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NOTES

In Vitro Activities of Azithromycin, Clarithromycin, Erythromycin, and Tetracycline against 13 Strains of *Chlamydia pneumoniae*

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Thirteen strains of *Chlamydia pneumoniae* were evaluated for their in vitro susceptibilities to azithromycin, clarithromycin, erythromycin, and tetracycline. The MIC ranges were 0.125 to 0.5 μg/ml for azithromycin, 0.031 to 1.0 μg/ml for clarithromycin, 0.125 to 1.0 μg/ml for erythromycin, and 0.125 to 1.0 μg/ml for tetracycline. The ranges for the minimal lethal concentrations were 0.125 to 0.5 μg/ml for azithromycin, 0.031 to 1.0 μg/ml for clarithromycin, 0.125 to 1.0 μg/ml for erythromycin, and 0.25 to 1.0 μg/ml for tetracycline. Clarithromycin and azithromycin were the most active antibiotics against *C. pneumoniae* in vitro.

Chlamydia pneumoniae has been recognized as a significant intracellular pathogen that causes respiratory infections such as pneumonia, bronchitis, and pharyngitis (8, 9). Administration of tetracycline or doxycycline has been the treatment of choice for chlamydial infections, with erythromycin being recommended as an alternative antibiotic regimen. Azithromycin is an azalide which has recently been recommended by the Centers for Diseases Control and Prevention as an alternative antibiotic for the treatment of genital *C. trachomatis* infection (5). Clarithromycin is another macrolide antibiotic which is effective for the treatment of most respiratory infections; it is rapidly distributed from serum to tissues, where high concentrations are achieved as the concentrations in serum and tissue are balanced. This study compared the in vitro activities of azithromycin, clarithromycin, erythromycin, and tetracycline against 13 strains of *C. pneumoniae.*

C. pneumoniae TW183, AR39, and AR388 were obtained from the Washington Research Foundation, Seattle. *C. pneumoniae* 2023, 2043, and VR1310 were obtained from the American Type Culture Collection, Rockville, Md. *C. pneumoniae* T2364, BAL15, BAL16, and BAL37 were received from Margaret R. Hammerschlag, State University of New York, Brooklyn. *C. pneumoniae* CWL-011 and CM-1 (Atlanta strains) and FML-16 (a Norwegian strain) were obtained from Carolyn M. Black, Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Ga. The antimicrobial agents tested were tetracycline hydrochloride, erythromycin lactobionate (Sigma, St. Louis, Mo.), azithromycin (Pfizer Central Research, Groton, Conn.), and clarithromycin (Abbott Laboratories, Chicago, Ill.). The antimicrobial agents were supplied as powders and solubilized according to the manufacturers' instructions. Susceptibility testing was performed in antibiotic-free HEp-2 cell monolayers in 96-well microtiter plates. The cells were inoculated with 0.1 ml of 10^3 inclusion-forming units of a chlamydial strain. Cultures were centrifuged for 60 min at 600 × *g* and 37°C and then incubated at 35°C with 5% CO_2 for 30 min (16). The inoculum in each well was aspirated and replaced with 0.2 ml of overlay medium, which consisted of 1.0 μg of cycloheximide (Sigma) per ml, and serial two-fold dilutions of an antimicrobial agent (17). The antimicrobial agents were diluted from 1.0 to 0.031 μg/ml. Each of six inoculated wells was overlaid with an antibiotic at each dilution (12). Plates were then incubated for 72 h at 37°C with 5% CO_2 in air. Following incubation, the culture medium was aspirated and three wells per dilution were fixed and stained with a genus-specific fluorescein-conjugated monoclonal antibody to *C. pneumoniae* (Sanofi Diagnostics Pasteur, Chaska, Minn.). Inclusion bodies were counted for each antimicrobial dilution, and the MIC (the lowest concentration at which complete inhibition of inclusion formation was observed) was determined (17). The remaining wells were passed, and the cultures were incubated for another 72 h. After staining with fluorescein-conjugated monoclonal antibody, the minimal lethal concentration (MLC) (the lowest concentration of antimicrobial agent preventing inclusion formation after passage) was determined (17).

The in vitro activities of azithromycin, clarithromycin, erythromycin, and tetracycline against 13 *C. pneumoniae* isolates are shown in Table 1. Clarithromycin and azithromycin were the most active antimicrobial agents against *C. pneumoniae*, followed by tetracycline and erythromycin. As the concentrations of the antibiotics increased, the size and number of inclusions decreased. Of the 39 pairs of MIC and MLC data, 37 of the MLCs were 0, 1, or 2 doubling concentrations above the correlating MIC. This indicates that the drugs were chlamydicidal at the tested concentrations and under the conditions of the experiment. For one isolate, VR1310, the MLC of clarithromycin was 3 doubling concentrations above the MIC (0.25 versus 0.031 μg/ml). The second case was isolate T2364; for tetracycline, the MLC was 3 doubling concentrations above the MIC (1.0 versus 0.125 μg/ml).

