Resistance to Sulbactam-Durlobactam in Clinical Isolates of Acinetobacter baumannii is Rare and Maps to PBP3

Samir H. Moussa, Adam B. Shapiro, Sarah M. McLeod, Alita A. Miller
Entasis Therapeutics, 35 Gatehouse Drive, Waltham, MA 02451 USA

Abstract

Effective treatments for infections caused by Acinetobacter baumannii (Abb) are desperately needed. These infections are considered a significant public health concern due to high mortality rates associated with multidrug resistance. Historically, sulbactam (SUL), a class B-lactamase inhibitor (BLI), could be used for the treatment of these infections due to its intrinsic antibacterial activity against Abb. However, its therapeutic utility has been severely compromised due to the emergence of resistance. We previously showed that laboratory-derived sul MIL resistance mapped to residues proximal to the active site of Abb at very low frequencies, and were associated with a fitness cost (1). Durlobactam (DUR, previously known as ETX2514), a broad-spectrum inhibitor of Class A, C, and D serine β-lactamases (2), is currently in Phase 3 clinical testing in combination with SUL for the treatment of carbapenem-resistant Abb. Multiple, unbiased, global surveillance studies have shown that DUR restores SUL susceptibility (MIC ≤ 4 mg/L) in nearly 99% of clinical isolates (>3600 tested to date). 59% of these were found to be carbapenem-resistant (CARB-R) Abb. Whole genome sequencing analyses revealed that the rare SUL-DUR-resistant Abb strains encoded either A515V, T526S or I343F variants of PBP3 (3). These mutant alleles were cloned, purified and compared to wildtype PBP3 for its relative binding to SUL and other β-lactams (BLs). The T526S mutant showed an almost complete loss of SUL binding, whereas A515V and I343F mutants showed only ~2-fold reduction. All three mutant PBP3s had a significant reduction in imipenem binding. Membrane binding to PBP3 was lowered by A515V and T526S but not I343F. Aztreonam binding was modestly affected in all three mutants. Taken together, these results show that: (1) the vast majority (~99%) of SUL resistance in Abb is due to β-lactamase mediated. (2) β-lactam binding, which can be mitigated by combining with DUR and (3) specific mutations in PBP3, while very rare, can confer resistance to SUL-DUR. This is in contrast to recently approved BLBs such as AvyCaz and Zerbila, where pre-existing clinical resistance was reported to be as high as 20% in certain target pathogens, such as CARB-R NSP. A. aerogenes (4).

Methods

• Broth microdilution susceptibility testing was conducted according to CLSI guidelines using cation-adjusted Mueller-Hinton broth. SUL-DUR was tested by dilution of sulbactam in the presence of a fixed concentration of 4 mg/L durlobactam. Testing of 2648 global A. baumannii-ca sacoalcosus complex isolates from 2016-2018 was performed at IHMA laboratories. Resistant mutants were analyzed by whole genome sequencing at Entasis Therapeutics on an Illumina MiSeq.

• Site-directed mutagenesis of the A. baumannii and A. bavlyi PBP3 genes was performed using standard laboratory protocols. Transformation of ADP1 was performed as previously described (5).

• The A. baumannii PBP3 proteins (wildtype, I343F, S390T, S395F, T511S, A515V, and T526S) were purified using a 2-step purification scheme as previously described (1). PBP acylation rate constants were determined by competition with BOCILLIN FL in fluorescence polarization assays (6).

Results

Table 1 Global Surveillance of 2648 A. baumannii-ca sacoalcosus complex (ABC) isolates (2016-2018) tested by IHMA

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sequenced PBP3 allele</th>
<th>SUL</th>
<th>DUR</th>
<th>SUL-DUR</th>
<th>IMI</th>
<th>AMO</th>
<th>TET</th>
<th>COL</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. baumannii ADP1</td>
<td>16 0.25 0.06 0.32</td>
<td>1 0.5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ADP1 + wild type</td>
<td>S390T</td>
<td>128 64 32</td>
<td>0.06 0.16 0.25</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>ADP1 + A515V</td>
<td>S395F</td>
<td>128 64 16</td>
<td>0.15 0.15 0.25</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>ADP1 + T526S</td>
<td>T526S</td>
<td>16 12 8</td>
<td>0.06 0.15 0.25</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>ADP1 + T526S</td>
<td>S395F</td>
<td>32 16 8</td>
<td>0.25 0.15 0.25</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td></td>
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</table>

In addition to genetic confirmation of sulbactam resistance being driven by mutations in PBP3, the wild type and various PBP3 alleles were cloned, expressed, and purified. The effects of these mutations on the reactivity with sulbactam, aztreonam, imipenem, and meropenem were determined.

Conclusions

• The novel β-lactamase inhibitor durlobactam restores sulbactam antibacterial activity against a large (n = 2648) and globally diverse set of A. baumannii isolates collected from 2016 – 2018.

• All mutants of PBP3 associated with sulbactam resistance map near the active site of A. baumannii (PBP3) or PDB: 3UE3

References


Acknowledgements

We thank the team at International Health Management Associates, Inc (IHMA) for conducting surveillance studies.