

Bactericidal Efficacy of ABI-0043, a Novel Rifamycin, in a Murine Pneumococcal Pneumonia Model

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Abstract Novel rifamycins such as ABI-0043, ABI-0369, and ABI-0699 had comparable *in vivo* bactericidal activity with ceftriaxone against a penicillin-G-resistant, *mefA*-positive *Streptococcus pneumoniae* in a murine pneumonia model. ABI-0043 demonstrated a dose-dependent response with a high correlation to bacterial kill (+0.1 to $-3.7 \log_{10}$ CFU).

Keywords rifamycin, pneumonia, mouse, *Streptococcus pneumoniae*

Streptococcus pneumoniae is an important bacterial pathogen in pneumonia, meningitis, sinusitis, and otitis media [1]. At least 500,000 cases of pneumonia are estimated to be caused by *S. pneumoniae* every year in the United States [2]. According to a North American surveillance, the current resistant rate of *S. pneumoniae* to penicillin is 16.7% among community acquired isolates and 12.1% among hospital acquired isolates [3].

ABI-0043 is a novel rifamycin derivative with potent activity against staphylococci and streptococci [4, 5]. Compared with rifampin, ABI-0043 also has improved activity against rifamycin-resistant strains of these pathogens. This improved activity, however, may not be sufficient to warrant its use as a clinical monotherapeutic agent for systemic infections [6]. Unlike other ansamycins, such as rifampin, ABI-0043 does not significantly induce or inhibit cytochrome P450 enzyme systems *in vitro* [7].

For new compounds, like ABI-0043, that have potent anti-pneumococcal activity in *in vitro* studies, the assessment of their bactericidal activity in a murine pneumococcal pneumonia model can provide an estimate of future clinical success. This study was reported in part at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 16 to 19 December 2005.

The purpose of this study was to evaluate the antibacterial activity of ActivBiotics' compounds ABI-0043, ABI-0369 and ABI-0699 in comparison to rifampin and/or ceftriaxone administered intraperitoneally once to thrice daily over time ranging from 6 to 24 hours in the *S. pneumoniae* murine pneumonia model.

Drug substance of New Chemical Entities (NCE) (ABI-0043, ABI-0369, and ABI-0699) was supplied by ActivBiotics, Inc. (Fig. 1); ceftriaxone was obtained from Roche Pharmaceuticals (Nutley, NJ); rifampin was obtained from Bedford Laboratories (Bedford, OH).

Three clinical isolates of *S. pneumoniae* were utilized for *in vitro* susceptibility testing: one penicillin-G-susceptible (SPN 21), one *mefA* (SPN 100; penicillin-resistant), and one *ermB* (SPN 95; penicillin-susceptible) isolate. The phenotypic and genotypic profile for these isolates has been previously confirmed [8~10]. All isolates were maintained in skim milk medium (BD Biosciences, Sparks, Md.) at -80°C and subcultured twice on trypticase soy agar with 5% sheep blood (BD Biosciences) before use in *in vitro* and *in vivo* experiments [8]. MICs of NCEs and rifampin were determined against *S. pneumoniae* strains SPN 21, SPN 95, and SPN 100 in triplicate using standard CLSI (previously

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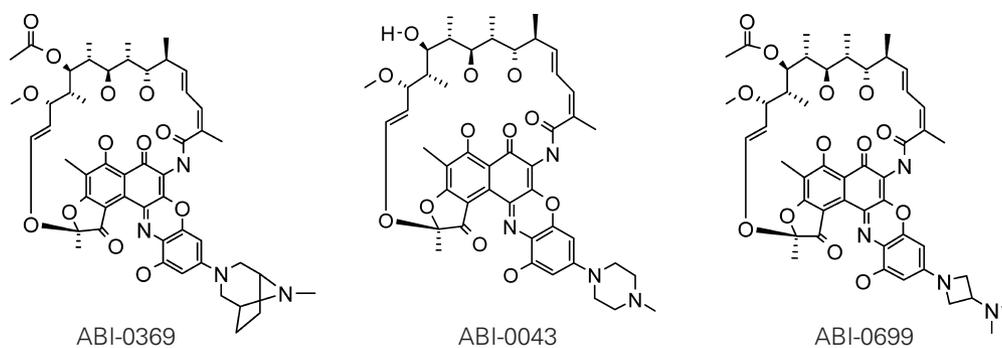


Fig. 1 Chemical structures of rifampin analogs.

Table 1 MIC ($\mu\text{g/ml}$) values of study compounds for *S. pneumoniae* isolates

Compound	SPN strain		
	21	95	100
Rifampin	0.06	0.06	0.015
ABI-0043	0.001	0.0005	0.0005
ABI-0369	0.00012	0.00025	0.00025
ABI-0699	0.00025	0.00025	0.00012
Penicillin G ^a	0.03 (S)	0.03~0.06 (S)	4 (R)
Genotypic variations or known resistance ^b	Gatifloxacin MIC=1 mg/ml	<i>ermB</i>	<i>mefA</i>

^a Sensitivity interpretations according to Clinical and Laboratory Standards Institute [9]: S=susceptible; I=intermediate; R=resistant. ^b As reported in references [8]~[10].

