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In Vitro Activity of a Novel Diaminopyrimidine Compound, Iclaprim, against *Chlamydia trachomatis* and *C. pneumoniae*

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The in vitro activities of iclaprim, a novel dihydrofolate reductase inhibitor, azithromycin, and levofloxacin were tested against 10 strains of *Chlamydia trachomatis* and 10 isolates of *Chlamydia pneumoniae*. For *C. trachomatis* and *C. pneumoniae*, the iclaprim MIC and minimal bactericidal concentration at which 90% of isolates were inhibited (MIC_{90} and MBC_{90}) were 0.5 μg/ml, compared to an azithromycin MIC_{90} and MBC_{90} of 0.125 μg/ml and levofloxacin MIC_{90}s and MBC_{90}s of 1 μg/ml for *C. trachomatis* and 0.5 μg/ml for *C. pneumoniae*.

Chlamydia trachomatis infection is the most common sexually transmitted infection in the United States, causing more than 3 million cases of cervicitis and urethritis every year. *Chlamydia pneumoniae* is a frequent cause of community-acquired respiratory infections, including pneumonia and bronchitis, in adults and children. Dihydrofolate reductase (DHFR) inhibitors, specifically trimethoprim, have been used for many years to treat infections due to a wide range of bacteria, usually in combination with sulfonamides. The antimicrobial activity against bacterial pathogens is mediated through inhibition of thymidylate synthesis and therefore nucleic acid synthesis. Since mammalian cells do not synthesize folic acid, human purine synthesis is not affected significantly. Even though chlamydiae synthesize folate, trimethoprim (TMP) or TMP-sulfamethoxazole cannot be used for the treatment of chlamydial disease, because trimethoprim is not active against chlamydiae (1).

Iclaprim (formerly AR-100), a novel DHFR inhibitor, has potent activity against gram-positive and gram-negative bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Escherichia coli* (C. G. Gemmell and G. Middlemas, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-2022, 2002; C. E. Good, A. Windau, S. Bajaksouzian, M. R. Jacobs, and P. C. Appelbaum, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-2023, 2002; R. L. Then, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-2021, 2002; M. R. Jacobs, A. Windau, S. Bajaksouzian, and P. C. Appelbaum, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-2026, 2002; A. Windau, S. Bajaksouzian, P. C. Appelbaum, and M. R. Jacobs, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-2025, 2002). There are no data on the activity of iclaprim against *Chlamydia* spp.; however, trimethoprim has previously been reported to have no significant activity against *C. trachomatis* in vitro (1). Therefore, we compared the activity of iclaprim, a new DHFR inhibitor, with the activities of azithromycin and levofloxacin against *C. trachomatis* and *C. pneumoniae.*

We tested 10 strains of *C. trachomatis*, including four clinical endocervical isolates: N16/CX, N17/CX, N18/CX, N19/CX, and ATCC strains HUW-43/CX (VR-879), JUW-36/CX (VR-886), IUW-12/UR (VR-880), LGV 434 (VR-902B), FICCAL3/CX (VR-346), and E-BOUR (VR-384B). Isolates of *C. pneumoniae* tested included three reference isolates, TW183 (VR-2282), CM-1 (VR-1360), and AR39 (ATCC 53592), and seven clinical isolates from adults and children with pneumonia, W6805, T2023 (ATCC VR-1310), T2043 (ATCC VR-1355), BAL-15, BAL-16, BAY255, and BAY493.

Antimicrobial agents were supplied as powders and solubilized according to manufacturers' instructions. Iclaprim (Arpida, Basel, Switzerland), azithromycin (Pfizer, New York, N.Y.), and levofloxacin (Ortho Pharmaceuticals, Raritan, N.J.) were used. In addition, TMP (Roche, N.J.) was tested against two isolates of *C. pneumoniae* (TW183 and CM-1). Susceptibility testing of *C. pneumoniae* was performed in cell culture by using HEp-2 cells grown in 96-well microtiter plates as previously described (3). Each experiment was set up in duplicate plates. Each well was inoculated with 0.1 ml of the test organism diluted to yield 10^3 to 10^4 inclusion-forming units per ml, 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, the cultures in one plate were fixed and stained for inclusions with fluorescein-conjugated antibody to the lipopolysaccharide genus antigen (Pathfinder; Bio-Rad Labs, Hercules, Calif.). 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 of the second plate, washing wells twice with phosphate-buffered saline, and adding antibiotic-free medium. Cultures were frozen at −70°C, thawed, passed onto fresh 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 assays were performed in triplicate.

