In Vitro Evaluation of CP-62,993, Erythromycin, Clindamycin, and Tetracycline against *Chlamydia trachomatis*

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The antimicrobial activity of CP-62,993 against four *Chlamydia trachomatis* isolates was compared with those of erythromycin, clindamycin, and tetracycline. The MIC of CP-62,993 was 0.26 to 1.02 µg/ml for 100% inclusion inhibition and 0.031 to 0.063 µg/ml for 50% inclusion inhibition. With pharmacokinetic and antimicrobial studies demonstrating prolonged half-life and in vitro effectiveness, CP-62,993 may make possible a single, short-course treatment regimen for *C. trachomatis* infection.

Chlamydial infection is now recognized as the most common sexually transmitted bacterial disease in the United States, with an estimated annual incidence of 3 million to 5 million infected people (7, 10). The diagnosis and treatment for genital chlamydial infections is frequently complicated by coinfection with several other sexually transmissible pathogens, including *Neisseria gonorrhoeae* and *Ureaplasma urealyticum*. Therefore, therapy should be administered with antimicrobial agents that have a broad spectrum of activity (2, 3, 6).

CP-62,993 is a 15-membered-ring macrolide antibiotic recently developed by Pfizer Central Research, Groton, Conn. Preliminary in vitro susceptibility studies have indicated superior activity for CP-62,993 compared with erythromycin when tested against *Haemophilus influenzae*, *Legionella pneumophila*, *U. urealyticum*, *Mycoplasma hominis*, and *N. gonorrhoeae* (S. C. Aronoff, C. Laurent, and M. R. Jacobs, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 931, 1986; R. W. Ryan, I. Kwasnik, and R. C. Tilton, 26th ICAAC, abstr. no. 932, 1986). In this study, a comparison was made of the in vitro activities of CP-62,993, erythromycin, tetracycline, and clindamycin against *Chlamydia trachomatis* serovars E and L₂ and two recent cervical isolates. The MICs and MBCs of all four antimicrobial agents were examined (1, 5).

Antibiotics in powder form were obtained from U.S.P.C. Inc., Rockville, Md.; The Upjohn Co., Kalamazoo, Mich.; and Pfizer, Inc.

The antimicrobial effects on *C. trachomatis* in vitro were determined by McCoy cell tissue culture assay in microtiter plates. Briefly, DEAE-dextran (Sigma Chemical Co., St. Louis, Mo.)-treated McCoy cells were inoculated with 10² to 10³ inclusion-forming units of each chlamydial isolate per well, centrifuged for 60 min, and incubated at 35°C in a 3.5% CO₂ atmosphere for 30 min (11). The inoculum was then replaced with culture media (0.2 ml per well) containing serial twofold dilutions of each antimicrobial agent and 0.1 µg of cyclohexamide (Sigma) per ml (1, 4, 11). Tetracycline, erythromycin, and CP-62,993 were diluted from 1.0 to 0.31 µg/ml. Clindamycin was diluted from 8.0 to 0.25 µg/ml. The same chlamydial strain or isolate without antibiotics served as a positive control for each assay. Inoculum for negative

controls consisted of RPMI 1640 medium without antibiotics.

After 48 h of incubation, three wells per dilution were fixed and stained with a fluorescein-conjugated monoclonal antibody to *C. trachomatis* (Syva Corp., Palo Alto, Calif.) (8). The MIC was defined as the lowest concentration at which complete inhibition of inclusion formation was observed.

The remaining wells were passed and incubated for another 48 h in culture media without antibiotics (4). The MBC was defined as the lowest concentration of antimicrobial agent preventing inclusion formation after passage (5).

CP-62,993 exhibited antimicrobial activity similar to that of erythromycin against all chlamydial strains tested (Table 1). Complete inhibition of inclusion formation was achieved with both antibiotics between 0.26 and 1.02 µg/ml for all strains tested except the L₂ serovar. The MIC of tetracycline ranged from 0.06 to 0.51 µg/ml for all strains. Clindamycin was the least active antibiotic, with complete inhibition of inclusion formation at a concentration of 2 µg/ml.

Endpoint titrations for the MICs of tetracycline, erythromycin, and CP-62,993 are shown in Table 2. CP-62,993 exhibited in vitro activity similar to that of tetracycline at the 50% inclusion inhibition level (0.031 to 0.063 µg/ml). Erythromycin, however, showed 50% suppression between 0.125 and 0.51 µg/ml.

