Efficacy of Novel Rifamycin Derivatives against Rifamycin-Sensitive and -Resistant *Staphylococcus aureus* Isolates in Murine Models of Infection[▽]

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Novel rifamycins (new chemical entities [NCEs]) having MICs of 0.002 to 0.03 µg/ml against *Staphylococcus aureus* and retaining some activity against rifampin-resistant mutants were tested for in vivo efficacy against susceptible and rifampin-resistant strains of *S. aureus*. Rifalazil and rifampin had a 50% effective dose (ED₅₀) of 0.06 mg/kg of body weight when administered as a single intravenous (i.v.) dose in a murine septicemia model against a susceptible strain of *S. aureus*. The majority of NCEs showed efficacy at a lower i.v. dose (0.003 to 0.06 mg/kg). In addition, half of the NCEs tested for oral efficacy had ED₅₀s in the range of 0.015 to 0.13 mg/kg, i.e., lower or equivalent to the oral ED₅₀s of rifampin and rifalazil. NCEs were also tested in the septicemia model against a rifampin-resistant strain of *S. aureus*. Twenty-four of 169 NCEs were efficacious when administered as a single oral dose of 80 mg/kg. These NCEs were examined in the murine thigh infection model against a susceptible strain of *S. aureus*. Several NCEs dosed by intraperitoneal injection at 0.06 mg/kg caused a significant difference in bacterial titer compared with placebo-treated animals. No NCEs showed efficacy in the thigh model against a highly rifampin-resistant strain. However, several NCEs showed an effect when tested against a partially rifampin-resistant strain. The NCEs having a 25-hydroxyl moiety were more effective as a group than their 25-*O*-acetyl counterparts. These model systems defined candidate NCEs as components of potential combination therapies to treat systemic infections or as monotherapeutic agents for topical applications.

Rifalazil [3'-hydroxy-5'-(4-isobutyl-1-piperazinyl) benzoxazinorifamycin], also referred to as KRM-1648 or ABI-1648, is a rifamycin derivative with exceptionally low MICs against gram-positive bacteria (7, 19), *Helicobacter pylori* (1), and *Chlamydia* (9, 18, 21, 22). The potency of rifalazil and the other rifamycins derives from their specific inhibition of bacterial RNA polymerase (6). Preclinical animal studies suggest that rifalazil has efficacy against *Chlamydia pneumoniae* (9), *Clostridium difficile* (2), *Mycobacterium tuberculosis* (8, 19, 20), and *Staphylococcus aureus* (7). In addition, rifalazil has been tested in phase 2 human clinical trials for the treatment of tuberculosis (5) and for nongonococcal urethritis (B. W. Batteiger, W. McCormack, W. Stamm, and the Rifalazil Study Group, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. L-992b, 2004). One of the attributes of rifamycins, including rifalazil, is the propensity for resistance to develop as a result of the occurrence of mutations in the *rpoB* gene. These mutations cause modifications in the binding site of the target enzyme, the β subunit of RNA polymerase, in pathogens such as

M. tuberculosis (13, 17, 25, 26), *S. aureus* (23, 24), and *Streptococcus pyogenes* (3). Thus, rifamycins have been confined primarily to multiple-drug therapy where resistance development is less of an issue.

Recently, we described a collection of over 700 novel rifamycins which are related in structure to rifalazil. More than 50% of these new chemical entities (NCEs) are more active against gram-positive bacteria such as *S. aureus* than rifalazil or rifampin. Moreover, some of these NCEs retained considerable activity against highly rifampin-resistant isolates of *S. aureus* (15). These resistant strains contain single-nucleotide changes in the *rpoB* gene that resulted in ~100,000-fold increase in the MIC of rifampin. The retention of activity against rifampin-resistant strains supports the possibility of the clinical use of our NCEs as monotherapeutic agents.

To further characterize the NCEs, we have undertaken in vivo studies with both wild-type and rifampin-resistant strains of *S. aureus* as the infecting agents. The experimental murine model of septicemia (4) was used to determine whether NCEs having potent in vitro antibacterial activity also exhibit in vivo efficacy. In addition, we have utilized this septicemia model to determine whether NCEs were orally bioavailable.

