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SUSCEPTIBILITY

In Vitro Activity of Omadacycline against *Chlamydia pneumoniae*

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ABSTRACT The *in vitro* activities of omadacycline, azithromycin, doxycycline, moxifloxacin, and levofloxacin were tested against 15 isolates of *Chlamydia pneumoniae*. The minimum inhibitory concentration at which 90% of the isolates of *C. pneumoniae* were inhibited by omadacycline was 0.25 μg/ml (range, 0.03 to 0.5 μg/ml).

KEYWORDS *Chlamydia pneumoniae*, omadacycline, tetracyclines

Chlamydia pneumoniae is a frequent cause of community-acquired respiratory infections, including pneumonia and bronchitis, in adults and children (1). Antibiotics commonly used to treat *C. pneumoniae* respiratory infections include macrolides, quinolones, and tetracyclines, specifically doxycycline (2).

Omadacycline (PTK 0796) is a new aminomethylcycline with potent *in vitro* antibacterial activity against a broad range of bacteria that cause respiratory infections, including *Streptococcus pneumoniae* (3) and *Mycoplasma pneumoniae* (4). We compared the *in vitro* activity of omadacycline with those of azithromycin, doxycycline, moxifloxacin, and levofloxacin against 15 isolates of *C. pneumoniae*.

Tested isolates of *C. pneumoniae* included 2 isolates from ATCC (Manassas, VA), i.e., TW-183 (VR-2282) and CM-1 (VR-1360), and 13 human isolates from patients with community-acquired pneumonia, including bronchoalveolar lavage specimens from patients with pneumonia from the United States. Omadacycline, azithromycin, levofloxacin, moxifloxacin, and doxycycline were provided as powders and solubilized according to the manufacturers' instructions. Sterile stock solutions of 1,280 μg/ml were made and frozen at −80°C. Aliquots of the stock drug suspensions were diluted each time the assay was run. *C. pneumoniae* isolates were expanded to concentrations of 10^7 to 10^8 inclusion-forming units (IFU) per milliliter by serial passage in tissue culture with antibiotic-free medium as previously described (5). Isolates were purified by centrifugation at 500 rpm to bring down the cell debris. The chlamydia-containing supernatant was pelleted at 17,000 × *g* for 1 h. The pellet containing the chlamydia was then resuspended in sucrose phosphate glutamate (SPG) and centrifuged through a discontinuous renografin gradient. The chlamydial elementary body (EB)-containing band was then washed 3 times and resuspended in SPG. The titers of the EB suspension were determined in HEp-2 cells (ATCC CCL-23).

Susceptibility testing of *C. pneumoniae* isolates was performed in cell culture by using HEp-2 cells grown in 96-well microtiter plates as previously described (6). Each well was inoculated with 0.2 ml of the test strain diluted to yield 10^4 IFU/ml; the plates were centrifuged at 1,700 × *g* for 1 h and incubated at 35°C for 1 h. Wells were then aspirated and overlaid with medium containing 1 μg/ml of cycloheximide and serial 2-fold dilutions of the test drugs. After incubation at 35°C for 72 h, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the chlamydial lipopolysaccharide genus-specific antigen (Pathfinder Chlamydia Culture Confirmation System; Bio-Rad, Hercules, CA). The MIC was the lowest antibiotic concentration at which no inclusions were seen. The minimal bactericidal concentration (MBC) was

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TABLE 1 Activities of omadacycline and comparators against 15 isolates of *C. pneumoniae*

Drug	MICs (mg/ml)			MBCs (mg/ml)	
	Range	MIC_{50}	MIC_{90}	Range	MBC_{90}
Omadacycline	0.03–0.5	0.06	0.25	0.06–0.5	0.5
Azithromycin	0.03–0.06	0.06	0.06	0.06–0.25	0.25
Levofloxacin	0.25–0.5	0.5	0.5	0.25–2	2
Moxifloxacin	0.25–1.0	0.5	1.0	0.5–1.0	1.0
Doxycycline	0.06–0.25	0.125	0.125	0.25–0.5	0.5

determined by aspirating the antibiotic-containing medium, washing wells twice with phosphate-buffered saline, and adding antibiotic-free medium. The infected cells were frozen at −70°C, thawed, passed onto new cells, incubated for 72 h, and then fixed and stained as described above. The MBC was the lowest antibiotic concentration that resulted in no inclusions after passage. All tests were run in duplicate. Positive (infection ± equal volumes drug carrier without active drug added) and negative (addition of medium ± active drug, not containing any bacteria) infection controls were included with each experiment.

The MICs and MBCs for *C. pneumoniae* are shown in Table 1. The MIC_{90} of *C. pneumoniae* isolates to omadacycline was 0.25 μg/ml (range, 0.03 to 0.5 μg/ml). The MBC_{90} of the isolates to omadacycline was 0.5 μg/ml (range, 0.6 to 0.5 μg/ml).

The *in vitro* activity of omadacycline against *C. pneumoniae* infection was comparable with the other antibacterial drugs tested, including doxycycline. The MIC_{90} and MBC_{90} were within 2 dilutions of the other agents. However, *in vitro* activity may not necessarily predict microbiological efficacy *in vivo* against *C. pneumoniae* infection (2).

The *in vitro* activity of omadacycline is comparable to those of several antibiotics with proven clinical efficacy. The results presented in this report therefore suggest that omadacycline would be effective for the treatment of infections due to *C. pneumoniae* isolates, depending on the concentrations achieved at the site of infection. Omadacycline has been demonstrated to achieve high, sustained concentrations in plasma, epithelial lining fluid, and alveolar cells, suggesting that it may be effective in the treatment of pulmonary infections, including those caused by intracellular organisms such as *C. pneumoniae* (7). A recent phase 3 trial found that a single daily dose of omadacycline was noninferior to moxifloxacin for treatment of community-acquired pneumonia caused by *C. pneumoniae*, based on clinical response (8).

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