The MBCs of CP-62,993 and erythromycin for cervical isolates 3012 and 3014 were between 0.51 and 1.02 µg/ml (Table 1). The MBCs of tetracycline for those isolates were 0.13 and 0.51 µg/ml, respectively. CP-62,993 had an MBC of 1.02 µg/ml when assayed with serovar E; however, chlamy-

TABLE 1. In vitro activity of four antibiotics against four *C. trachomatis* isolates^a

Serovar or isolate	Clindamycin		CP-62,993		Erythromycin		Tetracycline	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E	2.0	4.0	0.51	1.02	0.51	>1.02	0.26	>1.02
3012	2.0	>8.0	0.51	1.02	1.02	1.02	0.13	0.13
3014	2.0	4.0	0.26	0.51	0.51	1.02	0.06	0.51
L ₂	2.0	>8.0	1.02	>1.02	>1.02	>1.02	0.51	>1.02

^a MICs and MBCs are expressed in micrograms per milliliter.

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TABLE 2. Percent inhibition of inclusion formation (MIC)^a

Concn of antibiotic ($\mu\text{g/ml}$)	% Inhibition of inclusion formation for indicated serovar or isolate by:											
	Tetracycline				CP-62,993				Erythromycin			
	3012	3014	E	L ₂	3012	3014	E	L ₂	3012	3014	E	L ₂
0.00 (no antibiotic)	0	0	0	0	0	0	0	0	0	0	0	0
0.031	0	39.7	30	0	18.2	53.5	27.7	0	31.8	13.2	17.3	0
0.063	99.3	100	99.7	96.1	49.2	91.1	91.3	53.2	18.2	2.3	33.9	15.6
0.125	100	100	99.7	99.2	90.9	99.4	99.3	99.5	29.4	5.5	52.5	68.1
0.26	100	100	100	99.7	99.8	100	99.8	99.7	22.0	76.7	89.0	99.3
0.51	100	100	100	100	100	100	100	99.9	96.2	100	100	99.9
1.02	100	100	100	100	100	100	100	100	100	100	100	99.9

^a Quantitative results for clindamycin were not done. Results are averages for three wells.

ial replication was still observed at that concentration for tetracycline and erythromycin. Clindamycin had an MBC of 4.0 $\mu\text{g/ml}$ when assayed with serovar E and isolate 3014 but was beyond the range of concentrations tested when assayed with isolate 3012. Within the range of concentrations tested, MBCs were not obtained for any of the antibiotics when assayed with serovar L₂.

Tetracycline and erythromycin are the drugs of choice for treating chlamydial infections (2, 3, 6). The results of this study concur with those of previous studies demonstrating complete suppression of three genital strains, with the exception of the L₂ lymphogranuloma venereum strain, at readily achievable drug concentrations (<1.0 $\mu\text{g/ml}$) (4, 5). Despite this in vitro activity, occasional treatment failures have occurred, primarily due to lack of compliance by the patient (3, 4, 6). One additional problem with these antibiotics is that neither is recommended for the treatment of concurrent gonococcal infection (3).

Initial in vitro and in vivo studies have demonstrated that CP-62,993 differs structurally from erythromycin by a methyl-substituted nitrogen at position 9A in the macrolide ring. Because of this structural difference, the spectrum, potency, half-life, and tissue distribution of CP-62,993 are also different from those of erythromycin. CP-62,993 was also shown to be significantly more effective (MIC, <0.031 to 0.25 $\mu\text{g/ml}$) than erythromycin against penicillinase-producing *N. gonorrhoeae* in vitro (Ryan et al., 26th ICAAC).

After a 500-mg dose of CP-62,993 in vivo, the peak levels in serum are slightly lower than those achieved with erythromycin, but the concentrations of CP-62,993 in tissue are 400 times that achieved in serum. Because of high tissue affinity, the drug has a long half-life, with detectable concentrations in urine 7 to 14 days after a single dose. The mean peak concentration of CP-62,993 in serum was 0.37 $\mu\text{g/ml}$ compared with 1.73 $\mu\text{g/ml}$ for erythromycin, and the serum half-life of CP-62,993 was 7 h compared with 1.0 h for erythromycin (A. R. English, A. E. Girard, C. Cimo-chowski, J. Faiella, and J. A. Retsema, 26th ICAAC, abstr. no. 933, 1986).

The results of this study indicate that CP-62,993 is also effective against *C. trachomatis* in vitro, with MICs similar to those of erythromycin and tetracycline. Clindamycin MICs were higher than those observed with the other three drugs, although within a range routinely achieved during in vivo administration (8 to 16 $\mu\text{g/ml}$). Clindamycin was tested in this study because it is often used in the treatment of pelvic inflammatory disease (9).

It therefore appears on the basis of this and previous studies that CP-62,993 might be an effective antimicrobial agent against *C. trachomatis* as well as against other sexually transmissible pathogens. This drug may offer an alternative to other treatments as a one-drug regimen for the treatment of concurrent infections. The prolonged half-life and extremely high tissue levels indicate a regimen of one dose per day, as well as a shorter course of therapy, which might also result in greater patient compliance and convenience. With these advantages, clinical trials of this agent in men and women with chlamydial and gonococcal urethritis and cervicitis are now warranted.

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