The murine thigh infection model is a more rigorous test of in vivo efficacy and can generally predict a compound's potential for the treatment of skin and skin structure infections (10). We have utilized this model to measure efficacy against infections caused by rifampin-sensitive strains of *S. aureus* and by *S.*

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TABLE 1. Bacterial strains

Background strain and mutants	RpoB alteration	Source or reference
<i>S. aureus</i> 29213	None	ATCC
ABS-100	H481→Y	15
ABS-050	S464→P	15
ABS-052	A477→D	15
<i>S. aureus</i> Smith	None	ATCC
ABS-111	H481→Y	15
ABS-107	L466→S	15
ABS-112	S486→L	15

aureus strains with high and intermediate resistance to rifampin. These studies identify the most promising preclinical candidates and set realistic goals for their future use as therapeutic agents.

MATERIALS AND METHODS

Bacteria. *S. aureus* 29213 and *S. aureus* variant Smith (wild type) strains were obtained from ATCC. All mutant derivative strains were from the ActivBiotics strain collection (15) and are listed in Table 1.

Materials. Rifampin, tetracycline, and porcine gastric mucin were obtained from Sigma Chemical Company (St. Louis, MO). All benzoxazinorifamycin derivatives (NCEs) were from the ActivBiotics chemical collection and synthesized by either Kaneka Corporation, Japan, or by ActivBiotics scientists.

Animals. Swiss-Webster mice weighing 18 to 25 g were used in all experiments, which were approved by and conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Gwathmey, Inc., Cambridge, MA.

Murine septicemia model. Mice were treated as previously described (4). Briefly, mice were inoculated intraperitoneally (i.p.) with approximately 5×10^6 CFU of *S. aureus* variant Smith suspended in 5% hog gastric mucin. To obtain a comparable lethal inoculum with rifampin-resistant derivatives of *S. aureus* variant Smith, the inoculum was increased to 2×10^7 CFU. After 30 min, groups of five mice were treated with a dose of compound delivered either intravenously (i.v.) or orally (p.o.). At least three different doses encompassing a 1-log₁₀ difference in concentration, in half-log increments, were used per route of administration. The 50% effective dose (ED₅₀) was estimated graphically by plotting survival as a function of the concentration, assuming a linear relationship within the range of the concentration resulting in >50% survival, and the approximately threefold lower concentration tested.

Murine neutropenic thigh model. Mice were treated essentially as described previously (10), except that cyclophosphamide was administered at 3 days prior and 2 h prior to inoculation (11). Each thigh was inoculated with 0.1 ml containing 2×10^6 bacteria, and 1.5 h after inoculation, groups of three mice were treated with vehicle or a rifamycin. Because similar results were obtained using either i.v. or i.p. compound administration, the easier i.p. route was used. The number of CFU of bacteria from the two thighs was determined 24 h after dosing and also at 2 h after dosing when indicated. A difference in the number of CFU from animals treated with novel rifamycins compared with that of untreated or placebo-treated animals was evidence of an effect, and the lowest concentration resulting in at least a 1 log reduction was defined as the lowest effective dose. This metric was more sensitive than the determination of the reduction in titer compared with the inoculum, a method that is often used to determine efficacy (10, 11). Our approach was a more sensitive method of detecting an effect, especially when animals were inoculated with rifampin-resistant strains.