The MICs and MBCs for *C. trachomatis* and *C. pneumoniae* are shown in Tables 1 and 2. The MICs and MBCs at which 90% of isolates were inhibited (MIC_{90}s and MBC_{90}s) of iclaprim for *C. trachomatis* and *C. pneumoniae* were 0.5 μg/ml.

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TABLE 1. Activity of iclaprim and other antibiotics against 10 strains of *C. trachomatis*

Drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
Iclaprim	0.5	0.5	0.5	0.5	0.5
Levofloxacin	0.5–1	0.5	1	0.5–1	1
Azithromycin	0.062–0.125	0.062	0.125	0.062–0.125	0.125

The MIC_{90}s and MBC_{90}s of azithromycin and levofloxacin for *C. trachomatis* were 0.125 and 1 μg/ml, respectively. The MIC_{90}s and MBC_{90}s of azithromycin and levofloxacin for *C. pneumoniae* were 0.125 and 0.5 μg/ml, respectively. The MICs and MBCs of iclaprim for *C. trachomatis* and *C. pneumoniae* indicate that its activity is comparable to those of both azithromycin and levofloxacin. By contrast to the activity of iclaprim, trimethoprim was inactive. The MICs and MBCs of trimethoprim for *C. pneumoniae* TW183 and CM-1 were ≥128 μg/ml.

Iclaprim is a bactericidal drug that demonstrated activity comparable to those of both azithromycin and levofloxacin. MICs and MBCs were consistent for all strains and isolates tested, especially in view of their wide geographic distribution. Even though both trimethoprim and iclaprim target DHFR, iclaprim was much more active. Trimethoprim had poor activity against *C. pneumoniae* in this study, similar to what was previously reported for *C. trachomatis*, with MICs of ≥128 μg/ml (2). Whereas most bacteria are unable to utilize exogenous folate, Fan et al. demonstrated that *C. trachomatis* L_2, *C. psittaci* 6BC, and *C. psittaci* strain francis appeared to both synthesize and to transport folate from the host cell in various degrees, depending on the strain (1). The relevance of this folate transport mechanism for in vitro or in vivo susceptibilities to trimethoprim and sulfonamides is not clear. Sulfonamides block the bacterial folic acid metabolism at a different site and have been used in a fixed combination with trimethoprim for synergistic effect. Iclaprim was synergistic when tested in combination with sulfonamides against a wide range of gram-positive and gram-negative bacteria (S. Hawser, L. Weiss, M. Fischer, D. Gillessen, I. Kompis, and K. Islam, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-2019, 2002). Although *C. trachomatis* is susceptible to sulfonamides, *C. pneumoniae* is not (1). Earlier studies of the synergy of trimethoprim and sulfamethoxazole against *C. trachomatis* found the combined activity to be only additive, with most of the activity being due to the sulfonamide, but it may be useful to perform synergy studies with iclaprim and sulfonamides to look for similar effects on *C. trachomatis* (2). However, based on its in vitro activity in this study, using iclaprim as a single drug for the treatment of chlamydial infections could be adequate.

These data suggest that new DHFR inhibitors may have a potential role in the treatment of respiratory infections due to *C. pneumoniae* as well as genital infections caused by *C. trachomatis*. Studies to evaluate efficacy in patients using culture-based diagnostic methods are indicated.

TABLE 2. Activity of iclaprim and other antibiotics against 10 isolates of *C. pneumoniae*

Drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
Iclaprim	0.5	0.5	0.5	0.5	0.5
Levofloxacin	0.5–1	0.5	0.5	0.5–1	0.5
Azithromycin	0.062–0.125	0.062	0.125	0.062–0.125	0.125

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