ALX-009 (OSCN⁻/bLF) EFFICACY AGAINST EMERGENT CYSTIC FIBROSIS PATHOGENS
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INTRODUCTION

The genetic causes of CF are linked to the mutation of a single gene, the cfr (cystic fibrosis transmembrane conductance regulator) gene. This gene codes for the CFTR membrane protein involved in the ion exchange between the cell and the lumen. In the lung, ion equilibrium is important to preserve the protective action of the Airway Surface Liquid (ASL) and mucus. However in CF patients, ASL and mucus volumes and compositions are modified. The ASL normally contains bactericidal/bacteriostatic products in solution that are not or less present in the ASL of CF patients. In addition, the volume of the ASL of CF patients is reduced with increased salt content; both factors dehydrate the mucus that becomes thick and accumulates at the surface of cells.

OSCN and lactoferrin are among the bactericidal compounds that are deficient in the ASL of CF patients. OSCN is a potent large spectrum antibiotic whereas lactoferrin is a multifunctional protein that inactivates/kills bacteria (Fig. 1).

Presence of OSCN is significantly influenced by the CFTR function. Indeed, the precursor molecule SCN⁻ transits by the CFTR channel to the ASL where it reacts with H2O2 via a lactoperoxidase enzymatic reaction to produce OSCN⁻.

OSCN is a highly reactive compound that oxidizes free thiol radicals of proteins to create disulfide bonds that perturb the bacterial physiology. Lactoferrin may act by direct interaction with bacterial cell membranes or by depriving bacteria of iron due to its iron chelator activity. By providing both molecules, ALX-009, a fixed combination of OSCN and lactoferrin, will contribute to restore the natural capacity of the lung to fight against infections.

METHODS

Bacterial isolates

The bacterial isolates obtained from CF patients were kindly provided by several laboratories and national CF repositories around the world. The tested isolates were Achromobacter spp. (n=113), Burkholderia spp. (n=165), Pseudomonas aeruginosa (n=52), Multidrug Resistant (MDR) Pseudomonas aeruginosa (n=20) and Stenotrophomonas maltophilia (n=53). The MDR Pseudomonas aeruginosa isolates were selected according to the MDR criteria levels described by Magiorakos et al. (Clin Microbiol Infect 2012; 18:268-281).

Microbiological methods

1. Minimal Inhibitory Concentrations (MIC) for bLF, OSCN and ALX-009 were obtained with the microdilution method described in guideline M07-Ad of CLSI with slight modifications. Results are reported as the average values of three independent experiments.

2. Fractional Inhibitory Concentration Index (FICI) were obtained with a microdilution checkerboard method. Microdilution experiments were done as reported above. FICI ≤0.5 indicated synergy for the combination; 0.5<FICI ≤1.0: additive effect; 1.0< FICI ≤2.0: indifferent; and FICI >2.0 indicated antagonism between the components. Results are reported as the average values of three independent experiments.

3. Time kill curves for bLF, OSCN and ALX-009 were obtained with the microdilution method as described in guideline M07-Ad of CLSI. From each bacterial group, isolates were selected according to their FICI to test an equal number of synergistic, additive and indifferent isolates. Results are reported as the average values of three independent experiments.

4. Minimal Biofilm Inhibitory Concentration experiments were performed with the MBC™ assay (Innovotech, Vancouver, CA) technology as described by the manufacturer. Results are reported as the average values of three independent experiments.

Test compounds

OSCN solutions exempt of enzymes were produced by enzymatic reaction with Alaxia’s proprietary technology. bLF is a pharma grade bovine Lactoferrin produced by Alaxia.

RESULTS

Test compounds

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TABLE 1. Minimal Inhibitory Concentrations (MIC) for bLF, OSCN and ALX-009

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>OSCN µM</th>
<th>bLF µM</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Achromobacter</td>
<td>113 ± 1</td>
<td>20 ± 1</td>
<td>Additive</td>
</tr>
<tr>
<td>Burkholderia spp.</td>
<td>165 ± 2</td>
<td>20 ± 1</td>
<td>Additive</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>50 ± 2</td>
<td>20 ± 1</td>
<td>Additive</td>
</tr>
<tr>
<td>S. maltophilia</td>
<td>53 ± 2</td>
<td>20 ± 1</td>
<td>Additive</td>
</tr>
<tr>
<td>All isolates</td>
<td>403 ± 2</td>
<td>20 ± 1</td>
<td>Additive</td>
</tr>
</tbody>
</table>

Table 1. Minimal Inhibitory Concentrations (MIC) values for bLF, OSCN and ALX-009. MIC<sub>50</sub> and MIC<sub>90</sub> are given in µg/ml for OSCN and for bLF. MIC<sub>50</sub>: Median, Range; MDR: Multidrug Resistant; bLF: Bovine Lactoferrin; R: Resistant; S: Susceptible.

CONCLUSIONS

ALX-009 demonstrates a positive bactericidal potential on a large panel of multiresistant and emergent CF bacterial strains for which few or no therapeutic options exist.

The antibacterial activity of ALX-009, measured by time kill experiments, suggests that the efficacy of the investigational drug is independent of the drug-resistance strategies developed/acquired by cystic fibrosis pathogens.

The data presented here confirm the potential therapeutic use of ALX-009 for the treatment of multiresistant bacterial infections in CF lung.

ALX-009 phase I clinical trial is ongoing (NCT02598999).

ACKNOWLEDGMENTS