Dosage formulations. For doses below 4 mg/kg of body weight, the compounds were first dissolved at a concentration of 10 mg/ml in dimethyl sulfoxide (DMSO) and then further diluted into dissolution solution (5% Etocas NF grade [Croda, Inc., Edison, N.J.], 0.9% NaCl, and 0.1 mM Na₂HPO₄, pH 7.4), for a final dose volume of 0.1 ml for a 20-g animal. For doses above 4 mg/kg, stock solutions were prepared in DMSO as described below, and appropriate dilutions were made into diluted liquid fill. Liquid fill was prepared by combining 375 g of Etocas 35NF, 4.4 g of pluronic acid F68, 50.8 g of polyethylene glycol 400, and 10.8 ml of water and stirring overnight. Aliquots of 15 ml were stored at -80°C. On the week of an experiment, the liquid fill was thawed, and 33 ml of water was added to make a diluted liquid fill stock. For the 80 mg/kg dose, 50 mg/ml of DMSO stock solution of compound was prepared and diluted 1:4 in diluted liquid fill to

give a final dosage volume of 0.2 ml for a 20-g animal. For the 160 mg/kg doses, the same procedure was used except that the initial compound stock solution was prepared at 100 mg/ml in DMSO. Control noninfected animals injected with either vehicle showed no acute distress or obvious toxic effects. The number of CFU of bacteria from vehicle-treated infected animals was the same as from untreated infected animals.

RESULTS

Efficacy in the experimental murine septicemia model. A series of novel benzoxazinorifamycins (NCEs) have been described that have potent activity against the rifampin-susceptible strain of *S. aureus* (MIC of 0.002 to 0.03 µg/ml) (15). In order to determine if NCEs were effective in vivo, the murine septicemia model was employed. Compounds were tested by carrying out dose-response profiling with half-log₁₀ dilutions, as outlined in Materials and Methods. Experiments carried out with the parent compound, rifalazil, confirmed the reproducibility of the model. Ninety-five percent of the animals administered 0.1 mg/kg of rifalazil survived the *S. aureus* Smith challenge (a total of 38 of 40 mice from eight separate experiments). A dose of 0.03 mg/kg resulted in 7.5% survival (3 of 40 mice), indicating that the ED₅₀ of rifalazil was between 0.03 and 0.1 mg/kg. The ED₅₀ for rifalazil administered i.v., determined as described in Materials and Methods, was 0.063 mg/kg, which compares closely with the ED₅₀ of rifalazil reported previously (7) and was similar to the i.v. ED₅₀ of 0.062 mg/kg for rifampin (data not shown). Similarly, when rifalazil was administered orally at 0.2 mg/kg, 88% of mice survived (22 of 25 mice altogether, from five separate experiments), whereas 12% (3 of 25 mice) survived when a lower dose of 0.06 mg/kg was used.

Most NCEs tested in the septicemia model had i.v. ED₅₀s between 0.003 and 0.06 mg/kg, lower than rifampin and rifalazil (Table 2). The chemical structures of NCEs which are among the most effective in this model are shown in Table 3. The minority of NCEs that did not show efficacy approaching these concentrations (ED₅₀ of >0.1 mg/kg) (data not shown) were eliminated from further consideration.

Analysis of efficacy results also revealed a structure-activity relationship. In general, NCEs having a 25-hydroxyl group had better i.v. ED₅₀s than the 25-*O*-acetyl compounds (Table 2). This conclusion was reinforced when specific pairs of NCEs with identical structures except at position 25 were compared. The 25-hydroxyl NCE of the pair had a lower ED₅₀ compared with its 25-*O*-acetyl homolog eight of nine times (Table 2). The 25-hydroxyl compound was more effective in four of the pairs even though its MIC was the same or higher than the 25-*O*-acetyl congener.

Oral efficacy. The determination of efficacy following the administration by gavage in the murine septicemia model provided an estimate of oral bioavailability of NCEs. The "functional bioavailability" was calculated by comparing the oral dose required for 50% survival with the i.v. dose ([i.v. ED₅₀/oral ED₅₀] × 100) (Table 2). As an example, approximately twice the oral dose of rifalazil (ED₅₀ of 0.125 mg/kg) was required for efficacy compared to the i.v. dose (ED₅₀ of 0.063 mg/kg). The functional bioavailability of rifalazil was calculated to be 50% ([0.063/0.125] × 100).

