ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1983, p. 493–494
0066-4804/83/030493-02$02.00/0

Vol. 23, No. 3

In Vitro Activity of a Group of Broad-Spectrum Cephalosporins and Other β-Lactam Antibiotics Against *Chlamydia trachomatis*

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Received 27 September 1982/Accepted 29 December 1982

The activities of seven broad spectrum cephalosporins, four other β-lactam antibiotics, and one monobactam against *Chlamydia trachomatis* were measured in a cell culture system. The minimal inhibitory concentration of four of the seven cephalosporins was ≥128 μg/ml; those of the other three were from 16 to 32 μg/ml. Of the other agents, only mecillinam had activity against *C. trachomatis* comparable to that reported for ampicillin (minimal inhibitory concentration, ≤0.5 μg/ml).

A single dose of penicillin or ampicillin as commonly used in the treatment of uncomplicated gonococcal urethritis will usually not eradicate a concurrent chlamydial infection (5). Repetitive dosage is more effective; thus, amoxicillin given for 10 days eradicated *Chlamydia trachomatis* in 48% of men with chlamydial urethritis (2) and, given for 14 days, eradicated these organisms from the cervixes of pregnant women (E. R. Alexander, personal communication). To assess the potential activity of other β-lactam antibiotics, we tested seven of the new broad-spectrum cephalosporins, four other β-lactam antibiotics, and one monobactam against five *C. trachomatis* strains in a cell culture system.

McCoy cells treated with cycloheximide were used. The cells were grown in Eagle minimal essential medium to which 10% inactivated fetal calf serum, 2 mM glutamine, and 5% glucose were added. No antibiotics or antifungal agents except those under investigation were included in the medium. Seven-day-old monolayers were treated with trypsin and resuspended in medium to a concentration of 3×10^5 cells per ml. The cells were seeded onto 96-well microtiter plates with a volume of 0.2 ml per well and incubated at 35°C for 24 h.

One isolate of *C. trachomatis*, H/UW-4, which is well adapted to conditions in the laboratory; four clinical isolates from patients with urethritis, neonatal conjunctivitis, and from the cervix were used in our studies. These isolates had been passed three to five times in cells free of antibiotics.

The antimicrobial agents evaluated were moxalactam, cefmenoxime, cefotaxime, ceftriaxone, cefoperazone, *N*-formimidoyl thienamycin, cefsulodin, mecillinam, piperacillin, mezlocillin, azlocillin, and the monobactam Sch 29,482. Sch 29,482, an oral penem highly resistant to β-lactamase, has been reported to be highly active against both β-lactamase-positive and -negative strains of *Neisseria gonorrhoeae* and *Haemophilus influenzae* (C. Thornsberry and C. N. Baker, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 747, 1981).

The various antimicrobial agents were supplied as powders, solubilized according to instructions from the manufacturer, and diluted in medium containing 2 μg of cycloheximide per ml before use. One-day-old monolayers were inoculated with 0.1 ml of the test strain dilution to yield 10^3 to 10^4 inclusion-forming units per ml. The plates were then centrifuged for 60 min at $2,000 \times g$ at 25°C. After inoculation and centrifugation, the medium was aspirated and the wells were overlaid with 0.2-ml portions of medium containing the appropriate twofold dilutions of the test antibiotic. Each dilution was tested in quadruplicate. Appropriate controls without antibiotic were included with each assay. The cultures were incubated at 35°C for 48 h and then fixed and stained with iodine. The minimal inhibitory concentration (MIC) was the lowest concentration at which no inclusions were seen. To determine the minimal chlamydicidal concentration (MCC), the antibiotic-containing medium was removed, and after 48 h of incubation, the cells were washed twice and drug-free medium was added. The cultures were frozen at −70°C, thawed, passed onto new cells, incubated further for 48 to 72 h, and then fixed and

TABLE 1. Activities of various antibiotics against *C. trachomatis*

Antibiotic (no. of strains[a])	MIC (μg/ml)		MCC (μg/ml)
	Mean	Range	
Moxalactam (5)	≥128		
Cefmenoxime (4)	112	(64–128)	
Cefotaxime (5)	≥128		
Ceftriaxone (5)	19.2	(16–32)	32
Cefoperazone (5)	28.8	(16–32)	
Cefsulodin (3)	≥128		
N-Formimidoyl thienamycin (3)	32		
Mecillinam (5)	0.45	(0.25–0.5)	0.5
Piperacillin (5)	8		16
Mezlocillin (2)	8		
Azlocillin (2)	16		
Sch 29,482 (4)	28	(16–32)	

[a] Number of strains against which the agent was tested.

stained. The MCC was defined as the concentration that completely inhibited further development of inclusions.

Moxalactam, cefmenoxime, cefotaxime, and cefsulodin were either inactive or barely active at the highest test concentration (Table 1). Results were consistent, with no more than one dilution difference between isolates. Ceftriaxone, cefoperazone, and *N*-formimidoyl thienamycin all had an MIC ≤32 μg/ml, which may be within achievable serum concentrations. The activities of the other β-lactam antibiotics tested were greater than those of the cephalosporins. The most active drug was mecillinam, which had a mean MIC of 0.45 μg/ml and an MCC of 0.5 μg/ml. Overall, our results agree closely with those of previous studies (1, 3, 5, 6, 7), with the exception of piperacillin. Bowie (1) reported piperacillin MICs of ≥4,096 μg/ml by methods, including iodine staining, very similar to ours. Our results consistently showed an MIC of 8 μg/ml and an MCC of 16 μg/ml for several strains. Although we saw what appeared to be abnormal non-iodine-staining inclusions at higher concentrations of piperacillin, these inclusions did not result in viable chlamydiae on passage. Cefmenoxime and azlocillin had not previously been tested. The only other monobactam that has been tested against *C. trachomatis* is SQ 26,776 (5), which was reported to be inactive, with ≥100 μg/ml needed to suppress normal inclusions. We found Sch 29,482 to be more active, with a mean MIC of 28 μg/ml.

In vitro susceptibility testing of *C. trachomatis* is not standardized at present. Although our results are similar to those previously reported, prior studies have been carried out with different methods and a very limited number of strains. Hobson et al. (5) used only one isolate of *C. trachomatis* and examined 10-fold dilutions of the test antibiotic. Muytjens and Heessen (6) used three isolates but examined antibiotic concentrations of only 1 to 16 μg/ml. Both of these groups also used Giemsa staining of the tissue cultures.

The evaluation of antibiotics being considered for the treatment of gonorrhea and other sexually transmitted diseases for activity against *C. trachomatis* is very important because of the prevalence of this organism in such infections. The limited data now available suggests that some of the newer broad-spectrum cephalosporins will be of limited use in the treatment of chlamydial infections (4). Treatment with cefotaxime failed to eliminate concurrent chlamydial infection in men and women with uncomplicated gonorrhea (4). These results are not unexpected, considering that the MIC of cefotaxime against *C. trachomatis* is ≥128 μg/ml. However, other cephalosporins and β-lactam antibiotics, such as ceftriaxone, cefoperazone, and mecillinam, which were found to have activity in the intermediate range (MICs ≤32 μg/ml), may have a role in chlamydial infection therapy, although perhaps not in single-dose regimens.

This work was supported in part by grants from Hoffmann-LaRoche Inc., Nutley, N.J., Abbott Laboratories, North Chicago, Ill., and Lederle Laboratories, Pearl River, N.Y.

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