In vitro pharmacodynamics of novel rifamycin ABI-0043 against Staphylococcus aureus

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Objectives: ABI-0043 is a novel benzoxazinorifamycin derivative, which derives its potent bactericidal activity by the specific inhibition of bacterial RNA polymerase. We evaluated the in vitro pharmacodynamics and bactericidal activity of ABI-0043 against clinical isolates of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible S. aureus (MSSA).

Methods: Using time–kill studies at a wide range of concentrations of ABI-0043, we evaluated the killing activity against four clinical isolates of S. aureus over 24 h. An integrated pharmacokinetic/pharmacodynamic area measure was applied to all cfu data and was fitted to a Hill-type mathematical model to evaluate pharmacodynamics.

Results: Bacterial killing for ABI-0043 occurred rapidly and in a concentration-dependent manner. Bactericidal activity was achieved within 4 h at >16× MIC against all isolates. Bacterial reductions were greatest at >64× MIC against MRSA and MSSA isolates, as a >4 log10 cfu/mL reduction was observed as early as 2 h, and sustained throughout 24 h. The pharmacodynamics of ABI-0043 was well described by a Hill-type model, with a steep sigmoidicity constant and a low EC50 against all isolates.

Conclusions: ABI-0043 displayed rapid and sustained bactericidal activity against S. aureus clinical isolates. ABI-0043 represents a promising antibacterial agent to combat serious S. aureus infections. Further, pharmacokinetic, pharmacodynamic and in vivo studies are warranted to determine its ultimate place in antibacterial therapy.

Keywords: S. aureus, rifalazil, benzoxazinorifamycin

Introduction

ABI-0043 is a novel benzoxazinorifamycin that derives its potent bactericidal activity by the specific inhibition of bacterial RNA polymerase.1,2 ABI-0043 is closely related to rifalazil, also referred to as KRM-1648 or ABI-1648, but displays more potent in vitro activity against Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Propionibacterium acnes and Chlamydia spp. and has improved activity against rifampicin-resistant isolates of S. aureus and S. pyogenes.3–4 ABI-0043 has displayed efficacy against S. aureus in several animal models including the neutropenic murine thigh model,1 the mouse septicaemia model3 and a foreign body infection model.5 Limited data exist on the pharmacodynamics and bactericidal activity of ABI-0043 against clinical S. aureus isolates.

Materials and methods

Clinical isolates utilized in the study included methicillin-resistant S. aureus (MRSA; S285 and S293) and methicillin-susceptible S. aureus (MSSA; S288 and S290). All isolates were obtained from the blood of infected patients from the VA Medical Center (Buffalo, NY, USA) and were susceptible to rifampicin. S. aureus ATCC 29213 was utilized as a control.
Pharmacodynamics of ABI-0043

ABI-0043 was provided by Activbiotics Inc., Lexington, MA, USA. ABI-0043 powder was dissolved in 100% dimethyl sulfoxide (DMSO) (Sigma Chemical Company, St Louis, MO, USA) by bath sonication for 30 min to prepare a stock solution of 50 mg/mL. One volume of stock solution was added to four volumes of diluted liquid fill, resulting in a dosing solution containing ABI-0043 in 20% DMSO. The 20% DMSO diluent alone showed no antibacterial effect on S. aureus. Fresh working solutions of ABI-0043 were made prior to each experimental run.

MICs and minimum bactericidal concentrations (MBCs) were determined in quadruplicate using a microdilution technique in accordance with CLSI criteria. Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA) supplemented with calcium (25 mg/L) and magnesium (12.5 mg/L) (SMHB) was used for all broth microdilution susceptibility testing and selective time–kill experiments. The bactericidal activity of ABI-0043 was assessed by time–kill experiments. Briefly, fresh bacterial colonies from an overnight growth were added to normal saline and adjusted spectrophotometrically to provide a standard suspension. This suspension was diluted with SMHB and a standard antibiotic stock solution to achieve a starting inoculum of ~10^6 cfu/mL. Each 10 mL culture was placed in test tubes that were incubated in a water bath at 35°C with constant shaking, and 0.1 mL samples were withdrawn for determination of bacterial counts at 0, 1, 2, 4, 8 and 24 h. The following time–kill experiments were evaluated: growth control and 0.5×, 2×, 8×, 16×, 64× and 128× MIC of ABI-0043. Colony counts were determined by plating 50 μL of each diluted sample onto Trypticase soy agar [TSA; with 5% sheep blood (Becton–Dickinson, Sparks, MD, USA)] with an automated spiral dispenser (WASP; Don Whitley Scientific Limited, West Yorkshire, UK) and incubated at 35°C to confirm colony counts. This method resulted in a lower limit of detection of 2.0 log_{10} cfu/mL. Antimicrobial carryover was minimized by serial dilution (10–to-100 000-fold) and centrifuging the bacterial samples at 10 000 g for 5 min and reconstituting with sterile normal saline to their original volumes. All time–kill experiments were completed in duplicate to quadruplicate.

