Gene-silencing antisense oligomers inhibit *Acinetobacter* growth in vitro and in vivo

Running Title: Antisense Therapeutic for *Acinetobacter*

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Background: Peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs) are synthetic DNA/RNA analogs that silence expression of specific genes. We studied whether PPMOs targeted to essential genes in *Acinetobacter lwoffii* and *A. baumannii* are active in vitro and in vivo.

Methods: PPMOs were evaluated in vitro using MIC and viability assays, and in vivo using murine pulmonary infection models with intranasal PPMO treatment.

Results: MICs of PPMOs ranged from 0.1 and 64 μM (~0.6 to 38 μg/ml). The most effective PPMO tested was $(RXR)_4$-AcpP, which is targeted to *acpP*. $(RXR)_4$-AcpP reduced viability of *A. lwoffii* and *A. baumannii* by > 10^3 cfu/ml at 5 to 8 x MIC. Mice treated with 0.25 mg/kg or more of $(RXR)_4$-AcpP survived longer and had less inflammation and bacterial lung burden than mice treated with a scrambled-sequence PPMO or PBS. Treatment could be delayed after infection and still increase survival.

Conclusions: PPMOs targeted to essential genes of *A. lwoffii* and *A. baumannii* were bactericidal and had MICs in a clinically relevant range. $(RXR)_4$-AcpP increased survival of mice infected with *A. lwoffii* or *A. baumannii*, even when initial treatment was delayed after infection. PPMOs could be a viable therapeutic approach in dealing with multidrug resistant *Acinetobacter* species.

Key words: Acinetobacter, lwoffii, baumannii, MIC, antisense, oligomer, PMO, morpholino, mouse, infection, respiratory infection, phosphorodiamidate morpholino oligomer

Introduction

The *Acinetobacter* genus includes over 30 species, of which *A. baumannii* is the most prevalent cause of nosocomial infections [1], and *A. lwoffii* appears to be an emerging, opportunistic pathogen [2-4]. Strains from both species often have an impressive number of antibiotic- and toxin-resistance genes [1, 5-10]. Infections due to these pathogens are becoming increasingly difficult to treat [8]. With increasing rates of resistance in *Acinetobacter* and other Gram-negative pathogens, there is an urgent need for new approaches to therapeutics.

PPMOs are synthetic oligomers that mimic the structure of nucleic acid [11]. They are composed of the same 4 bases as DNA, but have a modified backbone that makes them resistant to nucleases [12-14]. Because of this resistance to nucleases, they can be used therapeutically without being degraded. At one end of the oligomer, a short peptide is covalently attached. The peptide is designed with a repeating sequence of cationic and nonpolar amino acid residues that enables the oligomer to penetrate the Gram-negative outer membrane [15-16].

PPMOs are gene-specific, and those that are targeted to essential genes inhibit growth of various bacteria [15-18]. Moreover, PPMOs targeted to specific, essential bacterial genes reduce infection and improve survival in mouse models of infectious diseases [16-19].

In this report, PPMOs were designed and targeted to specific genes, which are essential for viability in the closely related *Acinetobacter baylyi* [20] and assumed to be essential in *A. lwoffii* and *A. baumannii*. The PPMOs were then tested both in vitro and in vivo for their antibacterial activity against *A. lwoffii* and *A. baumannii*.

Methods

Bacteria. All strains of *Acinetobacter* were purchased from ATCC (Manassas, VA), except *A. baumannii* AB0057, which was kindly provided by Todd Hoopman (University of Texas

