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In Vitro Activities of Rifamycin Derivatives ABI-1648 (Rifalazil, KRM-1648), ABI-1657, and ABI-1131 against *Chlamydia trachomatis* and Recent Clinical Isolates of *Chlamydia pneumoniae*

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ABI-1648 (rifalazil) is a semisynthetic rifamycin with potent bactericidal activity against intracellular respiratory bacteria, including *Mycobacterium tuberculosis*, and a long half-life (~60 h) and thus can be administered once weekly. We therefore tested the in vitro activities of ABI-1648, its derivatives ABI-1657 and ABI-1131, azithromycin, and levofloxacin against 10 strains of *Chlamydia trachomatis* and 10 recent clinical isolates of *Chlamydia pneumoniae*. The MICs at which 90% of the isolates were inhibited and the minimal bactericidal concentration at which 90% of the isolates were killed for ABI-1648, ABI-1657, and ABI-1131 were 0.0025 μg/ml for *C. trachomatis* and 0.00125 to 0.0025 μg/ml for *C. pneumoniae*. ABI-1648, ABI-1657, and ABI-1131 were 10- to 1,000-fold more active than azithromycin and levofloxacin.

Although rifamycins, including rifampin, have been demonstrated to have excellent activity against *Chlamydia pneumoniae* and *Chlamydia trachomatis* in vitro, because of concern about the potential rapid development of resistance, these compounds have not been evaluated for the treatment of human chlamydial infections (3, 4, 7, 8, 10, 11). ABI-1648 (rifalazil, KRM-1648) is a semisynthetic rifamycin which has demonstrated potent activity against a variety of bacteria, including *Mycobacterium tuberculosis, Mycobacterium avium* complex, gram-positive bacteria including *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*, and most recently *Helicobacter pylori* (1, 5). ABI-1648 is also distinguished by having a long half-life, approximately 60 h, which has allowed once-weekly dosing in patients with pulmonary tuberculosis (2). Acquired drug resistance did not occur in any patient in this trial (2). We therefore tested the in vitro activities of ABI-1648, its derivatives ABI-1657 and ABI-1131, azithromycin, and levofloxacin against *C. trachomatis* and recent clinical isolates of *C. pneumoniae*.

Strains of *C. trachomatis* tested included E-BOUR (ATCC VR-384B), F-IC-CAL3 (ATCC VR-346), H-UW-43 (ATCC VR-879), J-UW-36 (ATCC VR-886), $L_2$434 (ATCC VR-902B), and five clinical cervical isolates. Isolates of *C. pneumoniae* tested included reference isolates TW183 and AR-39 (Washington Research Foundation, Seattle; ATCC VR-2282 and ATCC 53592), six recent clinical isolates (109, 453, 493, 912, 08002, and 08016) from adults enrolled in a multicenter community-acquired pneumonia treatment study conducted in the United States, and W6805 and J21, clinical isolates from Wisconsin and Japan, respectively. ABI-1648, ABI-1657, and ABI-1131 (ActivBiotics, Cambridge, Mass.), azithromycin (Pfizer, New York, N.Y.), and levofloxacin (Ortho Pharmaceuticals, Raritan, N.J.) were supplied as powders and solubilized according to the manufacturers' instructions. Susceptibility testing of *C. trachomatis* and *C. pneumoniae* was performed in cell culture with HEp-2 cells grown in 96-well microtiter plates as previously described (6). 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 for a multiplicity of infection of 1:1, 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 Laboratories, 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. Three replicates were conducted for each assay.

The MIC and MBCs for *C. trachomatis* are shown in Table 1. The MIC and MBC at which 90% of isolates were inhibited or killed (MIC_{90} and MBC_{90}, respectively) of ABI-1648, ABI-1657, and ABI-1131 were 0.0025 μg/ml, compared to 0.125 and 1.0 μg/ml for azithromycin and levofloxacin, respectively. The MICs and MBCs for *C. pneumoniae* are shown in Table 2. The MIC_{90} and MBC_{90} of ABI-1648 and ABI-1657 were 0.00125 μg/ml, and those of ABI-1131 were 0.0025 μg/ml, compared to 0.125 and 1.0 μg/ml for azithromycin and levofloxacin, respectively. The MICs of ABI-1648, ABI-1657, and ABI-1131 for *C.*