Most NCEs shown in Table 2 had lower oral ED₅₀s compared with rifalazil (Table 2). Among other NCEs tested, four

TABLE 2. Comparison of i.v. and oral ED₅₀s in the septicemia model

NCE ^a	Position 25 ^b	MIC (μg/ml) vs. <i>S. aureus</i> Smith	i.v. ED ₅₀ (mg/kg)	p.o. ED ₅₀ (mg/kg) ^c	Functional bioavailability (%) ^d
Compound pairs					
ABI-0043	OH	0.002	0.003	0.015	18
ABI-1131	O-Ac	0.004	0.006	0.035	16
ABI-0420	OH	0.004	0.004	0.045	9
ABI-0418	O-Ac	0.008	0.018	0.130	13
ABI-0322	OH	0.002	0.005	0.053	8
ABI-0306	O-Ac	0.004	0.027	0.176	15
ABI-0361	OH	0.004	0.005	ND	
ABI-0354	O-Ac	0.008	0.023	>.3	<7
ABI-0280	OH	0.004	0.013	0.113	12
ABI-0279	O-Ac	0.004	0.044	0.147	30
ABI-0376	OH	0.008	0.013	0.130	10
ABI-0375	O-Ac	0.008	0.027	ND	
ABI-0302	OH	0.002	0.013	0.282	5
ABI-0299	O-Ac	0.008	0.017	0.260	6
ABI-0063	OH	0.030	0.018	0.400	4
ABI-0046	O-Ac	0.008	0.022	0.350	6
ABI-0055	OH	0.030	0.018	ND	
ABI-0039	O-Ac	0.004	0.027	ND	
Other compounds					
ABI-0370	OH	0.008	0.003	0.040	6
ABI-0045	OH	0.004	0.004	0.045	9
ABI-0038	O-Ac	0.004	0.007	0.040	18
ABI-0273	OH	0.004	0.007	0.065	11
ABI-0094	OH	0.008	0.013	0.020	65
ABI-0338	OH	0.004	0.013	0.045	29
ABI-0269	OH	0.002	0.013	0.045	30
ABI-0204	O-Ac	0.008	0.013	0.083	16
Rifampin	O-Ac	0.015	0.062	0.130	48
Rifalazil	O-Ac	0.015	0.063	0.126	50

^a Specific NCEs with identical structures except at position 25 (either 25-O-acetyl or 25-hydroxyl) were compared in pairs; values for other NCEs are also shown.

^b OH, hydroxyl; O-Ac, O-acetyl.

^c ND, not done.

^d Functional bioavailability was calculated as (i.v. ED₅₀/oral ED₅₀) × 100.

25-O-acetyl NCEs and two 25-hydroxyl NCEs had relatively poor oral efficacy of >1 mg/kg (data not shown). The data again suggest that the 25-hydroxyl compounds, as a group, may have lower oral ED₅₀s (Table 2). All NCEs except for ABI-0094 had lower functional bioavailability than rifampin and rifalazil (Table 2). Thirteen NCEs had functional bioavailabilities at or above 10%, with ED₅₀s as low as 0.015 mg/kg (Table 2), suggesting that, clinically, oral dosing is a possibility.

Efficacy against mutant strains. NCEs showing efficacy in vivo against fully rifampin-resistant strains might be successful monotherapeutic agents (12). We thus used *S. aureus* strains that were resistant to >128 μg/ml of rifampin (15) as the inoculum in the murine septicemia model. ABI-0418 and ABI-0420, two NCEs identical in structure except at position 25 (Table 3), were highly active against susceptible *S. aureus* and had MICs of 2 μg/ml against highly rifampin-resistant strains. The collective results of several experiments using these compounds against the three highly rifampin-resistant strains are

TABLE 3. Structure of NCEs where R1 is hydroxyl or an *o*-acetyl group at position 25

Compound (ABI)	R1 ^a	R2
1648 (rifalazil)	O-Ac
0043	OH
0094	OH
0369	O-Ac
0418	O-Ac
0420	OH
0699	O-Ac
1131	O-Ac

^a OH, hydroxyl; O-Ac, O-acetyl.

