Novel Polymyxin Derivative CA824: Efficacy in Neutropenic Mouse Thigh and Lung Infection Models

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Abstract

Background: Polymyxins are an important class of antibiotics for therapy of Gram-negative bacterial infections as they retain activity against many MDR strains, however their utility is limited by a narrow therapeutic index, particularly with respect to renal toxicity. CA824 is a new semi-synthetic derivative of polymyxin B with a similar microbiological profile but reduced cytotoxicity against a human renal proximal tubule cell line. Here we report on the efficacy of CA824 in neutropenic mouse thigh and lung models.

Methods: Efficacy was determined in comparison to polymyxin B in a neutropenic mouse lung model against Pseudomonas aeruginosa ATCC 27853, and in both neutropenic mouse thigh and lung models against the carbapenem-resistant Acinetobacter baumannii NCTC 13301. Polymyxin B or CA824 were dosed subcutaneously 2, 5 and 10 h post-infection to determine the bacterial burden 16 h post-infection.

Results: Both CA824 and polymyxin B reduced the bacterial burden in the mouse thigh model (A. baumannii) by 3 log10 CFU/g at 30 mg/kg/dose. CA824 on the other hand reduced the bacterial burden in the mouse lung model (P. aeruginosa) by 4 log10 CFU/ml for strains ATCC 27853 and NCTC 13301.

Conclusions: CA824 is a new polymyxin-derivative with superior efficacy to polymyxin B in murine lung models.

Materials & Methods

Synthesis and formulation:
Test articles were prepared as sterile vials and dissolved in saline for injection for in vivo administration (except for the P. aeruginosa mouse lung study where CA824 was dosed in water for injection). CA824 (Figure 1) was dosed (mg/kg) so as to give the same level of free base as for PMB when dosed as PMB sulphate.

Susceptibility testing:
Minimal inhibitory concentrations (MICs) were determined by microbroth dilution using cation-adjusted Mueller-Hinton broth (Oxoid, CM0405) in 96-well polystyrene microtitre plates based on CLSI guidelines1.

Animals:
Male ICR (CD-1, Charles River) mice weighting 21.5 – 27.5 g were used in these studies. Neutropenia was induced with cyclophosphamide by intraperitoneal injection. Animals used in the thigh infection model received 150 mg/kg and 100 mg/kg cyclophosphamide at 4 days and 1 day before infection respectively. Mice used in the lung infection model received 200 mg/kg and 150 mg/kg cyclophosphamide respectively. All animal experiments were performed in accordance with UK Home Office regulations and with local ethical committee clearance.

Mouse Thigh Infection Model:
Mice were infected while under inhalant anaesthesia by intramuscular injection into both right and left thigh muscles. Each thigh received ~1.4 x 107 cfu/g. At 30 mg/kg/dose, CA824 also achieved increased reduction in bacterial burden compared to polymyxin B against P. aeruginosa in the lung at 20 mg/kg/dose (5.3 x 2.9 log10 cfu/g).

Mouse Lung Infection Model:
Groups of 4 mice (6, vehicle only (end group)) were administered test article by intratracheal instillation of 106 CFU A. baumannii NCTC 13301. Polymyxin B or CA824 were dosed (mg/kg SC, 2, 6, and 10 h, harvest at 16 h) at 4 days and 1 day before infection respectively. Mice used in the thigh infection model received 150 mg/kg  and 100 mg/kg cyclophosphamide for 24 h.

Results Table 1: MIC values (µg/ml) against strains used in infection models

<table>
<thead>
<tr>
<th>Compound</th>
<th>A. baumannii ATCC 27853</th>
<th>P. aeruginosa OHOH NHOH NHOH NHOH NHOH NHOH OHOH</th>
<th>P. aeruginosa ATCC 27853</th>
</tr>
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<tbody>
<tr>
<td>Polymyxin B</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>CA824</td>
<td>1</td>
<td>1</td>
<td>1</td>
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Results (cont.)

Summary

- CA824 is a semi-synthetic PMB analogue generated through replacement of the N-terminal fatty acyl and diaminobutyate at position 1 with an iso-butyl piperazine-2-carboxylate.
- The in vivo antibacterial activity of CA824 is comparable to PMB whilst an in vitro cytotoxicity assay against HK-2 cells has improved IC50 compared to PMB (F-735, P<0.001).
- In mouse lung infection models the antibacterial activity of CA824 was superior to PMB against A. baumannii NCTC 13301 and P. aeruginosa ATCC 27853.
- In a mouse thigh infection model the antibacterial activity of CA824 against A. baumannii NCTC 13301 was comparable to PMB.

References

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