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

S. A. Kohlhoff,1* P. M. Roblin,1 T. Reznik,1 S. Hawser,2 K. Islam,2 and M. R. Hammerschlag1

Department of Pediatrics, Downstate Medical Center, State University of New York, Brooklyn, New York,1 and Arpida, Ltd., Munchenstein, Switzerland2

Received 19 November 2003/Returned for modification 24 December 2003/Accepted 29 January 2004

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 are obligate intracellular pathogens, they contain DHFR and require folic acid. Trimethoprim has been effective against many isolates of Chlamydia trachomatis, including four clinical isolates: N16/CX, N17/CX, N18/CX, N19/CX, and ATCC strains HUW-43/CX (VR-879), IUW-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 10^9 to 10^4 inclusion-forming units per ml, centrifuged at 1,700 x 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 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 (MIC90 and MBC90) of iclaprim for C. trachomatis and C. pneumoniae were 0.5 μg/ml.


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 10^9 to 10^4 inclusion-forming units per ml, centrifuged at 1,700 x 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 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 (MIC90 and MBC90) of iclaprim for C. trachomatis and C. pneumoniae were 0.5 μg/ml.

* Corresponding author. Mailing address: Department of Pediatrics, Box 49, SUNY Downstate Medical Center, 450 Clarkson Ave., Brooklyn, NY 11203-2908. Phone: (718) 270-7588. Fax: (718) 270-3210. E-mail: stephan.kohlhoff@downstate.edu.


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 10^9 to 10^4 inclusion-forming units per ml, centrifuged at 1,700 x 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 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 (MIC90 and MBC90) of iclaprim for C. trachomatis and C. pneumoniae were 0.5 μg/ml.
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* L2, *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.

### REFERENCES