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Activity of a novel extended-spectrum β-lactamase inhibitor, AAI101, combined with cefepime against β-lactamase-producing Enterobacteriaceae in a neutropenic murine pneumonia model

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ABSTRACT

Background: AA101 is a novel β-lactamase inhibitor with potent activity against ESBLs and other β-lactamas. AA101 combined with cefepime has completed Phase 2 clinical trials and was granted Qualified Infectious Disease Product and Fast Track designations by the FDA. The ability to restore cefepime activity towards cefepime-resistant Enterobacteriaceae with defined constellations of β-lactamas was examined in a neutropenic mouse pneumonia model.

Materials/methods: MICs of test isolates were determined according to CLSI guidelines. Pharmacokineti profiles in male mice were obtained following intravenous (IV) administration of single doses of cefepime/AA101 (100 mg/kg). Intranasal mice were infected by nasal instillation with 10^7 CFUs of NCTC13434 co-producing TEM-β-lactamase, MUM-1, and HSA-1 (class D) β-lactamas. Vehicle, cefepime/AA101 (1:1), or meropenem was administered IV beginning 2 h post-infection. At 26 h post-infection lungs were excised and their pulmonary burdens determined by quantitative culture. Data were analyzed using the Kruskal-Wallis test, with the Conover-Iman post-hoc test for all pairwise comparisons between groups.

Results: Modal MICs (µg/mL) for the test strains were as follows:

- **K. pneumoniae HMA1280740**: 0.25
- **E. coli NCTC13434**: 0.125

In plasma and lung epithelial lining fluid (ELF), the elimination half-life for cefepime was ca. 11 min, and for AA101 ca. 14 min. Lungs penetration relative to plasma of cefepime and of AA101 was ca. 38% and 76%, respectively. A pneumoniae HMA1280740 and E. coli NCTC13434 each demonstrated robust proliferation and growth in mouse lungs. A dose-response effect for cefepime/AA101 was observed over the range 2.5-25.0/10-50.0 ìg/mL for both test strains. For the same concentration of cefepime, combined treatment showed statistically significant superior efficacy to cefepime monotherapy (P < 0.001) against both strains, and equivalent or superior efficacy to meropenem.

Conclusions: The experimental extended-spectrum β-lactamase inhibitor AA101 restored efficacy to cefepime in a neutropenic murine model of pneumonia caused by cefepime-resistant Enterobacteriaceae on producing ESBL and OXBS-type β-lactamase plus an AmpC or an OXA-β-lactamase. AA101 in combination with cefepime represents a promising new therapy model for treatment of infections caused by drug-resistant Enterobacteriaceae.

Evaluating Enterobacteriaceae isolates were classified as follows: Enterobacteriaceae: Aeromonas, Escherichia coli, K. pneumoniae, P. aeruginosa, and S. marcescens.

INTRODUCTION

Bacteria: The bacterial pathogens used in this study are listed in Table 1. Efficacy: These were obtained, following CLSI guidelines, for cefepime, alone, cefepime combined with fixed concentrations of 4 µg/mL and 8 µg/mL of AA101, and meropenem. Mouse strain: ICR mice (8 mice per dosing regimen per pathogen) were used.

PK study: Following single IV doses of cefepime/AA101 60/30 mg/kg, blood and bronchoalveolar lavage (BAL) samples were collected over 8 h. Cefepime, AA101, and urine concentrations were determined and PK parameters calculated.

Immunosuppression: Cyclophosphamide was administered intraperitoneally at day 4 and at day 1, to induce neutropenia throughout the infection period.

Infection: Mice were rendered unconscious using ketamine and xilazine. Bacterial suspension was administered intranasally.

Treatment: IV, q4h, commenced 2 h post-infection. Groups included pre-treatment, vehicle, cefepime, cefpime/AA101, and meropenem. Lungs were harvested at 2 and 26 h post-infection, homogenized and the infecting pathogens cultured quantitatively.

METHODS

RESULTS

Table 1: Modal MICs of cefepime, cefepime + AA101, and meropenem (23 replicates).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cefepime</th>
<th>Cefepime + AA101</th>
<th>Meropenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae HMA1280740</td>
<td>&gt;128</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>E. coli NCTC13434</td>
<td>&gt;128</td>
<td>0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Table 2: Single-dose (IV) parameters for cefepime and AA101 in mouse plasma & ELF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
<th>ELF Cefepime (µg/mL)</th>
<th>Plasma Cefepime (µg/mL)</th>
<th>ELF AA101 (µg/mL)</th>
<th>Plasma AA101 (µg/mL)</th>
<th>ELF/Plasma ELF Ratio</th>
<th>Plasma/Plasma AA101 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.008</td>
<td>0.02</td>
<td>0.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Cefepime</td>
<td>8.05</td>
<td>0.02</td>
<td>0.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>AA101</td>
<td>0.25</td>
<td>0.02</td>
<td>0.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3: Time point-specific ELF/plasma ratios for cefepime and AA101 in mice.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>ELF Cefepime/Plasma Cefepime</th>
<th>Plasma AA101/Plasma Cefepime</th>
<th>ELF/Plasma ELF Ratio</th>
<th>Plasma/Plasma AA101 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.008</td>
<td>0.02</td>
<td>0.12</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
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<td>0.00</td>
</tr>
<tr>
<td>0.25</td>
<td>0.02</td>
<td>0.12</td>
<td>0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

CONCLUSIONS

- Cefepime/AA101 proved highly efficacious at reducing lung bioburden in the neutropenic murine pneumonia model.
- AA101 restored the efficacy of cefepime against ESBL-producing Escherichia coli and Klebsiella pneumoniae also co-producing other β-lactamase classes.
- Efficacy of cefepime/AA101 was achieved using clinically feasible cefepime AA101 treatment regimens. AA101 achieved excellent penetration into lung ELF (70-90% of plasma levels).
- These studies support continued clinical development of cefepime combined with AA101 to treat infections caused by MDR Enterobacteriaceae expressing extended-spectrum β-lactamas.