89 Southwestern Medical Center, Dallas, TX) and *A. baumannii* M9, which was kindly provided
90 by Robert Bonomo (Cleveland Veterans Administration Hospital, Cleveland, OH).
91
92 ***Minimal Inhibitory concentration (MIC).*** MIC was determined using the microdilution
93 method of the Clinical Laboratory Standards Institute [21]. Each PPMO or antibiotic was
94 assayed at least 4 times. Antibiotics were purchased from Sigma, except aztreonam (MP
95 Biochemicals, Santa Ana, CA) and imipenem (LKT laboratories, St. Paul, MN).
96
97 ***Bactericidal measurements.*** Stationary phase cultures were diluted to 5 x 10^5 cfu/ml in
98 Mueller-Hinton II broth, and PPMOs were added to various concentrations as indicated in
99 the figure legends. Cultures were grown aerobically at 37ºC. For cultures of *A. lwoffii* 17976
100 and *A. baumannii* AYE (also named ATCC-BAA-1710), aliquots were removed at various
101 times as indicated in the figure legends, diluted, and spread on LB agar plates. For cultures
102 of *A. baumannii* AB0057, overnight cultures were washed 3 times with 0.15 M NaCl
103 (centrifuged at 5,000 x g, 10 min) to reduce clumping, and then diluted to the starting
104 concentration as above. Aliquots were removed and processed as for the other strains.
105
106 ***PPMO.*** All PPMOs were synthesized and purified at Sarepta (Corvallis, OR) as described
107 [15]. Sequences are shown in Table 1.
108
109 ***Mouse experiments.***
110 ***Preparation of cultures.*** Fifty ml cultures of *A. lwoffii* 17976 or *A. baumannii* AYE
111 were grown aerobically in LB broth at 37ºC to exponential phase. Cells were collected by
112 centrifugation (3,900 x g, 15 min, 20ºC), and washed 3 times in 50, 10 and 5 ml phosphate
113 buffered saline (PBS). Washed cells were resuspended in PBS to 6 x 10^9 cfu/ml and used
114 to infect the mice.
115 ***Dose-response*****:** Groups of 3 to 5, 7 to 10 week old female A/J mice were infected
116 intranasally under isoflurane anaesthesia with 3 x 10^8 cfu in 50 µl PBS (2 x LD_{50}) of either *A.*

Iwoffii 17976 or *A. Baumannii* AYE. Mice were treated intranasally under isoflurane anaesthesia with various doses of $(RXR)_4$-AcpP (NG-08-0163) or $(RXR)_4$-Scr (NG-06-0078) in 25 µl PBS, or 25 µl PBS alone at 5 min and 18 h post-infection. Mice infected with *A. Iwoffii* were further treated daily thereafter for 6 days, whereas mice infected with *A. baumannii* received no further treatment with PPMO after 18 h. Body temperature was recorded using a tympanic infrared thermometer (Braun ThermosScan Pro 4000, Kaz USA, Southborough, MA). The experiment was repeated at least twice.

***Fixed end point*:** Groups of 5 mice were infected as described above with *A. Iwoffii* 17976, and treated intranasally once at 5 min post-infection with 50 µg of either NG-08-0163 or NG-06-0078 in 25 µl PBS, or 25 µl PBS alone. Mice were euthanized at 18 h post-infection and samples were collected from each lung, immediately frozen on dry ice, and stored at -85ºC. Lung samples were thawed, weighed, and diluted with 300 µl PBS. A stainless steel bead was added, and the tissue was homogenized in a Tissue Lyser (Qiagen) for 3 min at 20 Hz. Homogenates were immediately diluted in PBS and spread on LB agar plates. The plates were incubated 15 h and colonies were counted. Cfu/ml was adjusted for differences in lung weight per sample. The experiment was done twice.

***Delayed treatment*:** Groups of 3 mice were infected with *A. Iwoffii* 17976 and initially treated with 50 µg PPMO or PBS at 1, 2, or 18 h post-infection as described above. Mice initially treated at 1 or 2 h post-infection were treated again at 18 h post-infection. All surviving mice were then treated daily for 6 days post-infection.

***Institutional approval*:** All procedures were approved by the Oregon State University Institutional Animal Care and Use Committee, approval numbers 4128 and 4355, and comply with all local, state and federal laws.

Cytokine analysis. Lung homogenates were assayed for cytokines using Flow Cytomix bead system (eBioscience, San Diego, CA) according to manufacturer's protocol.

Statistical analysis. Data were analyzed statistically using GraphPad Prism 6.0 software.

Differences in body weight and temperature were analyzed by t test. Bacteria in lung samples were analyzed by non-parametric (Mann-Whitney) t test. Survival was analyzed by log rank test (Mantel-Cox).

Results

Screen PPMOs for MIC using various strains. PPMOs were designed and synthesized to bind complementary bases in mRNAs of various (putative) essential genes in *Acinetobacter* (Table 1). Each PPMO was 11 bases in length, positioned near the start codon, and conjugated to one of two membrane-penetrating peptides: $(RFF)_3R$ or $(RXR)_4$. Previous work with other Gram-negative bacteria indicates that these criteria are optimal for growth inhibition [16, 22]. The assumption that each targeted gene was essential for viability was based on homologs from essential genes in the closely related *A. baylyi* and *E. coli* [20, 23-24]. The sequence of each PPMO was 100% complementary to its targeted mRNA in all strains of *A. baumannii* and *A. lwoffii* available in GenBank (data not shown), except one sequence targeting acpP that has a 2-base mismatch in *A. lwoffii* as indicated in Table 1.

