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In Vitro Activity of Novel Rifamycins against Gram-positive Clinical Isolates

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Abstract We describe novel rifamycins that have improved activity, compared with rifampin, against clinical isolates of staphylococci and streptococci, with MIC_{90}s of 0.008 and 0.0005 μg/ml, respectively. This enhanced antibacterial activity, along with their potential lack of drug–drug interactions, are considerations that suggest the potential of these novel rifamycins in combination therapy to treat serious Gram-positive infections.

Keywords rifampin, rifalazil, ABI-0043, staphylococci, streptococci, MIC

Introduction

Rifamycins such as rifampin have been used primarily in multiple drug therapy to treat tuberculosis [1], and serious Gram-positive infections [2～4]. The benzoxazinorifamycins, including rifalazil (3′-hydroxy-5′-(4-isobutyl-1-piperazinyl) benzoxazinorifamycin, also known as KRM-1648 and ABI-1648) and derivatives (new chemical entities, NCEs) that have been recently described [5], have a similar mode of action to rifampin in that they inhibit bacterial RNA polymerases [6].

The use of rifamycins has been limited to combination therapy due to their propensity to select for resistant mutants when used as single agents [7～9]. The mutations responsible for resistance reside in the *rpoB* gene, which encodes the β subunit of RNA polymerase (RpoB) [10～12]. Amino acid positions altered as a result of these mutations are thought to interact directly with rifampin as revealed by X-ray crystallography studies [13].

One exception to this resistance phenomenon is rifalazil's activity and efficacy against Chlamydia. Rifalazil retains activity against rifampin-resistant Chlamydia strains [14, 15], and is efficacious as a monotherapeutic agent in patients with non-gonococcal urethritis [16]. However, the evaluation of other species, including *Mycobacterium tuberculosis* [1], *Staphylococcus aureus* [5], and *Streptococcus pyogenes* [17] indicates that strains resistant to rifampin are also cross-resistant to rifalazil.

In this study we investigated the activities of rifalazil and NCEs against clinical isolates of Gram-positive pathogens. These NCEs were previously shown to be efficacious in experimental *S. aureus* infection models [18], and to have good activity against rifamycin-resistant *S. aureus* [5] and *S. pyogenes* [17] strains when compared with rifampin.

Materials and Methods

Bacterial Strains

A total of 60 isolates of *S. aureus* were tested, including 20 methicillin-susceptible (MSSA), 20 methicillin-resistant (MRSA), and 20 quinolone and methicillin resistant (QMRSA) strains. A total of 40 isolates of *Staphylococcus epidermidis* were tested, including 20 methicillin-susceptible and 20 methicillin resistant strains. A total of 40 isolates of *Streptococcus pneumoniae* were tested, including 20 penicillin-suscpetible and 20 penicillin-resistant strains. Twenty isolates of *S. pyogenes* and 21 isolates of *Streptococcus agalactiae* were tested. A total of

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20 isolates of *Enterococcus faecalis* and 20 isolates of *E. faecium* were tested, including 10 vancomycin-susceptible and 10 vancomycin-resistant strains of each species. All of these isolates were from the Eurofins Medinet, Inc. strain collection. In addition, 9 vancomycin-intermediate *S. aureus* (VISA) strains were tested from the NARSA (Network on Antibacterial Resistance in *S. aureus*) collection and a group of 20 confirmed rifampin-resistant strains of *S. aureus* (Eurofins Medinet, Inc.). The Clinical Laboratory and Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards) recommended quality control organisms *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, and *S. pneumoniae* ATCC 49619 were used throughout testing [19].

Materials

Rifampin B, oxacillin, penicillin, and vancomycin were purchased from Sigma Chemical Co. (St. Louis, MO). Rifalazil (3′-hydroxy-5′-(4-isobutyl-1-piperazinyl) benzoxazinorifamycin) and new chemical entities (NCEs) were provided by ActivBiotics. Inc. (Lexington, MA).

Antimicrobial Susceptibility Testing Methods

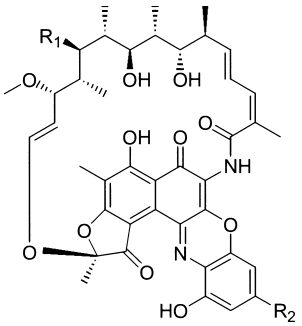
Test compounds and control antibiotics rifampin, oxacillin (staphylococci), penicillin (streptococci), and vancomycin (enterococci) were tested over doubling dilution concentrations in panels prepared on the day of susceptibility testing. MICs were interpreted as susceptible, intermediate, or resistant using the CLSI guidelines (M100-S16, 2006), where available [19]. In making rifalazil and dilutions of NCEs using pipettors, tips were changed after every three dilutions to prevent carryover.