NCCLS) methods [11]. The *in vitro* activity of NCEs was superior to that of rifampin and penicillin G (Table 1) and comparable to that of each other, especially for strains SPN 95 and SPN 100. The penicillin-resistant strain SPN 100 was chosen for *in vivo* studies.

Specific pathogen-free, female ICR (approximately 22 g) mice (Harlan, Inc., Indianapolis, IN) were managed and utilized according to National Research Council recommendations and were provided food and water ad libitum [12]. Mice were rendered neutropenic by IP injection of cyclophosphamide (Cytoxan; Bristol-Myers Squibb, Princeton, N.J.) 150 mg/kg at four days and 100 mg/kg at 1 day prior to inoculation [13, 14]. Pneumonia was induced *via* oropharyngeal inoculation of a 0.05 ml suspension of SPN 100 prepared from an inoculum adjusted to approximately 10^8 CFU/ml as described previously [8]. By 8 to 16 hours post inoculation, all animals showed signs of infection including rough coat appearance and limited mobility. IP treatment with antibacterial or normal saline (control) began 12~14 hours

post inoculation for groups of 6 mice as follows (also see Fig. 2): QD doses of ABI-0043 at 0.4, 4.0, 40 and 80 mg/kg, ABI-0369 and ABI-0699 at 80 mg/kg, ceftriaxone at 4.0 mg/kg and 40 mg/kg and rifampin at 10 mg/kg. All mice treated QD were sacrificed at 6 hours post treatment. ABI-0043 was also dosed at 120 and 240 mg/kg/day (q8h, 3 doses of 40 and 80 mg/kg, respectively) and the mice in these groups were sacrificed at 24 hours. The drug vehicle was as follows: 375 g of Etocas 35NF, 4.4 g pluronic acid F68, 50.8 g PEG 400, and 10.8 ml of water. Dimethyl sulfoxide (>99.9%, Sigma-Aldrich Corp., St. Louis, MO) was added to the powder and mixed with vehicle. A group of 6 control mice was sacrificed just prior to initiation of dosing and at the time of lung harvest (at 6 hours for the single dose groups and at 24 hours for groups receiving 3 doses) for each treatment regimen. Lungs were aseptically removed and individually homogenized in 1.0 ml of normal saline as described previously [8]. Serial dilutions of homogenate were plated on trypticase soy agar with 5.0% sheep blood and subsequently incubated at 35°C for 24

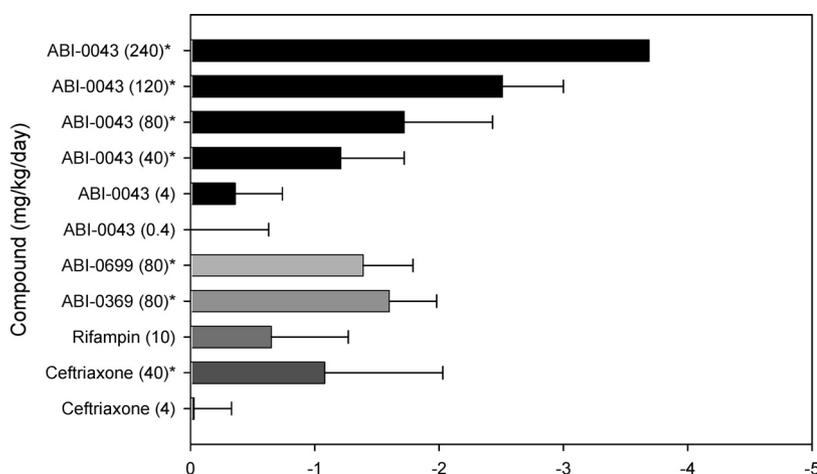


Fig. 2 *In vivo* antibacterial activity of rifamycin analogs and comparators against SPN100.

* $p < 0.05$ between log₁₀ CFU changes in infected mice treated with regimens and infected mice treated with placebo (normal saline). ABI-0043 240 mg/kg/day and 120 mg/kg/day were given as 80 mg q8h and 40 mg q8h. The rest of compounds were given as once daily dose.

hours in 5.0% CO₂ for CFU determination.

In the untreated control groups, both the 0 and 6 hours CFU levels in lung tissue were consistent between runs, starting at 10⁶ to 10⁷ CFU with an increase by about 1 log 24 hours post inoculation. The change in bacterial density in the lung tissues was calculated by subtracting the mean log₁₀ CFU of the control mice sacrificed just prior to dosing from the mean log₁₀ CFU of the drug treated and control mice at the end of 6 or 24 hours of therapy. Antibiotic-carry over was observed upon culturing the lung homogenates after the 3 doses of ABI-0043 80 mg/kg q8h, and as such, the limit of detection was 2 × 10⁴ CFU for this group.

