for Q fever endocarditis; and the risk of acquired resistance of C. burnetii to quinolones. As for other rickettsioses, it was previously shown that the in vitro efficacy of the quinolone ciprofloxacin against Rickettsia conorii (9) was in agreement with clinical results (8). We suggest careful clinical assays to evaluate quinolone efficacies in patients with chronic Q fever.

LITERATURE CITED