shown in Table 4. The i.v. ED₅₀ of the 25-hydroxyl NCE, ABI-0420, was approximately 12.5, 12.6, and 11.8 mg/kg against strains ABS-111, ABS-107, and ABS-112, respectively. Its oral ED₅₀ was estimated to be 33 mg/kg against strain ABS-111. The 25-O-acetyl NCE ABI-0418 had lower i.v. efficacy against strains ABS-111 and ABS-107, and less than 50% survival was observed for the highest i.v. dose that could be

TABLE 4. Survival in mouse septicemia model against strains resistant to rifampin carrying the indicated mutations

Compound	Position 25 ^a	MIC (μg/ml)	i.v. dose (mg/kg)	No. of survivors/ total no. treated i.v.	p.o. dose (mg/kg)	No. of survivors/ total no. treated p.o.
Against <i>S. aureus</i> ABS-111 (H481→Y)						
ABI-0418	O-Ac	2	20	11/30	160	5/5
			6	1/30	80	10/10
			2	1/20	24	1/10
ABI-0420	OH	2	20	29/30	160	5/5
			6	3/30	80	10/10
			2	1/20	24	4/10
Rifampin	O-Ac	>128	20	0/5	160	0/5
Against <i>S. aureus</i> ABS-107 (L466→S)						
ABI-0418	O-Ac	2	20	7/25		
			6	1/25		
			2	0/20		
ABI-0420	OH	2	20	23/25		
			6	3/25		
			2	1/20		
Rifampin	O-Ac	>128	20	0/5		
Against <i>S. aureus</i> ABS-112 (S486→L)						
ABI-0418	O-Ac	2	20	23/25		
			6	3/25		
			2	1/20		
ABI-0420	OH	2	20	23/25		
			6	5/25		
			2	0/20		
Rifampin	O-Ac	>128	20	0/5		

^a OH, hydroxyl; O-Ac, O-acetyl.

delivered due to solubility limitations. ABI-0418 had equivalent i.v. efficacy against strain ABS-112 (Table 4). ABI-0418 also showed lower oral efficacy than ABI-0420 when 24 mg/kg doses were compared against strain ABS-111 (Table 4).

We screened 167 additional NCEs for oral efficacy at 80 mg/kg in the septicemia model against the resistant strain ABS-111. These included NCEs that had good efficacy against rifampin-susceptible *S. aureus* (Table 2) and other NCEs that had MICs of ≤4 μg/ml against highly rifampin-resistant mutants but which had not been tested for efficacy against wild-type *S. aureus*. Of these 169 compounds, 24 were found to be effective, using a cutoff of at least three of five survivors in each of two independent tests.

As was the case against susceptible *S. aureus*, a higher proportion of 25-hydroxyl NCEs were efficacious against the rifampin-resistant strain. Seventeen 25-hydroxyl NCEs out of 70 were positive (24%), whereas only 7 of 99 25-*O*-acetyl NCEs (7%) were positive. Furthermore, when eight pairs of compounds identical except at position 25 were examined, the 25-hydroxyl NCE of the pairs was more efficacious against the strongly rifampin-resistant strain (e.g., ABI-0420/ABI-0418 in Table 4). Only in two compound pairs was the 25-*O*-acetyl member more efficacious (data not shown.)

Using the murine neutropenic thigh infection model as a screen to evaluate NCEs. The neutropenic murine thigh infection model is typically used to test the potential of antibiotics for treatment of skin and skin structure infection (10). The 24 NCEs which showed oral efficacy against highly rifampin-resistant *S. aureus* in the murine septicemia model were investigated in the thigh model. Each of the 24 NCEs was administered i.p. to mice which had been infected with rifampin-susceptible

bacteria. The bacterial titer was determined 24 h after a single dose of 0.2 mg/kg delivered i.p., as described in Materials and Methods. Treatment with NCEs ABI-0043, ABI-0369, and ABI-0699 resulted in an effect in that a substantial difference was observed compared with placebo-treated animals (greater than a 1 log reduction in bacterial titer). NCEs ABI-0420, ABI-0094, and ABI-0089 showed a more modest difference compared with titers of placebo-treated control mice for the 24-h time point (Fig. 1). Further testing of ABI-0043 revealed that i.p. administration of 0.06 mg/kg resulted in a diminished bacterial titer compared with placebo-treated mice (Fig. 1B). In addition, ABI-0558 dosed at 0.2 mg/kg showed a substantial difference compared with placebo, and ABI-0205 and ABI-0598 showed a more modest difference at 24 h (data not shown).

