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NOTES

In Vitro Activity of A-56268 (TE-031) and Four Other Antimicrobial Agents against *Chlamydia trachomatis*

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The in vitro activity of A-56268 (TE-031), a new macrolide antibiotic, against 11 strains of *Chlamydia trachomatis* was determined and compared with that of four other antibiotics. A-56268 was the most active drug tested. Doxycycline, trimethoprim-sulfamethoxazole, and erythromycin had good activity but were less active in vitro than A-56268.

A-56268 (TE-031) is a new macrolide antibiotic which is structurally related to erythromycin. Since erythromycin has both in vitro and in vivo activities against *Chlamydia trachomatis* (2, 6, 7, 9–12), we tested the activity of A-56268 against 11 strains of *C. trachomatis* (2 laboratory and 9 clinical strains). The susceptibilities of these strains to doxycycline, erythromycin, trimethoprim-sulfamethoxazole, and ciprofloxacin were also determined.

A-56268 and erythromycin base (Abbott Laboratories, North Chicago. Ill.), trimethoprim-sulfamethoxazole (Burroughs Wellcome Co., Research Triangle Park, N.C.), ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.), and doxycycline hydrochloride (Sigma Chemical Co., St. Louis, Mo.) were prepared as stock solutions at concentrations of 1,280 μg/ml according to their stated potency, except for sulfamethoxazole, which was prepared at a concentration of 25,600 μg/ml. Stock solutions were stored at −70°C for a maximum of 2 weeks. On the day of use, the drugs were diluted to the appropriate concentration with medium containing Hanks balanced salt solution, amino acids, vitamins, 1% glutamine, 10% inactivated fetal calf serum, 5.4 g of glucose per liter, and 1 μg of cycloheximide per ml. Trimethoprim-sulfamethoxazole was prepared at a ratio of 1:20. The 11 strains of *C. trachomatis* tested included strains D (ATCC VR-885) and LGV-II (ATCC V9-902-B), and 9 clinical strains (conjunctival or genital origin). The clinical strains had been passed fewer than 10 times in the laboratory. All isolates were passed an additional two times in antibiotic-free medium before susceptibility testing and were stored at −70°C until the time of use.

In vitro susceptibility studies were performed with McCoy cells which had been serially passed at least six times in antibiotic-free medium. McCoy cell monolayers were treated with trypsin and suspended with antibiotic-free medium to a concentration of about 2×10^6 cells per ml. Cell suspensions (100-μl portions) were seeded into 96-well microtiter plates and incubated at 37°C in 5% CO_2 for 24 h. Duplicate plates were prepared for each drug. The monolayers were inoculated with 0.05 ml of a dilution of the *C. trachomatis* test strain known to yield 100 to 1,000 inclusions per well. The plates were centrifuged at 1,000 × *g* at 25°C for 60 min and then overlaid with 0.1 ml of each drug to yield appropriate twofold dilutions. Each dilution was tested three times. Appropriate antibiotic-free controls were included on each plate. Cultures were incubated for 48 h at 37°C in 5% CO_2, then fixed with absolute ethanol, and stained with 0.02 ml of fluorescein-conjugated mouse monoclonal antibody to *C. trachomatis* (Ortho Diagnostics, Inc., Raritan, N.J.) according to the directions of the manufacturer. The MIC was defined as the lowest concentration of antibiotic without inclusions. To determine the MBC, the duplicate tray for each antibiotic was seeded and incubated as described above for the MIC. At 48 h, the antibiotic-containing medium was removed, and the wells were washed twice with antibiotic-free medium. Antibiotic-free medium (100 μl) was added to each well and the cells were scraped. Volumes (50 μl) of the scraped cell suspensions were inoculated onto 24-h-old McCoy cell monolayers grown in antibiotic-free medium. The plates were then incubated for 48 to 72 h, fixed, and stained as described above. The MBC was defined as the lowest concentration of antibiotics yielding no inclusions after passage.

A-56268 was the most active drug tested (MIC for 90% of the strains [MIC_{90}], 0.008 μg/ml; MBC_{90}, 0.008 μg/ml) (Table 1). The MBC/MIC ratio of A-56268 was 1 for all the isolates (except for LGV-II, which had a ratio of 64 [MBC, 0.25 μg/ml; MIC, 0.004 μg/ml]). Doxycycline was also very active (MIC_{90}, 0.03 μg/ml; MBC_{90}, 0.125 μg/ml) as were trimethoprim-sulfamethoxazole (MIC_{90}, 0.06 μg of trimethoprim per ml; MBC_{90}, 0.125 μg of trimethoprim per ml), and erythromycin (MIC_{90}, 0.06 μg/ml; MBC_{90}, 0.25 μg/ml). Ciprofloxacin was the least active antibiotic tested (MIC_{90}, 1.0 μg/ml; MBC_{90}, 2.0 μg/ml). The MBC/MIC ratio of these four antibiotics was 1 or 2 for all isolates (except LGV-II, for which the ratio ranged from 16 [doxycycline] to more than 64 [erythromycin]). Each antibiotic caused gross morphological changes in the inclusions. At 1 to 2 dilutions below the MIC, inclusions became fewer, smaller, and pycnotic. Passage of

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TABLE 1. Antichlamydial activity of A-56268 and four other antibiotics against 11 strains of *C. trachomatis*

Antibiotic	MIC (μg/ml)			MBC (μg/ml)		
	Range	50%	90%	Range	50%	90%
A-56268	<0.002–0.008	0.004	0.008	<0.002–0.25	0.004	0.008
Erythromycin	0.03–0.125	0.06	0.06	0.03–>4.0	0.125	0.25
Doxycycline	0.008–0.06	0.03	0.03	0.008–0.5	0.03	0.125
Trimethoprim-sulfamethoxazole[a]	<0.004–0.125	0.015	0.06	0.008–2.0	0.015	0.125
Ciprofloxacin	0.5–>4.0	0.5	1.0	0.5–>4.0	0.5	2.0

[a] Trimethoprim-sulfamethoxazole concentrations were 1:20; only the trimethoprim concentration is shown.

the cells containing these abnormal inclusions into antibiotic-free cell monolayers resulted in inclusions with normal morphology. These abnormal inclusions probably represent a failure of the reticulate bodies to develop into normal elementary bodies (4). The clinical significance of these abnormal inclusions is unclear.

Bowie (1) reported higher MICs and MBCs of A-56268 against two strains of *C. trachomatis* than we found. This may indicate geographical variation in susceptibility of *C. trachomatis* to A-56268. Nonetheless, our data showed that A-56268 was more active in vitro against the 11 strains of *C. trachomatis* than were doxycycline, erythromycin, or trimethoprim-sulfamethoxazole, three antibiotics that are clinically used to treat chlamydial infections. The MICs and MBCs of doxycycline, erythromycin, trimethoprim-sulfamethoxazole, and ciprofloxacin that we found against *C. trachomatis* are comparable to those obtained by other investigators (2, 3, 5, 7, 9, 12).

If the excellent in vitro activity of A-56268 against *C. trachomatis* is also seen for *Neisseria gonorrhoeae* and *Ureaplasma urealyticum*, and if results of animal and human pharmacokinetic and toxicity studies are favorable, then clinical trials of A-56268 in patients with urethritis, cervicitis, and pelvic inflammatory disease would be warranted. Similarly, although erythromycin is the drug of choice for neonatal chlamydial conjunctivitis, some authors have reported a high rate of failure and relapse with erythromycin therapy (8). If such reports are confirmed, alternate therapies, such as A-56268, should be investigated.

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