The MIC of each PPMO was measured using one strain of *A. lwoffii* (17976) and 5 strains of *A. baumannii* (17978, 19606, M9, AYE, and AB0057). The results show that *A. lwoffii* 17976 was more susceptible to all PPMOs than any tested strain of *A. baumannii* (Table 1). An apparent difference was not found in attaching the peptide to either end of the PMO. $(RXR)_4$ conjugates appear to have slightly lower MIC values than the comparable $(RFF)_3R$ conjugates in most strains. One of the most effective PPMOs against all strains tested was $(RXR)_4$-AcpP (NG-08-0163), which is targeted to the gene for acyl carrier protein. NG-08-0163 has MIC values from 0.1 to 8 μM, depending on which strain was used as indicator.

Measure bactericidal activity. The bactericidal activity of $(RXR)_4$-AcpP (NG-08-0163) was measured in pure cultures of *A. lwoffii* 17976, *A. baumannii* AYE, and *A. baumannii* AB0057.

A. lwoffii 17976 is relatively susceptible to standard antibiotics and PPMOs, whereas *A. baumannii* AYE and AB0057 are both multidrug resistant (Table 1). Overnight cultures were diluted into fresh broth, mixed with either 8 x MIC or various concentrations of NG-08-0163, and incubated aerobically for 24 h. Samples were taken at various times and viable cells were measured. Control cultures contained an equal concentration of scrambled base sequence PPMO (NG-06-0078) or were not treated with PPMO.

The results show that the viability of *A. lwoffii* increased slightly (3-fold) in the first 2 h post-treatment, but then decreased almost 4 orders of magnitude from 2 to 8 h post-treatment, and nearly 6 orders of magnitude by 24 h (Figure 1A). At the same time, the untreated culture or scrambled PPMO-treated cultures increased exponentially nearly 1000-fold within 4 h.

The viability of *A. baumannii* AYE decreased even more quickly than *A. lwoffii* in the presence of $(RXR)_4$-AcpP, dropping 3.4 orders of magnitude in 4 h compared to the starting concentration of cells (Figure 1B). From 4 to 24 h, viability of AYE decreased another 1.6 orders of magnitude. The scrambled PPMO slightly slowed the growth rate of AYE for 4 h post-treatment.

The viability of *A. baumannii* AB0057 decreased in proportion to the concentration of NG-08-0163 from 5 to 20 μM (Figure 1C). NG-08-0163 reduced viability of strain AB0057 at concentrations of 5 μM or greater, whereas at a concentration of 2.5 μM, growth was slowed compared to the untreated culture. Viability was reduced 1.4, 2.9, and 4.5 orders of magnitude by 24 h post-treatment at concentrations of 5, 10 or 20 μM, respectively. Scrambled PPMO at the highest concentrations tested had no significant effect on viability of AB0057. Similar results were found with additional strains of *A. baumannii* (data not shown).

Test PPMOs in vivo using an A. lwoffii infection model. $(RXR)_4$-AcpP (NG-08-0163) was tested in a mouse model of pneumonia. Mice were infected intranasally with *A. lwoffii* 17976 and treated intranasally with various amounts of PPMO at 5 min,18 h, and then daily

thereafter for 6 days post-infection. Control mice were treated with equal amounts of scrambled PPMO or PBS. The results show a dose-dependent effect (Figure 2).

Body weight steadily decreased about 20% over the first 3-4 days, and then steadily increased over the final 2-3 days (Figure 2A). There was no statistically significant difference ($P > 0.05$) between treatment and change in mean body weight.

Body temperature decreased sharply within 18 h post-infection, dropping 2.5, 3.1, 3.7, and 6.4°C in mice treated with 50, 15, 5, or 1.5 µg of NG-08-0163, respectively, and 5.2, 6.8, 10.0, 7.5, and 7.5 ºC in mice treated with 50, 15, 5, 1.5 or 0 µg of NG-06-0078, respectively (Figure 2B). The decrease in mean body temperature from 0 to 18 h post-infection was inversely proportional to the dose of PPMO, except for the mice treated with 5 µg of $(RXR)_4$-Scr, which experienced a greater drop in temperature than lower doses. There was statistical significance ($P < 0.01$) in the decrease of temperature between groups treated with equal doses of $(RXR)_4$-AcpP compared to $(RXR)_4$-Scr, except at the lowest dose tested. Group mean temperatures of surviving mice increased after 24 h, gradually returning to normal over the following 6 days.

