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In Vitro Activities of Thirteen β-Lactam Antibiotics Against *Chlamydia trachomatis*

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The in vitro activity (minimal inhibitory concentration and minimal lethal concentration) of 13 β-lactam antibiotics against two laboratory strains of *Chlamydia trachomatis* was compared. No useful activity could be detected.

Although it could be predicted that cell wall-active agents like β-lactam antibiotics would not be active against *Chlamydia trachomatis*, there is some clinical evidence that one of the older β-lactam antibiotics (amoxicillin) can eradicate *C. trachomatis* from the urethra (1). Therefore, we tested the in vitro activities of 13 β-lactam antibiotics against *C. trachomatis* in cell culture.

The antibiotics used were cephalothin and cefamandole (Lilly Research Centre Ltd., Windlesham, Surrey, England), moxalactam (Eli Lilly & Co., Indianapolis, Ind.), cefuroxime and ceftazidime (Glaxo Group Research Ltd., Greenford, Middlesex, England), cefoxitin and *N*-formimidoyl thienamycin (Merck Sharp & Dohme Research Laboratory, Rahway, N.J.), ceftizoxime (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan), ceforanide (Bristol-Myers B.V., Bussum, The Netherlands), cefotaxime (Roussel Uclaf, Paris, France), doxycycline and cefoperazone (Pfizer Corp., Brussels, Belgium), cefsulodin (Ciba-Geigy Ltd., Basel, Switzerland), and ceftriaxone (Hoffman-La Roche & Co. A.G., Basel, Switzerland).

C. trachomatis serotypes E and L2 were laboratory strains obtained from M. F. Michel, Department of Medical Microbiology, Erasmus University, Rotterdam, The Netherlands.

C. trachomatis types E and L2 were grown in HeLa 229 cells. Antibiotic-free chlamydial suspensions were prepared by culture during two passages in the absence of antibiotics (L2) or by two cycles of centrifugation and suspension in antibiotic-free maintenance medium (E). Maintenance medium was Eagle minimal essential medium with Earle salt solution, 375 mg of sodium bicarbonate per liter, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.5), 6 g of glucose per liter, and nonessential amino acids, to which 10% fetal bovine serum was added. Monolayers of antibiotic-free HeLa 229 cells in flat-bottom tubes were inoculated with 1 ml of a suspension of E or L2 organisms, diluted in maintenance medium to contain 100 to 1,000 inclusion-forming units. In the case of type E, monolayers were exposed to DEAE-dextran at 30 μg/ml for 30 min before inoculation and were centrifuged for 60 min at 2,200 × *g* and 35°C after inoculation. A 1-ml amount of medium with a dilution of one of the antibiotics tested or of doxycycline, which was used as a control, was added to each of four monolayers after 60 min. Doubling concentrations of from 1 to 16 μg/ml were used. After 72 h of incubation at 36°C, two monolayers of each antibiotic dilution were fixed with methanol and stained with Giemsa for determination of the presence or absence of chlamydial inclusions by using dark-ground illumination. The lowest concentration of each antibiotic which completely inhibited inclusion formation (minimal inhibitory concentration) was determined. The medium was removed from the two other monolayers of each antibiotic dilution and replaced by maintenance medium with antibiotics (100 μg of vancomycin, 50 μg of gentamicin, and 2.5 μg of amphotericin B per ml) as was used in our standard procedure for the isolation of *C. trachomatis*. Cultures were frozen and thawed once, and 1 ml was inoculated onto each of two new monolayers. Adsorption and incubation were performed as before, except that 0.5 μg of cycloheximide was added per ml of maintenance medium after the adsorption period. After 72 h, monolayers were fixed with methanol, stained with Giemsa, and evaluated for the presence of inclusions. The dilution of each antibiotic which completely inhibited further multiplication of *C. trachomatis* during one passage after removal of the drug (minimal lethal concentration) was determined.

None of the 13 antibiotics completely inhibited the multiplication of *C. trachomatis* except ceftriaxone and cephalothin, which inhibited multiplication of *C. trachomatis* serotype E at concentrations of 8 to 16 and 16 μg/ml, respectively. Although cefamandole and ceforanide (16

μg/ml) did not completely inhibit the formation of inclusions by *C. trachomatis* E in the first passage, further replication of this organism after removal of the drugs was inhibited. Doxycycline (used as control), to which all or most strains of *C. trachomatis* are susceptible, was inhibitory at 1 μg/ml, the lowest concentration used.

Although the results of in vitro assessments of activity are not always predictive of therapeutic efficacy (1), the performances of the 13 β-lactams included in this evaluation suggest that none would be of value in patients infected with *C. trachomatis*. However, there was enough difference in activity of members of this group to warrant testing of new β-lactams as they appear (2). Since there is little difference in the antibiotic susceptibility of *C. trachomatis* of different serotypes (3), these activity measurements can be limited with respect to serotype coverage, as they were in the present study.

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