Activities of Rifamycin Derivatives against Wild-Type and \textit{rpoB} Mutants of \textit{Chlamydia trachomatis}

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Rifalazil, a semisynthetic rifamycin, was shown previously to have exceptional potency against \textit{Chlamydia trachomatis} (MIC of 0.00025 \(\mu\)g/ml). We therefore tested 250 additional rifamycin derivatives and identified 12 with activities that are eightfold more potent than that of rifalazil. These compounds also showed exceptional activities against rifampin-resistant strains that carry missense mutations in the \textit{rpoB} gene. The antimicrobial potency and intracellular penetration of these agents suggest their potential in treatment of chlamydial infections.

Rifampin, a rifamycin derivative, is one of the most active antimicrobial agents against the obligate intracellular pathogen \textit{Chlamydia trachomatis}, but its tendency to select for resistant strains makes it less attractive than other drugs for routine treatment of chlamydial infections (3, 4, 10). Rifalazil (ABI-1648), a semisynthetic rifamycin which has potent activity against several clinically important bacterial pathogens, including \textit{Staphylococcus aureus}, \textit{Streptococcus pneumoniae}, \textit{Streptococcus pyogenes}, \textit{Helicobacter pylori}, \textit{Clostridium difficile}, and \textit{Mycoplasma tuberculosis}, has also been shown to be effective in cell culture against \textit{Chlamydia trachomatis} and \textit{Chlamydia pneumoniae}, with MICs in the range of 0.00025 \(\mu\)g/ml (5, 8, 9, 12). The antibacterial activities of rifamycin and rifampin derivatives, including rifalazil, result from inhibition of the bacterial RNA polymerase by binding to its \(\beta\) subunit encoded by the \textit{rpoB} gene (1, 2). With regard to \textit{Chlamydia} infections, rifalazil also has excellent tissue and cellular penetration and has had a good safety profile in human clinical trials to date (9). Unlike rifampin, interaction of rifalazil with the P450 system is insignificant, and thus it produces less interference with metabolism of other drugs (6, 7). Furthermore, rifalazil was shown recently to retain substantial activity against strains of \textit{C. trachomatis} that are highly resistant to rifampin (12). These features prompted us to evaluate additional rifamycin derivatives that are closely related to rifalazil for potency against wild-type \textit{C. trachomatis}, as well as against rifampin-resistant strains of \textit{Chlamydia trachomatis} containing missense mutations in the \textit{rpoB} gene (12).

Assays to determine the MICs of rifampin, rifalazil, and other rifamycin derivatives for \textit{C. trachomatis} were performed as previously described (11). Briefly, \textit{C. trachomatis} D/uw-3 was inoculated onto monolayers of McCoy cells in 96-well microtiter plates and centrifuged for 1 h. Immediately following centrifugation, wells were overlaid with growth medium containing serial twofold dilutions of the appropriate drug and incubated for 48 h. Wells were then fixed with methanol, labeled with fluorescein isothiocyanate-labeled anti-\textit{Chlamydia} lipopolysaccharide monoclonal antibody, and visualized by fluorescence microscopy as described previously (11). The MIC was defined as the minimum drug concentration at which no \textit{C. trachomatis} were detected while the organisms were exposed to the drug. To measure the minimum cidal concentration (MCC) of selected rifamycin derivatives, D/uw-3 was inoculated onto cell cultures exposed to the selected agents and incubated as described above. The cells were then subpassaged to drug-free cell cultures. The MCC for a given agent was defined as the minimum concentration at which no viable chlamydial organisms were detected in the first subpassage in drug-free cell cultures. The minimum concentrations at which no viable chlamydial organisms were detected in three consecutive passages in drug-free cell cultures (MCCs) were also determined (11).