Survival showed a similar pattern (Figure 2C). The percent survival of mice treated with 0, 1.5, 5, 15, or 50 µg NG-08-0163 was 0, 17, 62, 82 and 89%, respectively. Mice treated with 5, 15, or 50 µg NG-08-0163 survived significantly ($P < 0.001$) longer than mice treated with an equal amount of scrambled PPMO or PBS. The highest dose of scrambled PPMO tested also showed a statistically significant ($P < 0.001$) increase in median survival time (46 h) compared to mice treated with PBS (18 h).

Fixed end point analysis. $(RXR)_4$-AcpP was tested for its ability to reduce bacteria in the lungs of mice infected with *A. lwoffii* 17976. Mice were infected and treated intranasally with a single 50 µg dose of either $(RXR)_4$-AcpP (NG-08-0163) or the scrambled control (NG-06-0078), or PBS at 5 min post infection. At 24 h post-infection, lung samples were collected and analyzed for bacterial burden. The results indicate that a single dose of $(RXR)_4$-AcpP significantly ($P < 0.05$) reduced the cfu/g lung by 1.7 orders of magnitude (32-fold) compared

to treatment with PBS, and by 1.3 orders of magnitude (16-fold) compared to treatment with scrambled PPMO (Figure 3A). The results also indicate that $(RXR)_4$-Scr control reduced bacterial lung burden to a small degree (2-fold) compared to treatment with PBS ($P < 0.01$). TNF-α was significantly lower in lung homogenates from mice treated with $(RXR)_4$-AcpP compared to lung homogenates of mice treated with either $(RXR)_4$-Scr or PBS (Figure 3B; AcpP, 3.9 ng/ml; PBS, 6.2 ng/ml; Scr, 6.1 ng/ml). Similar trends were also found for IL-6 (data not shown). There was no significant ($P > 0.05$) difference in either cytokine between mice treated with $(RXR)_4$-Scr or PBS.

Delayed treatment in A. Iwoffii infection. $(RXR)_4$-AcpP was tested for its ability to reduce morbidity when the initial treatment was delayed after infection. Mice were infected intranasally with *A. Iwoffii* 17976 and treated intranasally with 50 μg $(RXR)_4$-AcpP, the scrambled control, or PBS at 1, 2, or 18 h post infection. Additional treatments were given daily for 6 days post infection. The results show that $(RXR)_4$-AcpP significantly improved the outcome even when administered up to 18 h post-infection (Figure 4). Body weight loss and recovery was similar in all groups (Figure 4A). However, there were trends in body temperature (Figure 4B). Mice treated with $(RXR)_4$-AcpP had less initial loss of body temperature than mice treated with $(RXR)_4$-Scr or PBS. Additionally, mice treated earlier (at 1 h post infection) with $(RXR)_4$-AcpP showed less initial loss in body temperature than those treated initially at later time points.

Mice initially treated with $(RXR)_4$-AcpP at 1, 2 or 18 h post-infection survived significantly ($P < 0.01$) longer than mice treated with PBS (Figure 4C). However, the same was true for mice treated with $(RXR)_4$-Scr. Nevertheless, mice initially treated at 1 h post infection with $(RXR)_4$-AcpP survived significantly ($P < 0.05$) longer than mice initially treated at 1 h post infection with $(RXR)_4$-Scr. In addition, there was a trend (although statistically not significant) in the 2 and 18 h initial treatment groups toward increased survival of mice treated with $(RXR)_4$-AcpP compared to $(RXR)_4$-Scr.

Test PPMOs in vivo using an A. baumannii infection model. $(RXR)_4$-AcpP was also tested against multidrug resistant *A. baumannii* AYE in a mouse model of pneumonia. Mice were infected and treated intranasally with a single 100 µg dose of either $(RXR)_4$-AcpP (NG-08-0163) or the scrambled control (NG-06-0078), or PBS at 5 min post infection. Mice were not treated thereafter. The results show that all of the mice that were treated with $(RXR)_4$-AcpP had reduced morbidity, as indicated by an improvement in weight and body temperature (Figure 5). The decrease in body temperature for mice treated with $(RXR)_4$-AcpP was significantly ($P < 0.05$) less at 18 h or more post-infection than mice treated with either the $(RXR)_4$-Scr or PBS. All mice treated with $(RXR)_4$-AcpP survived the duration of the experiment, whereas all mice treated with either the scrambled PPMO or PBS died, most within 24 h. The differences in survival between mice treated with $(RXR)_4$-AcpP or either $(RXR)_4$-Scr or PBS were statistically significant ($P < 0.001$). There was no statistical difference in survival ($P > 0.05$) between mice treated with $(RXR)_4$-Scr or PBS.