Efficacy of NCEs in the neutropenic murine thigh model infected with rifamycin-resistant *S. aureus*. NCEs were tested following infection with *S. aureus* ABS-100, a strain encoding the H481→Y alteration in RpoB that has high resistance to rifampin. The most potent NCEs had an MIC of 2 μg/ml, compared with 0.002 to 0.03 μg/ml against wild-type *S. aureus*. Based on the difference in MICs between strain ABS-100 and the wild type, it was likely that a higher dose would be required to observe an effect. We therefore treated mice i.p. with NCEs at a dose of 160 mg/kg. None of the candidate NCEs was found to have an effect in the murine neutropenic thigh model when the bacterial titers were measured 2 h or 24 h after treatment and compared with the titer of placebo-treated animals (data not shown). Despite the fact that no significant difference in titer was observed for the NCE compounds compared to pla-

FIG. 1. Screening for an effect against wild-type *S. aureus* strain 29213 using the mouse thigh model. (A) NCEs were administered i.p. at 0.2 mg/kg, and titers of bacteria per mouse ($n = 3$ per group) were determined 24 h after treatment; the mean titers of the placebo groups were 8.03 and 8.18 log units. (B) ABI-0043 was administered i.p. at lower doses, and titers were determined at 24 h; the mean titer of the placebo group was 8.65 log units. The dotted line indicates a 1-log reduction in the number of CFU compared with placebo-treated animals.

cebo, 60 mg/kg of the control drug tetracycline resulted in a reduction in titer of several logs₁₀.

Although NCEs showed no effect in the murine thigh infection against the resistant strain ABS-100, they did show an effect when the intermediate rifampin-resistant strain ABS-052 was the infecting agent. For example, ABI-0043 which has an MIC of 0.5 μ g/ml against ABS-052, showed an effect at 10 mg/kg in mice infected with this strain. (Fig. 2). An effect against strain ABS-052 was also observed when NCEs ABI-0418, ABI-0420, ABI-369, and ABI-370 were administered at 10 mg/kg (data not shown).

DISCUSSION

Novel benzoxazinorifamycins having significantly improved activity against rifamycin-resistant strains of *S. aureus* (15) were screened in mouse models of infection to examine the following key properties: (i) structure-function relationships

for efficacy among NCEs, (ii) oral efficacy and an estimate of bioavailability, and (iii) therapeutic potential and limitations of NCEs.

The mouse septicemia model was informative in showing that the majority of NCEs tested were very potent in vivo. Most compounds exhibited ED₅₀s below that of rifalazil (<0.063 mg/kg), and some NCEs had an ED₅₀ as low as 0.003 mg/kg (Table 2). The moiety at position 25 appeared to be an important determinant of efficacy. We do not presently have a clear explanation for the observation that 25-hydroxyl NCEs as a group showed better efficacy than 25-*O*-acetyl NCEs against both rifampin-susceptible and -resistant strains of *S. aureus*, as revealed by results in the murine septicemia model (Tables 2 and 4). One possibility is that the 25-hydroxyl compounds, which are more water soluble than their 25-*O*-acetyl counterparts, might have a higher free fraction or in other ways result in improved pharmacokinetic properties. However, solubility was not found to correlate strictly with in vivo activities (data not shown).

The septicemia model also provided a means of estimating bioavailability by comparing efficacy of an NCE dosed orally to efficacy following i.v. administration [(i.v. ED₅₀/p.o. ED₅₀) $\times$ 100]. Rifalazil and rifampin had a higher functional bioavailability than most of the NCEs. However, it should be noted that the functional bioavailability could be an underestimate of the absolute bioavailability of any specific NCE, because of the acute nature of the septicemia model system. The delay in systemic exposure that may occur for compounds delivered orally could result in diminished efficacy, compared with i.v. dosing that results in immediate exposure. In any case, approximately half of the NCEs tested were more effective orally than rifalazil and rifampin, with ED₅₀s as low as 0.015 mg/kg (Table 2), suggesting a potential for very potent, orally administered agents.