Development of resistance was evaluated at 24 h for all experiments in duplicate. Samples from each time point were plated on TSA plates containing 4- and 8-fold the MIC of the respective antibiotic to assess the development of resistance and examined for material density from the starting inoculum.

To accommodate all available data generated for each regimen tested and avoid conclusions based on cfu counts at a single time point, an integrated pharmacokinetic/pharmacodynamic area measure (log ratio area) was applied to all cfu data. For each regimen tested, the area under the cfu versus time curve from 0 to 24 h (AUCCfu_{0–24}) was calculated via the trapezoidal rule for both the growth control (AUCCfu_{growth control}) and drug-containing regimen (AUCCfu_{drug}). The AUCCfu_{0–24} was normalized by the AUCCfu_{growth control} of the growth control and the logarithm of this ratio was used to quantify the drug effect as shown in Equation (1). Additionally, the traditional approach (log ratio change) of comparing the changes in cfu/mL from 0 h (cfu_{0}) versus 24 h (cfu_{24}) was calculated as shown in Equation (2).

\[
\log \text{ ratio area} = \log_{10}(\text{AUCCfu}_{\text{drug}}/\text{AUCCfu}_{\text{growth control}})
\]

\[
\log \text{ ratio change} = \log_{10}(\text{cfu}_{24}/\text{cfu}_{0})
\]

Using non-linear regression, a four-parameter concentration–effect Hill-type model was fitted to the effect parameter Systat (Version 11, Richmond, VA, USA) using:

\[
E = E_0 - [(E_{\max} \times \text{C:MIC}^H)/(E_{50}^H + \text{C:MIC}^H)]
\]

Where the dependent variable (E) is either log ratio area or log ratio change, E_0 the measured effect at zero drug concentration, E_{max} the maximal effect, C:MIC the concentration of drug divided by the MIC, E_{50} the C:MIC for which there is 50% maximal effect and H the Hill or sigmoidicity constant.

Results

ABI-0043 displayed MICs and MBCs against all MRSA and MSSA isolates of 0.008 mg/L. The activity of ABI-0043 is depicted in Figure 1. At concentrations of 2× and 4× MIC, ABI-0043 displayed bacteriostatic activity, with a reduction in bacterial counts at 24 h of <3 log_{10} cfu/mL when compared with the starting inoculum against both MRSA and MSSA. Bactericidal activity was achieved within 4 h at concentrations ≥16× MIC against all isolates and was generally maintained through 8 h. However, against S293 and S288 isolates, a rebound in bacterial growth was observed at 24 h. With increasing ABI-0043 concentrations, a concentration-dependent trend towards greater bacterial killing was observed. Bacterial reductions were greatest at higher concentrations of 64× and 128× MIC against MRSA and MSSA isolates: a rapid reduction in bacterial counts >4 log_{10} cfu/mL was observed as early as 2 h, reaching undetectable limits, which was sustained throughout 24 h. Growth on resistance plates at 4× MIC was detected at concentrations 0.5× MIC against S285, S288, S290 and S293 providing a potential explanation for the regrowth that was demonstrated in time–kill experiments. Concentrations ≥2× MIC suppressed the development of resistance as no growth was detected on medium containing 4× or 8× MIC of drug.

Analysis of pharmacodynamics revealed excellent model fits of the data to the Hill model (Table 1 and Figure 1). R^2 values were slightly higher using the log ratio area approach, as all model fits were >0.99 versus >0.96 for the log ratio change approach. Bactericidal activity for ABI-0043 occurred in a concentration-dependent manner, as seen with the steep sigmoidicity constant (H) and low E_{50} against all isolates. Rapid concentration-dependent activity was most evident against MRSA S285, displaying the steepest H among all isolates and a low E_{50}. ABI-0043 against MSSA 288 displayed the shallowest H and highest E_{50}, partially explained by the different concentration-killing profiles, with slight regrowth and less activity at 16× MIC, versus other isolates where this exposure resulted in bactericidal activity. The maximal effect for ABI-0043 was a >7 log_{10} reduction in cfu/mL and a reduction of >4 log_{10} of area for ABI-0043 against all isolates.