Discussion

Infections with *Acinetobacter* are associated with significant morbidity and mortality. This is in large part due to the inherent level of antibiotic resistance that many *Acinetobacter* species and strains display, making treatment of these infections challenging [1, 4-10]. We have found that some PPMOs targeted to specific, (presumably) essential genes effectively kill *A. lwoffii* and *A. baumannii* in vitro. Importantly, strains that are resistant to multiple antibiotics, such as *A. baumannii* AYE, are susceptible to PPMOs in a clinically relevant range.

There was a large range of MIC values for the various PPMOs tested, from 0.1 µM to >256 µM, depending on the PPMO, the choice of target, and the strain. We note several trends in the efficacy of PPMOs for *Acinetobacter*: 1) Positioning the PPMO to overlap the start codon was more effective than positioning it 3' of the start codon; 2) $(RXR)_4$-conjugates may be slightly more effective than $(RFF)_3R$-conjugates for *Acinetobacter*; 3) There was no

difference in efficacy in vitro between equivalent 3'- and 5'-conjugates. This latter point is consistent with data from *Escherichia coli* [25].

$(RXR)_4$-AcpP (NG-08-0163) was bactericidal. Viability was reduced in proportion to the concentration of PPMO, and over 3 orders of magnitude in multiple strains. This result is similar to the bactericidal effects of PPMOs targeted to essential genes in other bacteria including *E. coli* and *Burkholderia cepacia* complex [15, 17]. Other PPMOs targeted to *acpP* and *ftsZ* in *Acinetobacter* have also been found to be bactericidal (B. Geller, unpublished). These results show that the inhibition of growth caused by PPMOs is the result of loss of viability and not simply a bacteriostatic effect.

In vivo, $(RXR)_4$-AcpP (NG-08-0163) reduced infection and increased survival in a mouse model of pulmonary infection. The response to treatment was proportional to dose. Body temperature decreased less and survival increased with higher doses of $(RXR)_4$-AcpP. Body weight was regained following treatment with $(RXR)_4$-AcpP, indicating an improvement following infection. By all three indicators, $(RXR)_4$-AcpP significantly reduced infection and improved the outcome of mice infected with either *A. lwoffii* or *A. baumannii* compared to mice treated with PBS or $(RXR)_4$-Scr.

Treatment with the control $(RXR)_4$-Scr also increased survival of mice infected with *Acinetobacter* compared to treatment with PBS. However, the improvement was significantly less than treatment with $(RXR)_4$-AcpP. This suggests that a small amount of the benefits of $(RXR)_4$-AcpP in vivo may be attributable to non-specific (sequence independent) effects of PPMO. Similar results have been reported with PPMOs used to treat a mouse model of infection with *Burkholderia cepacia* complex [17]. Because a non-specific effect is not observed in vitro, this suggests that the non-specific effect does not act directly on the bacteria.

Mice treated once with $(RXR)_4$-AcpP (NG-08-0163) showed a significant reduction in *A. lwoffii* in the lungs compared to mice treated with either $(RXR)_4$-Scr or PBS. In addition, the pro-inflammatory cytokine TNF-α was also significantly reduced in AcpP-PPMO-treated mice. These results reinforce the survival data, and taken together, show a probable cause

for the beneficial effects of PPMO treatment, namely that PPMOs directly inhibit bacterial growth and may also have secondary effects on host inflammatory responses.

Treating mice with PPMO 5 minutes post-infection probably does not accurately model the way a PPMO would be used to treat an established infection. In order to model a more clinically relevant situation, initial treatment was delayed following infection. It should be emphasized that this model of pulmonary infection is very aggressive. Mice become morbid within 4 h post infection as indicated by lethargy and a decrease in body temperature of about 3ºC. Within 8-24 h post infection the mice are moribund. Despite this challenging model, $(RXR)_4$-AcpP was able to increase survival even when first administered up to 18 h post-infection. Significantly improved survival is apparent if treatment was initiated 1 or 2 h post-infection. These results demonstrate that $(RXR)_4$-AcpP can improve an *in vivo* outcome under conditions that more closely mimic an established pulmonary infection.