The more rigorous mouse thigh model was very useful in defining both the potential and the limitations of these NCEs. The best NCEs diminished bacterial titers of wild-type *S. aureus* compared with placebo-treated animals at 0.2 mg/kg and, for some NCEs, at 0.06 mg/kg (Fig. 1). These NCEs also

FIG. 2. Effect of ABI-0043 against rifampin-resistant strain ABS-052 (MIC of 0.5 μ g/ml). Compounds were administered i.p. as described in Materials and Methods, and bacterial titers were determined 24 h after treatment. Nine, three, nine, and six mice were used for placebo, 40 mg/kg, 10 mg/kg, and 3 mg/kg ABI-0043 treatments, respectively. The mean titer for the placebo group was 8.30 log units. The dotted line indicates a 1-log reduction in the number of CFU compared with placebo-treated animals.

TABLE 5. Relationship of MIC and in vivo efficacy in the mouse thigh model for NCE ABI-0043

<i>S. aureus</i> strain	Mutation	MIC	Lowest ED (mg/kg) ^a	Lowest ED/MIC ^a
29213	None	0.002	0.06	30
ABS-052	A477→D	0.5	10	20
ABS-100	H481→Y	2	>160	>80

^a The lowest ED is the dose (mouse thigh) resulting in a 1-log difference in bacterial titer compared with placebo-treated animals.

showed an effect in this model against strain ABS-052, the moderately rifampin-resistant mutant, when the MIC was 0.5 µg/ml, at a dose of 10 mg/kg (Fig. 2). In fact, the ratio of the dose showing an effect in the mouse thigh model to the MIC was similar for the wild type and strain ABS-052 (Table 5). Comparable effects were observed 2 h after dosing as well (data not shown). However, no NCEs showed an effect against the highly rifampin-resistant strain ABS-100 (MICs of ~2 µg/ml), even when the dose was raised to 160 mg/kg, at either 2 or 24 h after dosing. The fact that no effect was detected against ABS-100 when the dose was increased 16-fold (Table 5) suggests that increasing the dose further would not compensate for deficits in activity and/or distribution of these NCEs. It will be interesting to carry out a thorough pharmacokinetic analysis, including the determination of protein binding as well as the concentration of ABI-0043 and other selected NCEs in the interstitial fluid, in order to verify this hypothesis.

Screening with these murine models of infection provided a means of selecting a small number of NCEs, including ABI-0043, ABI-0369, ABI-0699, ABI-0558, ABI-0420, ABI-0094, ABI-0089, ABI-0205, and ABI-0598 that performed best in efficacy studies either at 24 h (Fig. 1) or at 2 h after treatment (data not shown). These NCEs are lead clinical candidates as combination drugs in the treatment of serious gram-positive infections. Candidate NCEs show significant improvement in efficacy and potency compared with rifampin, which is currently used in antibiotic cocktails. In addition, they have been shown to have even more potent in vitro activities against *S. pyogenes* (14). Whereas rifampin can cause drug-drug interactions because it is a potent inducer of the P450 CYP3A4 enzyme (16), rifalazil does not induce CYP3A4 (19), and the NCEs appear to share this beneficial attribute (R. Farquhar, M. Xia, Y.-X. Chen, I. Meerts, L. Phillips, D. Buxton, M. Larsson, C. K. Murphy, and I. MacNeil, Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-2048, 2005). In addition, ABI-0043 and other selected NCEs are excellent candidates for monotherapy in topical applications, given their extraordinary potency against wild-type *S. aureus* (15) and *Propionibacterium acnes* (C. K. Murphy and J. van Duzer, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-2053, 2004) and their retention of activity against rifampin-resistant derivatives from these two species in the microgram/milliliter range.

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