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In Vitro Activity of a New Ketolide Antibiotic, HMR 3647, against *Chlamydia pneumoniae*

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The in vitro activities of HMR 3647, roxithromycin, erythromycin, and azithromycin against 19 strains of *Chlamydia pneumoniae* were tested. The MIC at which 90% of the isolates are inhibited and the minimum bactericidal concentration at which 90% of the isolates are killed of HMR 3647 were 0.25 μg/ml (range, 0.015 to 2 μg/ml). Nine recently obtained clinical isolates from children with pneumonia were more susceptible (MICs, 0.015 to 0.0625 μg/ml) than older strains that had been passaged more extensively.

The ketolides are a new class of macrolide antibiotics with a 3-keto function instead of the cladinose sugar. The ketolides are acid stable and have activity against a broad range of respiratory pathogens, including multiresistant pneumococci, *Haemophilus influenzae*, *Legionella* species, and *Mycoplasma pneumoniae* (1, 4, 5). *Chlamydia pneumoniae* is emerging as an important cause of community-acquired respiratory infection in adults and children worldwide. Clinically these infections cannot be readily differentiated from those caused by "atypical" pathogens, such as *M. pneumoniae*. Data on the activity of HMR 647 or other ketolides against *C. pneumoniae* are very limited; only two strains, TW 183 and IOL 207, have been tested so far (2). Therefore, we compared the in vitro activities of HMR 3647, roxithromycin, erythromycin, and azithromycin against 19 isolates of *C. pneumoniae*, including recently obtained clinical isolates.

Isolates of *C. pneumoniae* tested included a reference strain (TW 183), 15 isolates from children and adults with pneumonia from the United States (AR39, T2023, T2043, W6805, CDC8, T2337, 279, 327, 040, 015, 380, 117, 206, 032, and 428), an isolate from a child with pneumonia from Japan (J-21), and 2 strains from bronchoalveolar lavage specimens from patients with human immunodeficiency virus infection and pneumonia from the United States (BAL15 and BAL16). HMR 3647, roxithromycin, erythromycin, and azithromycin were provided as powders and solubilized according to the instructions of the manufacturers.

Susceptibility testing of *C. pneumoniae* was performed with cycloheximide-treated HEp-2 cells grown in 96-well microtiter plates (6). Each well was inoculated with 0.1 ml of the test strain diluted to yield 10^3 to 10^4 inclusion-forming units per ml; the plates were then centrifuged at 1,700 × *g* for 1 h and incubated at 35°C for 1 h. Wells were then aspirated and overlaid with 0.2 ml of medium containing 1 μg of cycloheximide per ml and serial twofold dilutions of the test drug. 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; Kallestad Diagnostics, Chaska, Minn.). The MIC was the lowest antibiotic concentration at which no inclusions were seen. The minimal bactericidal concentration (MBC) was 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 which resulted in no inclusions after passage. All tests were run in triplicate.

The results are shown in Table 1. The MIC at which 90% of the isolates are inhibited (MIC_{90}) of HMR 3647 was 0.25 μg/ml (range, 0.015 to 2 μg/ml); the MBC_{90} was also 0.25 μg/ml (range, 0.015 to 2 μg/ml). The MIC_{90}s for roxithromycin, erythromycin, and azithromycin were 0.5, 0.25, and 0.25 μg/ml, respectively. Nine recent clinical isolates of *C. pneumoniae* were more susceptible to HMR 3647 than older isolates; the MICs ranged from 0.031 to 0.0625 μg/ml (data not shown). The MIC for the least susceptible isolate, T2023, was 2 μg/ml. T2023 was first isolated from a child with pneumonia in 1987 and has been extensively passaged in our laboratory. This variability of susceptibility between recent clinical isolates and older isolates was not observed with the other antibiotics tested.

HMR 3647 had overall activity in vitro against *C. pneumoniae* comparable to those of the other macrolides tested. However, there was wide interstrain variation in susceptibility that appeared to be related to the age of the isolates. HMR 3647 was 10-fold more active against recent clinical isolates of *C. pneumoniae* than against older isolates that had been passaged more intensively in the laboratory. The older isolates, including T2023, had been passaged as many as 100 times, compared to only 5 to 6 times for the recent clinical isolates. HMR 3647 was also 10-fold more active against these isolates than were the other macrolides tested. This degree of variability was not observed with the other macrolides tested in this study or previously with other macrolides (3). Studies comparing the in vitro activities of several quinolones, including oflox-

TABLE 1. Activities of HMR 3647 and other antibiotics against 19 isolates of *C. pneumoniae*

Drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
HMR 3647	0.031–2	0.0625	0.25	0.031–2	0.25
Roxithromycin	0.0625–2	0.25	0.5	0.0625–2	0.5
Erythromycin	0.015–0.25	0.125	0.25	0.0625–0.5	0.25
Azithromycin	0.015–0.5	0.125	0.25	0.015–0.5	0.25

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acin, levofoloxacin, and trovafloxacin, against many of the same strains tested here found very little variability, with MICs ranging from only 0.5 to 1 μg/ml (3, 7, 8).

The available data on the activity of ketolides against *Chlamydia* species are very limited. Haider et al. evaluated the activity of another ketolide compound, RU 64004, against one strain of *Chlamydia psittaci* and two strains each of *Chlamydia trachomatis* and *C. pneumoniae* (2). The *C. pneumoniae* strains tested were two reference strains, TW 183 and IOL 207. The MICs and MBCs were ≤0.01 to 0.05 μg/ml for all three species. These results are dramatically different from those of the present study, where we found the MIC of HMR 3647 against TW 183 to be 0.125 μg/ml, 10-fold less than that found by Haider et al. We also tested RU 64004 side by side in vitro against the same isolates, and it was less active than HMR 3647, with a MIC_{90} of 0.5 μg/ml and an MBC_{90} of 1 μg/ml (data not shown). We also saw the same patterns of susceptibility seen with HMR 3647; recent clinical isolates were more susceptible, with MICs ranging from 0.015 to 0.25 μg/ml. One possible explanation for this discrepancy is the different tissue culture system used by Haider et al. They used McCoy cells, which we have found to be 3 log units less susceptible to infection with *C. pneumoniae* than HEp-2 cells (6). This might lead to an overall lower rate of infection in the McCoy cell monolayers, resulting in lower MICs.

HMR 3647 shows promising activity in vitro against *C. pneumoniae*, especially against recent clinical isolates. The potential role of this antibiotic in the treatment of respiratory infection due to *C. pneumoniae* will depend on the results of treatment studies utilizing culture for diagnosis and evaluation of microbiologic efficacy.

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