Given the dramatically increasing rate of multidrug resistance in *Acinetobacter* throughout the world [7-8], urgent new approaches to therapeutics are needed. Our results show efficacy of PPMOs in vitro in both *A. lwoffii* and *A. baumannii*. In addition, $(RXR)_4$-AcpP reduced pulmonary infection in mouse models using either of these pathogens. The effective dose in these models ranged from 5 µg (250 µg/kg) to 100 µg (5 mg/kg), which is in a clinically achievable range. Future studies involve screening of new gene targets as well as studying the efficacy of different routes of drug administration. PPMOs and their gene-specific silencing attributes could be a viable approach to developing novel antibacterials for these emerging pathogens.

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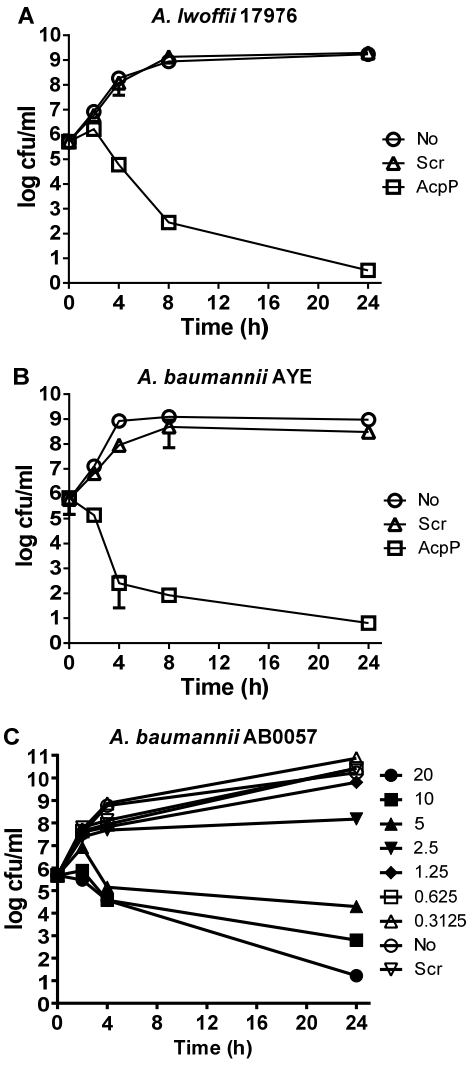

Figure 1. Bactericidal Activity of $(RXR)_4$-AcpP (NG-08-0163). $(RXR)_4$-AcpP or $(RXR)_4$-Scr (NG-06-0078) was added to growing cultures of *A. lwoffii* 17976 (A), *A. baumannii* AYE (B), or *A. baumannii* AB0057 (C). The concentration of PPMOs was 8 x MIC in (A) and (B), which was 1 μM and 32 μM, respectively. Various concentrations of $(RXR)_4$-AcpP were added to cultures in (C) as indicated (μM). $(RXR)_4$-Scr was added at 20 μM. Samples were taken at the indicated times, diluted, spread on agar plates, and grown overnight. Colonies were counted and viable cells from each sample were calculated. Each point n = 3 to 7. Error bars indicate standard deviation.

