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In Vitro and Vivo Activity of a Novel Antisense Compound Against Multi-Drug Resistant Acinetobacter baumannii

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Abstract

Background: Multidrug-resistant (MDR) *Acinetobacter baumannii* is a problematic pathogen in hospitals in New York City (NYC) and worldwide. The use of antisense nucleic acid analogs, which competitively bind targeted sequences of essential bacterial genes, is a promising strategy for the treatment of MDR bacterial infections. **Methods:** A peptide nucleic acid (PNA) containing the cell-penetrating peptide (RXR)4XB bound to a 10-mer nucleic acid was designed to target the *car*A gene of *A. baumannii*. This gene, which is necessary for pyrimidine synthesis, was previously shown to be an essential gene for *A. baumannii*. MICs were performed in chemically defined minimal medium against 4 clinical isolates representing the dominant strains of *A. baumannii* in the NYC region. The in vivo activity of the PNA compound was tested in a sepsis model using *Galleria mellonella* caterpillars. *A. baumanni* strain KB17, which possesses *bla*OXA23 and is susceptible only to polymyxins, was used in the in vivo model. Caterpillars were inoculated with 1x106 cfu of KB17 followed 30 min later with an inoculation of phosphate-buffered saline (PBS) or the PNA compound. Survival was observed over a period of 6 days. **Results:** The MIC of the PNA compound was 1.25 µM for the 4 strains of A. baumannii. When *Galleria mellonella* caterpillars were given a dose of the PNA compound expected to achieve a concentration of 5 µM in the caterpillars, no effect on mortality was seen. When given a dose expected to achieve a concentration of 20 µM, mortality was reduced 53% in the experimental group compared to the PBS controls (p = 0.015). **Conclusions:** A PNA compound targeting the *car*A gene of *A. baumannii* demonstrated both in vitro and in vivo activity against one of the dominant MDR *A. baumannii* strains in the NYC region. These findings are promising for the future use of antisense technology against MDR bacteria. More studies using other animal models and additional bacterial strains are needed to elucidate the true promise of this approach.

Objectives

To determine the efficacy of a novel peptide nucleic acid (PNA) against a multidrug-resistant *Acinetobacter baumannii* in a sepsis model using *Galleria mellonella* caterpillars.

Methods

The *Acinetobacter* isolate we used, KCAB17, was chosen because of its resistance to most commonly-used antibiotics. It possesses the carbapenemase OXA-23. Our PNA compound, Car-coil-PNA, is composed of the following peptide-linked, ten-base pair strand: {N-terminus (RXR) 4XB-cct cat att g C-terminus}. *Galleria mellonella* caterpillars were chosen based on a healthy appearance (moving, non-melanized) and a weight between 250mg and 350mg.

Inoculum determination: Preliminary studies were done to determine an inoculum that would kill the majority of our caterpillars within a 2-5 day window. Caterpillars were injected with varying bacterial concentrations ranging from 1x10^7 to 1x10^10 CFU/ml in a 10µL volume. Through this, a concentration of 1x10^8 CFU/ml (1x10^6 total CFU inoculum) was selected for the remainder of the experiment.

PNA concentration determination: Preliminary studies in minimal medium broth yielded an in vitro minimal inhibitory concentration (MIC) of 1.25 micromoles/L (µM) against our *Acinetobacter* isolate. Based on this MIC we attempted to achieve a concentration of 5 µM within the hemolymph of our caterpillar. After failure at the 5µM concentration, we repeated our experiment using a PNA inoculation that would achieve a 20 µM concentration in the caterpillars.

Experimental Design: Caterpillars were divided into three experimental groups: group 1 - two consecutive PBS injections (negative control); group 2 - Acinetobacter followed by PBS (positive control); group 3 - Acinetobacter followed by PNA. All injections were 10 microliters in volume. All injections were within one half hour of each other, directly into caterpillars' contralateral prolegs. Caterpillars that died within the six-day observation period were included in the mortality count.

Results

Group	Intervention (5 µM)	Mortality at End of Study Period
1	PBS only	1/12 (8%)
2	A.Baumanni followed by PBS	10/12 (83%)
3	A.baumanni followed by PNA	11/12 (92%)

Group	Intervention (20 µM)	Mortality at End of Study Period
1	PBS only	5/28 (18%)
2	A.Baumanni followed by PBS	19/28 (68%)
3	A.baumanni followed by PNA	9/28 (32%), p=0.015 vs. group 2

Results

The absolute reduction in mortality rate seen in the experimental group (group 3) when compared to the positive control of group 2 was 36% (p = 0.015, Fisher's Exact Test). While there was an 18% absolute mortality difference between our experimental group and the negative control that only received PBS, this was not significantly different (p = 0.36).

Conclusion

1. An antisense compound directed at the carA gene reduced mortality in a caterpillar model of MDR Acinetobacter sepsis.
2. Limitations include: size of the study, applicability of the caterpillar model to human infection, and the unexplained requirement for concentrations of the PNA that are much higher than the MIC determined in vitro.

Conclusion (cont.)

3. This study supports the concept of using an antisense approach to combat MDR organisms and suggests the need for additional studies.

References

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