Fig. 2 displays the change in log₁₀ CFU/ml for control and treatment groups. The IP dosing of ABI-0043 produced a dose-dependent killing effect. The bacterial density decreased proportionally with an increase of total dosage when compared with the bacterial density of the same hour control animals (+0.1 to -3.7 log₁₀ CFU). This relationship was confirmed by analysis of data with WinNonlin 5.0 (Pharsight Co., Cary, NC) and SigmaPlot 2001 (SPSS Inc., Chicago, IL) each of which gave a high correlation ($R^2 = 0.994$).

Statistical analysis was conducted using SigmaStat 2.03 (SPSS Inc., Chicago, IL) with statistical significance defined at p -value of 0.05 or less. Mann-Whitney Rank Sum test, t -test, and one-way ANOVA was applied where appropriate. The 40 mg/kg dose of ABI-0043 showed comparable activity to that of the 40 mg/kg dose of ceftriaxone ($P = 0.756$). The 10 mg/kg dose of rifampin was also similar in efficacy to ABI-0043 at 4 and 40 mg/kg

doses ($P = 0.147$).

The extent of *in vivo* antibacterial activity of ABI-0369 and ABI-0699 was similar to that of the ABI-0043 when 80 mg/kg dose groups were compared ($P = 0.596$), consistent with both potentially similar PK and the similar MICs determined in this study.

The close correlation between the ABI-0043 daily dosage and change in log₁₀ CFU in lung tissue suggested that ABI-0043 might have concentration-dependent killing.

In conclusion, these novel rifamycins demonstrated excellent *in vitro* potency against *S. pneumoniae* having diverse genotypic and phenotypic profiles. *In vivo*, the NCEs demonstrated significant bacterial killing, which increased in a dose dependent fashion and was similar to ceftriaxone at equal mg/kg doses. These data support the continued preclinical development of this class of compounds towards the indication of respiratory infection.

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References

1. Musher DM. *Streptococcus pneumoniae*. In Principles and Practice of Infectious Diseases, 5th ed. Ed., G. Mandell *et al.*, pp. 2128–2147. Churchill Livingstone Inc., New York (2000)
2. Jernigan DB, Cetron MS, Breiman RF. Minimizing the impact of drug-resistant *Streptococcus pneumoniae* (DRSP). A strategy from the DRSP Working Group. JAMA 275: 206–209 (1996)

3. Gordon KA, Biedenbach DJ, Jones RN. Comparison of *Streptococcus pneumoniae* and *Haemophilus influenzae* susceptibilities from community-acquired respiratory tract infections and hospitalized patients with pneumonia: five-year results for the SENTRY Antimicrobial Surveillance Program. *Diagn Microbiol Infect Dis* 46: 285–289 (2003)
4. Murphy CK, Mullin S, Osburne MS, Duzer J, Siedlecki J, *et al.* *In vitro* activity of novel rifamycins against rifamycin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 50: 827–834 (2006)
5. Mullin S, Rothstein DM, Murphy CK. Activity of novel benzoxazinorifamycins against rifamycin-resistant *Streptococcus pyogenes*. *Antimicrob Agents Chemother* 50: 1908–1909 (2006)
6. Rothstein DM, Farquhar RS, Sirokman K, Sondergaard KL, Hazlett C, *et al.* Efficacy of novel rifamycin derivatives against rifamycin-sensitive and -resistant *Staphylococcus aureus* isolates in murine models of infection. *Antimicrob Agents Chemother* 50: 3658–3664 (2006)
7. Farquhar R, Xia M, Meerts I, Phillips L, Buxton D, Larsson M, Chen Y, Murphy CK, Macneil I. Activity of novel rifamycins in assays for human cytochrome P450 induction. Abstracts of Papers of 45th Intersci Conf on Antimicrob Agents Chemother, F-2048, Washington, DC (2005)
8. Tessier PR, Kim MK, Zhou W, Xuan D, Li C, Ye M, Nightingale CH, Nicolau DP. Pharmacodynamic assessment of clarithromycin in a murine model of pneumococcal pneumonia. *Antimicrob Agents Chemother* 46: 1425–1434 (2002)
9. Mattoes HM, Banevicius MA, Li D, Turley C, Xuan D, Nightingale CH, Nicolau DP. Pharmacodynamic assessment of gatifloxacin against *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 45: 2092–2097 (2001)
10. Capitano B, Maglio D, Banevicius MA, Nightingale CH, Nicolau DP. Bactericidal effect of cethromycin (ABT-773) in an immunocompetent murine pneumococcal pneumonia model. *Intern J Antimicrob Agents* 22: 588–593 (2003)
11. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibilities tests for bacteria that grow aerobically-Fifteenth Informational Supplement. CLSI/NCCLS document M100-S15. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA. (2005)
12. Institute of Laboratory Animal Research: Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.
13. Andes D, Craig WA. *In vivo* activities of amoxicillin and amoxicillin-clavulanate against *Streptococcus pneumoniae*: application to breakpoint determinations. *Antimicrob Agents Chemother* 42: 2375–2379 (1998)
14. Joly-Guillou ML, Wolff M, Pocard JJ, Walker F, Carbon C. Use of a new mouse model of *Acinetobacter baumannii* pneumonia to evaluate the post-antibiotic effect of imipenem. *Antimicrob Agents Chemother* 41: 345–351 (1997)