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TABLE 1. Activities of ABI-1648, ABI-1657, ABI-1131, and other antibiotics against 10 isolates of *C. trachomatis*

Drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
ABI-1648	0.00125–0.0025	0.0025	0.0025	0.00125–0.0025	0.0025
ABI-1657	0.00125–0.0025	0.0025	0.0025	0.00125–0.0025	0.0025
ABI-1131	0.00125–0.0025	0.0025	0.0025	0.00125–0.0025	0.0025
Azithromycin	0.062–0.125	0.125	0.125	0.062–0.125	0.125
Levofloxacin	0.5–1.0	0.5	1.0	0.5–1.0	1.0

trachomatis and *C. pneumoniae* in the present study were very consistent, especially in view of the wide geographic distribution of the isolates tested. We also tested five of the *C. pneumoniae* isolates against rifampin; the MICs and MBCs were 0.015 μg/ml.

Rifampin and other rifamycins have been known for over 30 years to be very active in vitro against *C. trachomatis* and *C. pneumoniae*, with MICs ranging from 0.0075 to 0.03 μg/ml (7, 8, 10, 11). However, as early as 1973, Keshishyan et al. (8) demonstrated rapid single-step emergence of resistance to rifampin in *C. trachomatis* in eggs. Similar results were also obtained in tissue culture (11). All these studies have examined *C. trachomatis*; there are no data on the possible emergence of resistance in *C. pneumoniae.* However, because of the concern about the ease with which resistance to rifampin developed in vitro, few clinical trials evaluating rifampin or related drugs for the treatment of human chlamydial infections have been done. The risk of emergence of resistance may not occur with every rifamycin compound. Treharne et al. (11) found that although *C. trachomatis* rapidly developed resistance to rifampin after serial passage in subinhibitory concentrations, the organism remained susceptible to rifabutin even after 10 serial passages. There are no data on in vitro emergence of resistance in either *C. trachomatis* or *C. pneumoniae* with ABI-1648 or its derivative compounds, ABI-1657 and ABI-1131. Limited experience with the use of ABI-1648 for treatment of pulmonary tuberculosis in animals and humans has not documented emergence of resistance (2). A recent study examining in vitro resistance in *S. aureus* found that some isolates which developed high-level resistance to rifampin were still susceptible to ABI-1648 (12).

ABI-1648, ABI-1657, and ABI-1131 were the most active antibiotics against *Chlamydia* spp., with MICs and MBCs 10- to 1,000-fold lower than those of azithromycin and levofloxacin. These compounds were also 10-fold more active against *C. pneumoniae* than rifampin. The MIC_{90}s and MBC_{90}s in the present study were identical to those previously reported by Kuo et al. (9) in 1997, where they found the MIC and MBC of ABI-1648 for one isolate each of *C. pneumoniae* (TW-183) and *C. trachomatis* (B/TW-5/OT) to be 0.00125 to 0.0025 μg/ml. In addition, they found the in vitro activity of ABI-1648 in combination with azithromycin to be additive. Using a mouse model of *C. pneumoniae* pneumonitis, Kuo et al. (9) also found that ABI-1648 at a dose of 10 mg/kg for 3 days was 100% effective in eradication of the organism from the lungs of infected animals 5 days after the initiation of treatment. They suggested that the combination of ABI-1648 and other antibiotics might be even more effective. In addition, such combinations may prevent the development of resistance. Dresses-Werringloer et al. (3) and Freidank et al. (4) recently reported that the combinations of rifampin with azithromycin and/or ofloxacin and doxycycline were synergistic against *C. trachomatis* in vitro. Treatment with rifampin alone, at a concentration of 0.015 μg/ml (twice the MIC) for up to 20 days, resulted in emergence of resistance, with the MICs increasing to 4 to 256 μg/ml (3). No development of resistance occurred when rifampin was used in combination with the other antibiotics (3).