Discussion

ABI-0043 is a representative of the most recent series of benzoxazinorifamycins, which displays potent activity against S. aureus, including rifampicin-resistant isolates. ABI-0043 is one of a number of rifamycins, such as ABI-0369, ABI-0699 and rifalazil, which also displays extremely low MICs for staphylococci. Although rifampicin, the oldest representative...
rifamycin, possesses rapid concentration-dependent activity against staphylococci, its therapeutic use has been limited due to the rapid emergence of resistant organisms, poor tolerance and interactions with CYP3A4. Rifampicin has typically been utilized in combination with other agents in persistent, difficult-to-treat infections to overcome a high bacterial burden. However, numerous reports on the emergence of resistance, questionable efficacy and clinical failure raise uncertainty regarding its utility, especially in the treatment of S. aureus endocarditis where it has been used in combination with other agents. ABI-0043 possesses more potent activity than rifampicin in vitro and retains some activity against

Figure 1. Time–kill experiments evaluating the bactericidal activity of ABI-0043 versus MRSA S285 (a), MRSA S293 (b), MSSA 288 (c) and MSSA 290 (d), and the pharmacodynamic relationship between concentration and log ratio change or log ratio area for MRSA S285 (e and i), MRSA S293 (f and j), MSSA 288 (g and k) and MSSA 290 (h and l).
Pharmacodynamics of ABI-0043

Table 1. Model-fitted parameter estimates for ABI-0043 versus S. aureus clinical isolates

<table>
<thead>
<tr>
<th>Log ratio change</th>
<th>S285</th>
<th>S293</th>
<th>S288</th>
<th>S290</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_0^b$</td>
<td>3.15 (7.08)</td>
<td>3.32 (20.6)</td>
<td>3.38 (9.39)</td>
<td>3.16 (33.8)</td>
</tr>
<tr>
<td>$E_{max}^c$</td>
<td>7.27 (3.59)</td>
<td>7.74 (12.3)</td>
<td>8.59 (10.8)</td>
<td>7.40 (16.7)</td>
</tr>
<tr>
<td>$H^d$</td>
<td>1.67 (9.60)</td>
<td>1.00 (34.7)</td>
<td>0.55 (22.8)</td>
<td>1.96 (47.5)</td>
</tr>
<tr>
<td>EC$_{50}^e$</td>
<td>1.10 (8.46)</td>
<td>1.63 (36.0)</td>
<td>2.61 (44.7)</td>
<td>1.07 (33.8)</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.998</td>
<td>0.979</td>
<td>0.994</td>
<td>0.969</td>
</tr>
</tbody>
</table>

Log ratio area

| $E_0$            | 0.0195 (>100) | 0.0177 (>100) | 1.58 (91.3) | 0 (>100) |
| $E_{max}$        | 4.17 (2.46)   | 4.07 (5.97)   | 5.92 (25.4) | 4.00 (1.08) |
| $H$              | 2.84 (14.7)   | 2.89 (39.0)   | 1.07 (27.8) | 5.07 (20.8) |
| EC$_{50}$        | 0.669 (5.46)  | 0.656 (13.2)  | 0.256 (55.7) | 0.717 (10.3) |
| $R^2$            | 0.999        | 0.998        | 0.999        | 0.999        |

aData are reported as maximum likelihood parameter estimates (% standard errors are shown in parentheses).

$bE_0$, effect at concentration to MIC ratio of 0.

c$E_{max}$, maximal effect, values expressed in log$_{10}$ cfu/mL.

d$H$, Hill’s constant.

eEC$_{50}$, concentration to MIC ratio for which there is 50% maximal effect.

rifampicin-resistant strains. Comparable efficacy has also been established for ABI-0043 at lower doses than rifampicin in murine models of infection, and ABI-0043 has also displayed efficacy equivalent to rifampicin in combination, in an experimental model of foreign body infection. In addition, ABI-0043 does not induce the P450 system, whereas rifampicin is a strong inducer of CYP3A4 and other P450 isoenzymes. In the present study, ABI-0043 displayed rapid bactericidal activity against clinical isolates of S. aureus, at concentrations as low as $16 \times$ MIC. Concentrations above the MIC prevented the amplification of resistant mutants, although exposure to $0.5 \times$ MIC resulted in the development of resistance at 24 h. The pharmacodynamics of ABI-0043 were well described by a Hill-type sigmoidal, maximal effect model, displaying concentration-dependent activity against both MRSA and MSSA, where maximal effect ($>7 \log_{10}$ cfu/mL) was achieved at higher thresholds of $\geq 64 \times$ MIC. Our findings are also similar to other investigations in which ABI-0043 demonstrated a dose-dependent response with a high correlation to bacterial kill in a murine pneumococcal pneumonia model. Additionally, the pharmacokinetic profile of ABI-0043 is reported to have a prolonged terminal-phase half-life in animal studies, which may be consistent with once daily dosing in humans. In conclusion, ABI-0043 is a novel rifamycin that may provide an alternative option for the treatment of MRSA infections. Further pharmacokinetic and pharmacodynamic, resistance and clinical studies are warranted to determine its ultimate place in antibacterial therapy.

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References