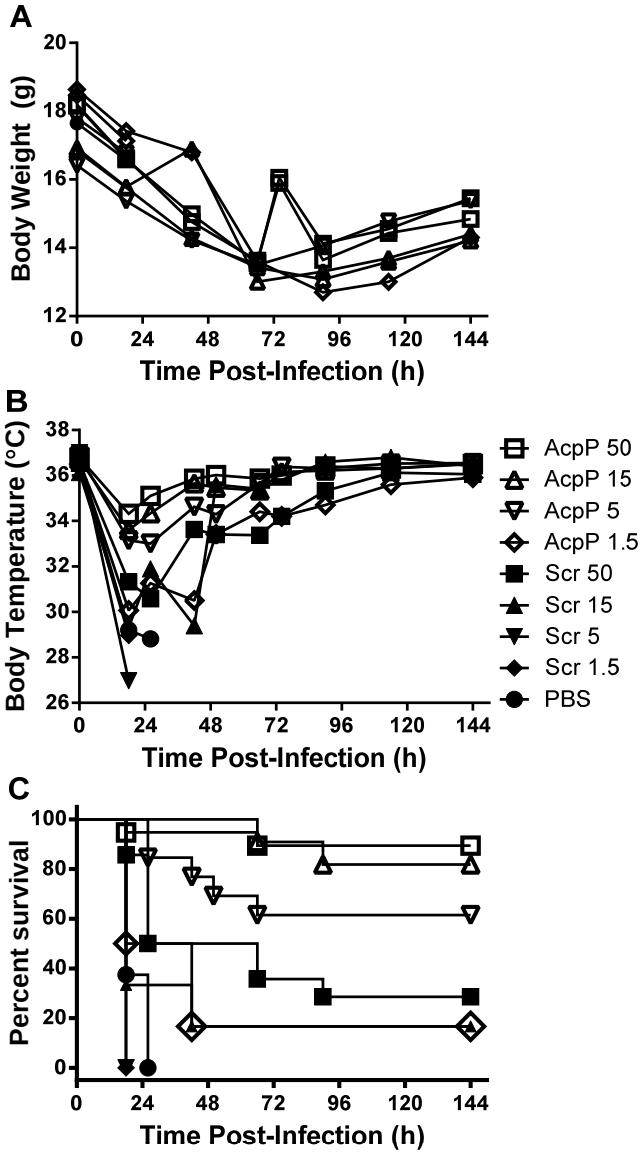

Figure 2. Dose-Response of $(RXR)_4$-AcpP (NG-08-0163) in a pulmonary infection model. Mice were infected intranasally with *A. lwoffii* 17976, and treated intranasally with various doses of either $(RXR)_4$-AcpP (labeled AcpP followed by the dose in μg), $(RXR)_4$-Scr (NG-06-0078, labeled Scr followed by the dose in μg), or PBS at 5 min and 18 h post-infection. Doses were the same for all three panels A, B, and C, and are indicated in μg. Mice were then treated daily thereafter for 6 days. N = 24 (PBS, ●), 19 (AcpP 50, □), 11 (AcpP 15, △), 13 (AcpP 5, ▽), 6 (AcpP 1.5, ◇), 14 (Scr 50, ■), 6 (Scr 15, ▲), 7 (Scr 5, ▼), and 3 (Scr 1.5, ◆). Error bars are not shown for clarity.

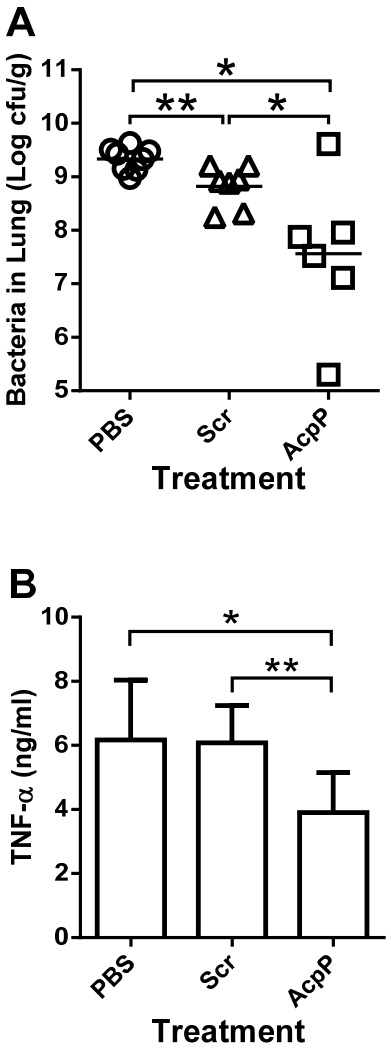

Figure 3. Fixed end point analysis.. Mice were infected intranasally with *A. lwoffii* 17976 and treated intranasally at 5 min post-infection with 50 µg of either $(RXR)_4$-AcpP (NG-08-0163) or $(RXR)_4$-Scr (NG-0078), or PBS. At 18 h post-infection, mice were euthanized and lung samples were collected and immediately frozen. Lung samples were thawed and homogenized in PBS. (A) Lung homogenates were diluted and spread on agar growth plates, and incubated for 15 h. Colonies were counted and bacterial concentration calculated. (B) TNF-α was measured in lung homogenates. * ($P < 0.05$); ** ($P < 0.01$) N = 8 (PBS), 7 (Scr), and 6 (Acp).