We determined the MICs of 250 rifamycin derivative compounds for \textit{C. trachomatis} D/uw-3. Overall, most of these compounds had MICs ranging from 0.000016 \(\mu\)g/ml to 0.01 \(\mu\)g/ml. The distribution of the number of compounds with a particular MIC is shown in Fig. 1. For points of reference, the MICs of rifampin and rifalazil were determined to be 0.004 and 0.00025 \(\mu\)g/ml, respectively. The 12 most potent compounds among the 250 rifamycin derivatives tested had MICs of 0.000032 \(\mu\)g/ml, with one compound, ABI-1662, showing an MIC of 0.000016 \(\mu\)g/ml. These extremely potent agents are, thus, new rifamycin derivatives with significantly improved antimicrobial activities compared with those of rifampin and rifalazil.

Rifalazil has unusual properties that may contribute to its activity against intracellular infections, such as chlamydiae. These include exceptional tissue penetration and high intracellular concentrations of compound (9). Interestingly, rifalazil is poorly soluble in aqueous solutions at a neutral pH (~1 \(\mu\)g/ml [unpublished results]). It is possible that more water-

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soluble compounds might not partition as extensively inside mammalian cells and might therefore have less activity against *C. trachomatis* than rifalazil. In fact, the trend was that the most water-soluble compounds were the least potent in terms of antichlamydial activity. When solubility exceeded 10 μg/ml, compounds were uniformly less active against *Chlamydia* (Fig. 2). However, there was not a strict inverse relationship between solubility and activity against *C. trachomatis*; 5 of the 14 more soluble compounds had strong activities, including 3 compounds having at least as much activity as rifalazil. Importantly, for potent compounds that were poorly soluble, the solubility exceeded the MIC for *C. trachomatis* by a factor of at least 4,000-fold.

A panel of four *C. trachomatis* L2 mutants that are highly resistant to rifampin was then tested for susceptibility to identify potent compounds (12). These mutants all carried missense mutations in the *rpoB* gene (Table 1). Significantly, we found that all four mutants were susceptible to the most active compounds. The MICs of rifalazil ranged from 0.002 to 0.064 μg/ml, considerably lower than the MICs of rifampin, which ranged from 0.5 to 512. Among the selected rifamycin derivatives listed in Table 1, ABI-0046, ABI-1131, and ABI-1662 demonstrated overall improved MICs for the mutants, even compared to rifalazil.

Besides measuring inhibitory potency, we also assessed these agents’ bactericidal potency by determining the MCCs and MCC3s for wild-type and rifampin-resistant strains (Table 2). These compounds demonstrated excellent potencies against *C. trachomatis*, especially compound ABI-1662.

Structures of the most potent compound against both wild-type and mutant strains of *C. trachomatis* (ABI-1662), as well as that of another potent compound (ABI-1131) (13) and rifalazil, are shown in Fig. 3. Although it is difficult to derive structure-activity relationships at this time, it is clearly possible to make subtle modifications outside of the ansa ring that can increase potency in cell culture. If the ability of rifalazil to penetrate tissue is an important component of potency, the propensity of these compounds to penetrate mammalian cells may lead to potent in vivo activities, as with rifalazil (5).

Compound ABI-0046 is noteworthy in that it is no more potent against wild-type *C. trachomatis* than rifalazil, but it demonstrated better potency against all four *rpoB* mutants tested. The attribute of having selectively more potent activities against rifampin-resistant mutant strains suggests possible differences in the affinities of these two compounds for mutant and wild-type RNA polymerases. This hypothesis should be tested.

In conclusion, our studies suggest that novel rifamycin derivatives, especially ABI-1662, ABI-1131, ABI-0046, and ABI-0204, are promising new antibiotics that have improved MICs, MCCs, and MCC3s for wild-type *C. trachomatis*. These compounds are also potent against rifampin-resistant strains, as indicated by their excellent MICs. Because of their extraordinary in vitro potencies against both the wild-type and rifampin-resistant variants of *C. trachomatis*, these compounds warrant further evaluation as to their usefulness for treatment of chlamydial infections in vivo. Further investigation may also illuminate the molecular mechanisms by which these potent compounds are effective against rifampin-resistant bacteria. Such studies may define whether the potencies they exhibit are due to a possible increased uptake and intracellular concentration.
an improved interaction with the mutant polymerases compared to rifampin, or both.

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