TABLE 2. Activities of ABI-1648, ABI-1657, ABI-1131, and other antibiotics against 10 isolates of *C. pneumoniae*

Drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
ABI-1648	0.00125	0.00125	0.00125	0.00125	0.00125
ABI-1657	0.00125–0.0025	0.00125	0.00125	0.00125–0.0025	0.00125
ABI-1131	0.00125–0.0025	0.00125	0.0025	0.00125–0.0025	0.0025
Azithromycin	0.062–0.125	0.062	0.125	0.062–0.125	0.125
Levofloxacin	0.5–1.0	0.5	1.0	0.5–1.0	1.0

The long half-life of ABI-1648, which is similar to that of azithromycin, also offers the potential for single-dose administration or weekly dosing, depending on the chlamydial species and type of infection. Given the high degree of activity in vitro, ABI-1648 and its derivatives should be evaluated for the treatment of human chlamydial infections.

REFERENCES

1. **Akada, J. K., M. Shirai, K. Fujii, K. Okita, and T. Nakazawa.** 1999. In vitro anti-*Helicobacter pylori* activities of new rifamycin derivatives KRM-1648 and KRM-1657. Antimicrob. Agents Chemother. **43:**1072–1076.
2. **Deitze, R., L. Teixeira, L. Márcia, C. Rocha, M. Palaci, J. L. Johnson, C. Wells, L. Rose, K. Eisenbach, and J. J. Ellner.** 2001. Safety and bactericidal activity of rifalazil in patients with pulmonary tuberculosis. Antimicrob. Agents Chemother. **45:**1972–1976.
3. **Dresses-Werringloer, U., I. Padubrin, H. Zeidler, and L. Köhler.** 2001. Effects of azithromycin and rifampin on *Chlamydia trachomatis* infection in vitro. Antimicrob. Agents Chemother. **45:**3001–3008.
4. **Freidank, H. M., P. Losch, H. Vögele, and M. Weidmann-Al-Ahmad.** 1999. In vitro susceptibilities of *Chlamydia pneumoniae* isolates from German patients and synergistic activity of antibiotic combinations. Antimicrob. Agents Chemother. **43:**1808–1810.
5. **Fujii, K., A. Tsuji, S. Miyazaki, K. Yamaguchi, and S. Goto.** 1994. The in vitro and in vivo activities of KRM-1648 and KRM-1657, new rifamycin derivatives. Antimicrob. Agents Chemother. **38:**1118–1122.
6. **Hammerschlag, M. R., K. K. Qumei, and P. M. Roblin.** 1992. In vitro activities of azithromycin, clarithromycin, L-ofloxacin, and other antibiotics against *Chlamydia pneumoniae*. Antimicrob. Agents Chemother. **36:**1573–1574.
7. **Jones, R. B., G. L. Ridgway, S. Boulding, and K. L. Hunley.** 1983. In vitro activity of rifamycins alone and in combination with other antibiotics against *Chlamydia trachomatis*. Rev. Infect. Dis. **5**(Suppl. 3)**:**S556–S561.
8. **Keshishyan, H., L. Hanna, and E. Jawetz.** 1973. Emergence of rifampin-resistance in *Chlamydia trachomatis*. Nature **244:**173–174.
9. **Kuo, C.-C., J. T. Grayston, T. Hidaka, and L. M. Rose.** 1997. A comparison of the in vitro sensitivity of *Chlamydia pneumoniae* to macrolides and a new benzoxazinorifamycin, KRM-1648. Infect. Dis. Ther. **21:**317–321.
10. **Schachter, J.** 1983. Rifampin in chlamydial infections. Rev. Infect. Dis. **5**(Suppl. 3)**:**S562–S564.
11. **Treharne, J. D., P. J. Yearsley, and R. C. Ballard.** 1989. In vitro studies of *Chlamydia trachomatis* susceptibility and resistance to rifampin and rifabutin. Antimicrob. Agents Chemother. **33:**1393–1394.
12. **Wichelhaus, T. A., V. Schäfer, V. Brade, and B. Böddinghaus.** 2001. Differential effect of *rpoB* mutations on antibacterial activities of rifampicin and KRM-1648 against *Staphylococcus aureus*. J. Antimicrob. Chemother. **47:** 153–156.