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TABLE 1. In vitro activities of azithromycin, clarithromycin, erythromycin, and tetracycline against *C. pneumoniae* isolates[a]

Strain	Azithromycin		Clarithromycin		Erythromycin		Tetracycline	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
TW183	0.25	0.5	0.25	0.5	0.5	0.5	0.125	0.5
AR39	0.5	0.5	0.5	0.5	0.25	0.5	0.25	0.25
AR388	0.5	0.5	1.0	1.0	1.0	1.0	0.5	0.5
VR1310	0.25	0.25	0.031	0.25	0.25	0.5	0.25	0.5
2023	0.125	0.125	0.0625	0.125	0.125	0.5	0.5	1.0
2043	0.125	0.125	0.0625	0.0625	0.25	0.5	1.0	1.0
CM-1	0.25	0.25	0.0626	0.125	0.5	1.0	0.25	1.0
CWL-011	0.25	0.5	0.0625	0.0625	0.25	0.5	0.25	0.5
BAL15	0.25	0.5	0.125	0.25	0.25	0.25	0.5	1.0
BAL16	0.125	0.125	0.0625	0.125	0.25	0.5	0.5	0.5
BAL37	0.125	0.125	0.0625	0.0625	0.125	0.125	0.125	0.25
FML-16	0.125	0.125	0.031	0.031	0.25	0.25	0.5	0.5
T2364	0.125	0.125	0.031	0.031	0.125	0.25	0.125	1.0
Mean	0.25	0.29	0.18	0.24	0.32	0.49	0.38	0.65

[a] MICs and MLCs are expressed in micrograms per milliliter of antibiotic.

Tetracycline and erythromycin historically have been the drugs of choice for the treatment of chlamydia infections, including *C. pneumoniae* infections. Doxycycline has replaced tetracycline because of improved patient compliance, and erythromycin is used for treatment when the patient is pregnant, is nursing a child, or has known adverse reactions or allergy to tetracycline. Our results suggest a role for the new macrolides in the treatment of *C. pneumoniae* infections. Previous studies have shown clarithromycin to be active against this organism (6). An MIC of 0.015 μg/ml and an MLC of 0.03 μg/ml were determined for strain TW-183, and the MICs for strains 2043 and 2023 were 0.03 and 0.015 μg/ml, respectively. Similar results were seen in another study (11) with clarithromycin as the most active agent tested. The MICs and MLCs for 90% of the 11 strains of *C. pneumoniae* were 0.03 μg/ml.

Clarithromycin is one of the most active antibiotics against *C. pneumoniae* in vitro, and it has excellent tissue and intracellular penetration (15). Azithromycin is also widely distributed in tissue, and it has a pharmacokinetic profile different from the profiles of other macrolides (15). Azithromycin has an elimination half-life of 57 h, and steady state is not reached for 12 days. In contrast, steady state for clarithromycin is reached in 15 to 20 h.

At steady state, azithromycin achieves a peak concentration in plasma of 0.41 μg/ml and a peak concentration in the lungs of 4.0 μg/g (2). While this ratio is 10:1, the value is meaningless unless one considers the MIC at which 90% of the isolates are inhibited (MIC_{90}) and evaluates the ratio between the concentration in plasma or the lungs and the MIC_{90}. Clarithromycin achieves a concentration in serum of 2.8 μg/ml and a concentration in the lungs of 17.47 μg/g (7). The ratios of the concentrations in the tissues and the MIC_{90}s are 1,165:1 for clarithromycin and 16:1 for azithromycin (1). Levels of clarithromycin in the alveolar macrophages and bronchial mucosa are 106 and 18.6 μg/g, respectively, while azithromycin levels in the alveolar macrophages and bronchial mucosa are 23 and 3.89 μg/ml, respectively (2). The MICs, MLCs, and MIC_{90}s presented are those measured under the conditions of this study. The pK_a is defined as the pH at which 50% of a drug exists in its ionized form and 50% exists in the ionized or active form. However, because the pK_a values of all four drugs studied are between 8.2 and 8.7, one would expect these values to change in parallel fashion relative to those of the other compounds, depending upon the pH of the in vitro experiment or the pH in different body sites. As the pH rises and falls, the amount of nonionized drug in each compartment will rise and fall for these basic drugs with higher pK_a values.

In a recent study (3), clarithromycin was compared with the ethylsuccinate salt of erythromycin. The results with clarithromycin and erythromycin were comparable: clinical success, 98 versus 95%, respectively; radiological success, 98 versus 94%, respectively; and eradication of *C. pneumoniae*, 79 versus 86%, respectively. The authors concluded that because either *C. pneumoniae* or *Mycoplasma pneumoniae* was detected in nearly 50% of the patients, a macrolide may be preferred for treatment of this uncomplicated pediatric pneumonia. In another comparison of the susceptibility of *C. pneumoniae* isolates to clarithromycin and erythromycin (14), Roblin et al. showed that patients improved clinically even though the organism persisted after treatment. Clarithromycin was 2- to 10-fold more active than erythromycin; with an MIC_{90} and MLC for 90% of the strains tested of 0.031 μg/ml, in contrast to 0.125 μg/ml for erythromycin. Frequently, the etiology of community-acquired pneumonia remains unknown, and few centers screen for *C. pneumoniae* (13). The difficulty of differential diagnosis of patients with community-acquired pneumonia on the basis of history, cough, fever, leukocyte count, and appearance of chest X ray is well-known. On the basis of currently available data, it appears that 2 to 3 weeks of doxycycline or erythromycin treatment is equivalent to 5 days of treatment with azithromycin (10).

Our study demonstrates that clarithromycin has in vitro activity comparable to the activities of azithromycin, erythromycin, and tetracycline. Clarithromycin penetrates well into bronchial epithelium and alveolar macrophages, at levels of penetration much higher than those of other macrolides and azalides. New macrolides such as clarithromycin and azithromycin appear to be better tolerated and have better activity than erythromycin against some of the organisms responsible for community-acquired pneumonia (4).

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