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Antimicrobial Activity of U-70138F (Paldimycin), Roxithromycin (RU 965), and Ofloxacin (ORF 18489) against *Chlamydia trachomatis* in Cell Culture

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The MICs of three new antimicrobial agents of different classes, U-70138F, roxithromycin (RU 965), and ofloxacin (ORF 18489), versus *Chlamydia trachomatis* in McCoy cell cultures were 0.25, 0.8, and 1.0 μg/ml, respectively. For each test drug, the MIC and MBC were identical or were within 1 dilution of one another. These drugs possessed sufficient activity in cell culture to suggest possible clinical effectiveness.

Chlamydia trachomatis genital infections have been recognized as an increasingly important public health problem in the United States and throughout the world (5). Although these infections can be treated with tetracycline, doxycycline, and erythromycin, all three of these drugs are usually given in 7-day regimens and often cause gastrointestinal intolerance, factors which may result in poor compliance. Accordingly, new agents for treatment of *C. trachomatis* infections are of potential interest. In this study, we screened three new antimicrobial agents of different classes—roxithromycin (RU 965, a macrolide), ofloxacin (ORF 18489, a quinolone), and U-70138F (paldimycin, a glycopeptide)—for their activity against *C. trachomatis* in cell culture. U-70138F is a novel antibiotic prepared from paulomycin by reaction with *N*-acetyl-L-cysteine. Its mode of action against *Staphylococcus aureus* appears to be via inhibition of protein synthesis (A. L. Laborde, S. E. Truesdell, and S. C. Linn, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 801, 1985).

Three laboratory strains of *C. trachomatis* (type E-UW/17, a urethral isolate; type C-UW/76B, a cervical isolate; and type L2/LGV432, a rectal isolate) were grown in McCoy cells in 96-well microtiter plates. All three strains have been extensively passaged in the laboratory before these studies. Antibiotic-free chlamydial suspensions were prepared by two cycles of centrifugation and subsequent suspension in antibiotic-free maintenance medium (Eagle minimal essential medium with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [pH 7.5], 6 g of glucose per liter, and 10% fetal bovine serum).

McCoy cells were maintained and passed 10 to 15 times in antibiotic-free medium to ensure that no extraneous antibiotic interaction could occur. Monolayers of antibiotic-free McCoy cells were then grown in 96-well microtiter plates seeded at a concentration of 3.0×10^5 cells per ml. After 48 h of incubation, monolayers were exposed to DEAE-dextran at 30 μg/ml for 30 min before inoculation. The three *C. trachomatis* strains used (serovars E, C, and L2) were then each inoculated onto monolayers at two dilutions (500 and 5,000 inclusion-forming units per well) to assess antimicrobial activity at high as well as at low inocula. After inoculation, monolayers were centrifuged for 60 min at 1,200 × *g*, aspirated, and overlaid with appropriate serial dilutions of the three drugs tested. Penicillin G and tetracycline were also inoculated as control drugs which we have previously studied on numerous occasions. All dilutions were made with the above-mentioned antibiotic-free maintenance medium containing a 1.0-μg/ml concentration of cycloheximide. Roxithromycin was found to be soluble in absolute methanol (100 mg of roxithromycin per 1.0 ml of methanol), and serial dilutions were then made. The purity of all drugs tested was assumed to be 100%. Roxithromycin was provided by Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.; ofloxacin was from Ortho Pharmaceutical Corp., Raritan, N.J.; and U-70138F was from The Upjohn Co., Kalamazoo, Mich.

Infected cells were incubated for 48 h in 2% CO_2 in air at 36°C, fixed in absolute methanol-acetone, and stained for enumeration of inclusions by using a fluorescein-conjugated monoclonal antibody (no. 8H109; SYVA Co., Palo Alto, Calif.). Each well was read as positive or negative based on the presence or absence of inclusions. The lowest concentration of each antibiotic which completely inhibited inclusion formation was defined as the MIC. Duplicate plates were incubated for 72 h, passed, and then stained at 48 h to determine the lowest concentration of antibiotic which completely inhibited any further inclusion formation after removal of the drug (MBC). All studies were done in triplicate; results did not vary greater than 1 dilution from one replicate reading to another.

The MICs of U-70138F, RU 965, and ofloxacin against *C. trachomatis* were 0.25, 0.8, and 1.0 μg/ml, respectively, while that of tetracycline was 0.1 μg/ml and that of penicillin G was >1,000 μg/ml. For each of the test drugs, the MIC and MBC were identical or were within 1 dilution of one another. Similarly, there were no differences in MIC or MBC when the high and low inocula were compared. There were no apparent differences among the three *C. trachomatis* strains tested in terms of susceptibility to the three test antimicrobial agents.

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Various methods have been used to assess the activity of antimicrobial agents against *C. trachomatis* in cell cultures (1–4, 6). Our method closely resembles that of Bailey and co-workers (2), which utilizes immunofluorescence staining to identify the aberrant inclusions induced by exposure of *C. trachomatis* to some antibiotics. Our MICs of tetracycline and ofloxacin with this method confirm their previously reported results (2).

Each of the three experimental drugs that we tested had sufficient activity against *C. trachomatis* in cell cultures to suggest possible clinical effectiveness. Rifampin, erythromycin, tetracycline, and doxycycline, all drugs that are clinically effective for *C. trachomatis* infection, have MICs as measured in prior studies of 0.1 to 1.0 μg/ml (2–4, 6). Ofloxacin, an oxyquinolone, had an MIC of 1.0 μg/ml in our study and an MIC of between 1 and 8 μg/ml in previous studies (1, 2, 6). In preliminary trials, the drug appears effective in the treatment of genital infections caused by *C. trachomatis* (P. Brust and W. E. Stamm, 2nd World Congr. Sex. Transm. Dis., Paris, France, June 1986). Roxithromycin, a macrolide, had an MIC of 0.8 μg/ml in our study and is being evaluated in preliminary clinical trials. The activity of U-70138F, a congener of paulomycin, against other genital pathogens in vitro has not yet been established. However, the drug appears highly active against *C. trachomatis* in cell culture, and studies of its clinical effectiveness are warranted.

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