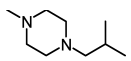
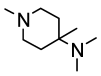
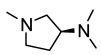
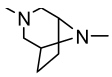
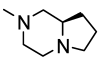
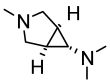
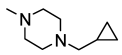
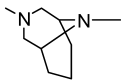
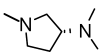
Results

Susceptibility

A total of 13 NCEs were tested against *S. aureus* and *S. epidermidis* isolates (ABI-0043, ABI-0045, ABI-0090, ABI-0094, ABI-0204, ABI-0273, ABI-0299, ABI-0322, ABI-0338, ABI-0370, ABI-0376, ABI-0418, and ABI-0420). In all, 10 of the 13 NCEs, (depicted in Table 1, data in Table 2) had better potency against *S. aureus*, defined as at least a four fold reduction in MIC_{90} compared with the MIC_{90} of both rifampin (0.06 μg/ml) and rifalazil (0.06 μg/ml). Similarly, the MIC_{90}s of 10 of the 13 NCEs for *S. epidermidis* were at least as low as that of rifalazil (MIC_{90} of 0.06 μg/ml), and were at least 4-fold reduced compared with the MIC_{90} of rifampin (0.25 μg/ml). Three NCEs that were tested more extensively, ABI-0043, ABI-0094, and ABI-0420, also had good antibacterial activity

Table 1 Structures of NCEs tested in this study, and MIC_{90} values for *Staphylococcus aureus* and *Streptococcus pyogenes*.



Compound	R_1	R_2	MIC_{90} (μg/ml)	
			Staphylococcus aureus	*Streptococcus pyogenes*
Rifalazil	a		0.06	0.002
ABI-0043	h		0.004	0.0005
ABI-0418	a		0.03	0.001
ABI-0420	h		0.008	0.002
ABI-0299	a		0.03	0.0005
ABI-0204	a		0.5	0.0005
ABI-0338	h		0.008	0.002
ABI-0370	h		0.008	0.002
ABI-0045	h		0.008	0.0005
ABI-0273	h		0.015	0.002
ABI-0090	a		0.008	0.001
ABI-0094	h		0.015	0.001
ABI-0376	h		0.015	0.002
ABI-0322	h		0.015	0.002

R_1 groups are *O*-acetyl (a) or hydroxyl (h).

Table 2 Susceptibility testing against Gram-positive pathogens

	MIC (μg/ml)					
	Antimicrobial	Species/Status	Total n	Range	MIC_{50}	MIC_{90}
Staphylococci	ABI-0043	*Staphylococcus aureus*	60	0.001～>4	0.002	0.004
	ABI-0094		60	0.004～>4	0.015	0.015
	ABI-0420		60	0.002～>4	0.004	0.008
	Rifalazil		60	0.008～>4	0.03	0.06
	Rifampin		60	0.015～>4	0.03	0.06
	Oxacillin		60	0.12～>16	>16	>16
	ABI-0043	*S. epidermidis*	40	0.00025～1	0.001	0.002
	ABI-0094		40	0.002～4	0.004	0.008
	ABI-0420		40	0.001～1	0.002	0.03
	Rifalazil		40	0.008～>4	0.008	0.06
	Rifampin		40	0.03～>4	0.06	0.25
	Oxacillin		40	0.06～>16	0.25	16
Streptococci	ABI-0043	*Streptococcus pneumoniae*	40	0.00012～0.001	0.00025	0.0005
	ABI-0094		40	0.00025～0.002	0.0005	0.001
	ABI-0420		40	0.00025～0.002	0.0005	0.001
	Rifalazil		40	0.00012～0.002	0.00025	0.001
	Rifampin		40	0.008～0.12	0.03	0.06
	Penicillin		40	0.008～4	0.12	4
	ABI-0043	*S. pyogenes*	20	0.00006～0.001	0.00025	0.0005
	ABI-0094		20	0.00012～0.002	0.0005	0.001
	ABI-0420		20	0.0005～0.002	0.001	0.002
	Rifalazil		20	0.00003～0.004	0.001	0.002
	Rifampin		20	0.03～0.25	0.12	0.12
	Penicillin		20	0.001～0.015	0.015	0.015
	ABI-0043	*S. agalactiae*	21	0.002～0.008	0.002	0.004
	ABI-0094		21	0.004～0.030	0.008	0.03
	ABI-0420		21	0.004～0.03	0.008	0.015
	Rifampin		21	0.003～0.012	0.06	0.12
	Penicillin		21	0.003～0.012	0.06	0.06
Enterococci	ABI-0043	*Enterococcus faecalis*	20	≦0.008～1	0.06	0.25
	ABI-0094		20	0.015～4	0.25	1
	ABI-0420		20	0.015～1	0.12	0.25
	Rifalazil		20	1～>8	4	8
	Rifampin		20	0.25～>8	2	8
	Vancomycin		20	0.5～>64	2	>64
	ABI-0043	*E. faecium*	20	≦0.008～>8	0.25	2
	ABI-0094		20	0.03～>8	0.12	4
	ABI-0420		20	0.015～>8	0.25	2
	Rifalazil		20	0.06～>8	1	>8
	Rifampin		20	0.03～>8	0.5	>8
	Vancomycin		20	0.25～>64	2	>64

against *S. agalactiae*, with MIC_{90}s in the 0.004 to 0.015 μg/ml range. The rifampin MIC_{90} for this organism is 0.12 μg/ml. Rifalazil and NCEs had particularly potent activity against *S. pneumoniae* and *S. pyogenes* strains. All 13 NCEs had at least an 8-fold reduction in the MIC_{90} against *S. pneumoniae* compared with the rifampin MIC_{90} of 0.06 μg/ml, and a 64-fold reduction against *S. pyogenes* compared with the rifampin MIC_{90} of 0.12 μg/ml. Although the NCEs and rifalazil had relatively less activity against enterococci, some NCEs were more potent than rifampin (Table 2).

