Letters to the Editor

Activity of Novel Benzoxazinorifamycins against Rifamycin-Resistant *Streptococcus pyogenes*

Serious gram-positive infections are frequently caused by *Staphylococcus aureus* or *Streptococcus pyogenes*. The rifamycin rifampin (RIF) is often used as part of the treatment for such infections, because it has potent bactericidal activity against both free-living and biofilm-associated gram-positive bacteria (13) and has favorable pharmacokinetics (2). Despite this, RIF's use as monotherapy has been limited because of treatment failure due to the emergence of rifamycin-resistant organisms (3, 4, 7, 8). Resistance results from amino acid substitutions within the conserved rifamycin resistance-determining region (RRDR) of the RpoB subunit of RNA polymerase, the target of rifamycins. Because of resistance emergence, RIF is used mainly as part of a multidrug treatment regimen in which the partner drug(s) is able to prevent outgrowth of resistant subpopulations.

A previous study described the activity of the benzoxazinorifamycins rifalazil (RFZ) and related new chemical entities (NCEs) against RIF-resistant *S. aureus* (10). The maximum MICs of the most potent NCEs were 2 to 4 µg/ml against the most rifamycin-resistant *S. aureus* strains. RIF and RFZ have MICs of >512 µg/ml against these same strains (10). Considering that a large proportion of serious gram-positive infections are caused by streptococci, we sought to determine in the present study whether NCEs had improved activity against rifamycin-resistant strains of *S. pyogenes*.

The two mutations conferring RIF resistance in clinical isolates of *S. pyogenes* (4, 7) and several mutations in other streptococci (1, 5, 6, 9, 12) all reside in the RRDR of RpoB in these organisms. *S. pyogenes* ATCC 19615 mutants with decreased susceptibility to RIF were selected for and characterized as

described previously (10). The resistance frequencies of *S. pyogenes* for RIF were 7.0×10^{-8} (at 0.24 µg/ml), 6.0×10^{-8} (at 0.12 µg/ml), and 2.9×10^{-8} (at 0.06 µg/ml). Thirty-three colonies with decreased susceptibility to RIF were isolated, and oligonucleotide primers SPY-F and SPY-R (5'-AACCGT CGTATCCGTGCCGTTGGT-3' and 5'-TGCCGTCGCAAC AGCAACTACCTG-3', respectively) were used to amplify and DNA sequence their RRDR regions, encoded by base pairs 1228 to 1884 (*S. pyogenes* MGAS315 coordinates; GenBank accession number NC 004070).

Seventeen discrete amino acid changes at 10 different positions within the *S. pyogenes* RRDR were identified (Table 1). Changes at H486, R489, and S491 correspond to positions that, when altered, confer the high levels of resistance to rifamycins in other bacteria (10). The H486N substitution was previously identified in a RIF-resistant clinical strain of *S. pyogenes* (7).

The MICs of RIF, RFZ, and NCEs for *S. pyogenes* ATCC 19615 and its rifamycin-resistant derivatives were determined using standard methods (11). *S. pyogenes* ATCC 19615 was 16- to 32-fold more susceptible to RFZ and NCEs than was *S. aureus* ATCC 29213 (Table 1) (10). MICs of the most potent NCEs (e.g., ABI-0043) against rifamycin-resistant *S. pyogenes* strains ranged from 0.00025 µg/ml to 0.25 µg/ml for the most resistant strain (Table 1) and 0.016 µg/ml to 4 µg/ml for the most rifamycin-resistant *S. aureus* strains (10). In fact, the MICs of NCEs against *S. pyogenes* mutants (Table 1) were consistently ≥ 8 -fold lower than those for *S. aureus* mutants containing mutations in the corresponding codon of the *rpoB* gene (10).

It is anticipated that NCEs having in vivo efficacy against

TABLE 1. Susceptibility of *S. pyogenes* RIF-resistant mutants to RIF, RFZ, and select NCEs

<i>S. pyogenes</i> RpoB alteration ^a (no. isolated)	MIC (µg/ml)					
	Rifampin	Rifalazil	NCE			
			ABI-0043	ABI-0370	ABI-0418	ABI-0420
None ^b	0.031	0.001	0.000125	0.00025	0.00025	0.00025
S472P (1)	0.5	0.004	0.001	0.002	0.00025	0.004
D476Y (2)	2	0.031	0.016	0.031	0.002	0.002
D476G (1)	0.25	0.008	0.001	0.00025	0.002	0.0005
D476N (2)	0.5	0.004	0.00025	0.00025	0.00025	0.00025
H486N (2)	4	0.016	0.008	0.016	0.001	0.016
H486Y (2)	128	>128	0.063	0.031	0.031	0.031
R488H (2)	0.25	0.002	0.001	0.002	0.00025	0.002
R489H (2)	2	0.063	0.063	0.125	0.008	0.063
S491Y (2)	64	>128	0.25	0.125	1	0.25
S491F (1)	128	>128	0.25	0.25	1	0.5
A492P (1)	0.125	0.001	0.00025	0.00025	0.00025	0.00025
H514P (1)	0.125	0.0005	0.00025	0.00025	0.00025	0.00025
P524S (9)	0.25	0.002	0.0005	0.001	0.00025	0.002
P524T (1)	0.25	0.004	0.001	0.002	0.00025	0.002
P524L (1)	128	>128	0.031	0.031	0.004	0.031
I532L (2)	0.5	0.008	0.004	0.004	0.00025	0.004
I532F (1)	64	>128	0.125	0.125	0.063	0.125

^a The changes denoted in boldface have been found in rifampin-resistant clinical strains of *S. pyogenes* (H486N only) and *S. pneumoniae* (all changes in boldface).

^b No alterations were present compared to the sequence from MGAS315.

rifamycin-resistant *S. aureus* strains would be effective against the most resistant *S. pyogenes* mutants. Experimental infection models using resistant strains from both of these species should verify this assumption.

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