Rifampin Resistance

The susceptibilities to NCEs did not change markedly among the *S. aureus* strains as a function of the resistance pattern of the isolates to antibiotics frequently used in therapy. The MSSA strains would generally be considered to be the earliest in origin because they have not yet been selected against by methicillin or quinolones. Historically, MRSA and QMRSA strains were likely selected for as a result of exposure to these drugs. As Fig. 1 indicates, susceptibility to rifampin, rifalazil, and a representative NCE, ABI-0043, remained similar across MSSA, MRSA, and QMRSA strains. However, VISA strains had a higher incidence of rifamycin resistance. The MICs against 3 of the 9 VISA isolates tested were >4.0 μg/ml for ABI-0043 and ABI-0094, and 4.0 μg/ml for ABI-0420 (not shown in Table 2). The NCEs had similar activity against the remaining 6 VISA strains when compared with other *S. aureus* strains (*i.e.*, at or below the MIC_{90}s shown in Table 2). The cause of this high proportion of rifamycin-resistance is not known. It is possible that patients infected with identified VISA strains are more frequently treated with multiple drug therapy, including rifampin.

Because resistance development is a concern for rifamycins, we also assessed rifalazil and NCE activity against 20 (non-VISA) clinical isolates of MRSA known to be resistant to rifampin (MIC >16 μg/ml) from the Eurofins Medinet, Inc strain collection. These NCEs were previously shown to retain some activity against a collection of rifamycin-resistant mutants of *S. aureus* ATCC 29213, with maximum MICs in the 1~4 μg/ml range [5]. When the 20 rifampin-resistant clinical isolates of MRSA were tested, the MICs of ABI-0043 against 6 strains exceeded 4.0 μg/ml (1 strain had an MIC of 8.0 μg/ml and 5 strains had MICs of 16 μg/ml). It is possible that the nature of the strain background among clinical isolates can contribute to elevated MICs of NCEs among rifampin-resistant isolates.

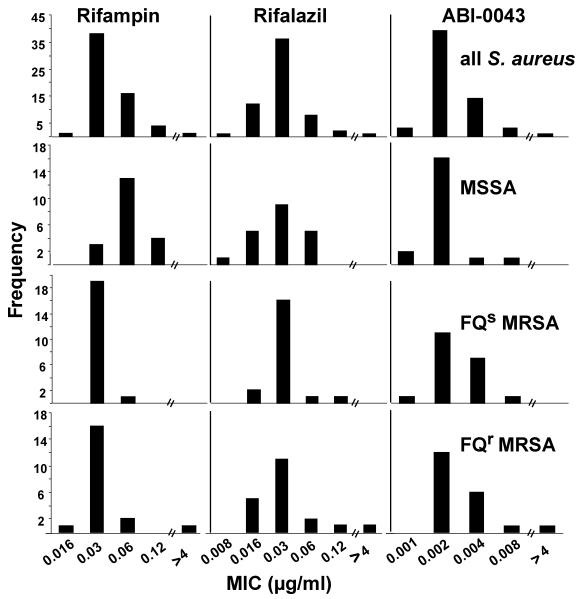


Fig. 1 Susceptibility pattern of *S. aureus* to rifampin, rifalazil and ABI-0043 based on resistance to other agents.

Conclusion

Our results show that NCEs are attractive candidates for the treatment of systemic infections caused by Gram-positive pathogens, particularly staphylococci and streptococci. The 10 most potent NCEs in this study may be prioritized further with regard to clinical potential by *in vivo* studies of efficacy in rigorous preclinical models [18, 20, 21], as well as safety and toxicological studies to be carried out in the future. The optimal therapeutic utility of NCEs may be their combination with other antibacterials for several reasons: 1) the high frequency of spontaneous rifamycin-resistant mutants [5, 17], 2) the relatively high MIC of NCEs for rifampin-resistant isolates determined in this study, and 3) the lack of efficacy of NCEs against rifamycin-resistant strains when tested *in vivo* [18]. However, the NCEs have two distinct advantages compared with rifampin when considering their potential in combination therapy. First, the NCEs showed increased potency compared with rifampin (Table 2). Second, in contrast to rifampin, NCEs do not affect P450 enzyme levels [20] and therefore would be unlikely to have drug–drug interactions.

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