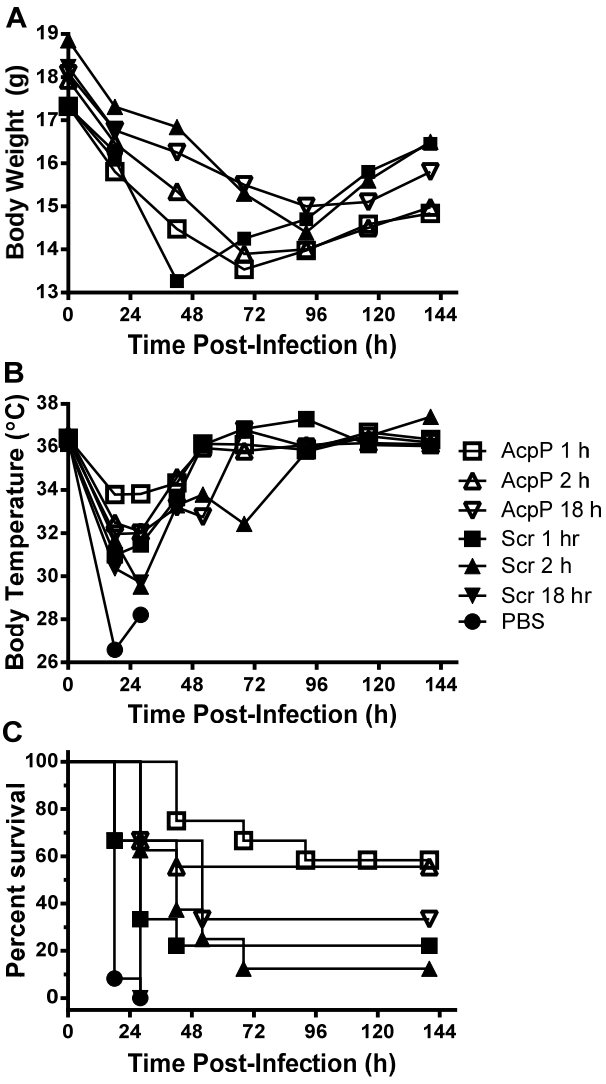

Figure 4. Delayed time of treatment post-infection. Mice were infected intranasally with *A. lwoffii* 17976, and treated intranasally with 50 µg of either $(RXR)_4$-AcpP (NG-08-0163, 1 h □, 2 h △, 18 h ▽ post-infection) or $(RXR)_4$-Scr (NG-06-0078,1 h ■, 2 h ▲, 18 h ▼post-infection), or PBS (●). Mice were then treated daily thereafter for 6 days. Initial treatment times and symbols are the same in all three panels A, B, and C. N = 12 (PBS, Acp 1 h), 9 (Acp 2 h, Scr 1h), 8 (Scr 2h), 3 (Acp 18 h, Scr 18 h). Error bars are not shown for clarity.

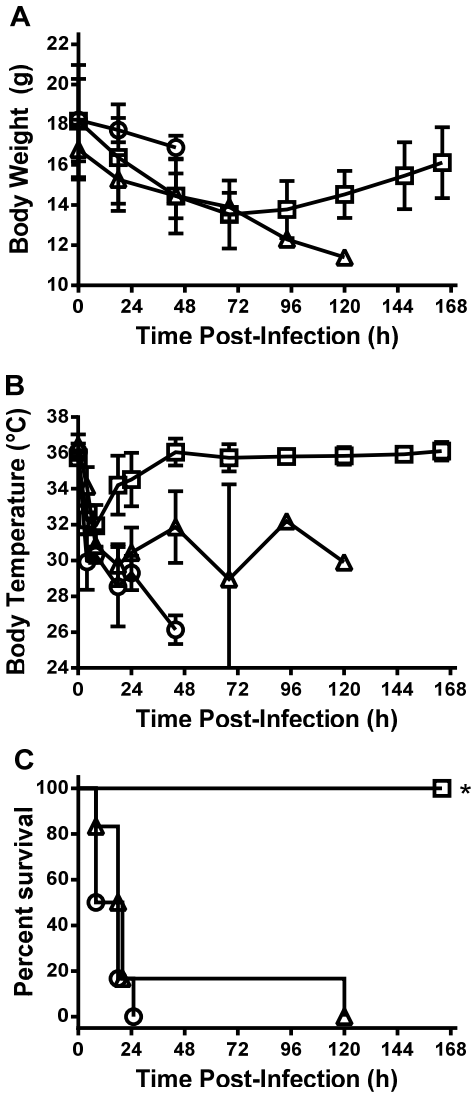

Figure 5. In vivo efficacy of $(RXR)_4$-AcpP against *A. baumannii* AYE. Mice were infected intranasally with *A. baumannii* AYE, and treated intranasally once with 100 µg of either $(RXR)_4$-AcpP (NG-08-0163, □), or $(RXR)_4$-Scr (NG-06-0078, △), or PBS (○) at 5 min post-infection. Error bars indicate standard deviation. N = 6 all treatment groups. *(P < 0.01).

Table 1. MIC of PPMOs and standard antibiotics

PPMO					MIC (μM)					
		Sequence	Target		Strain					
Target (gene ID)[1]	No. NG-	5'→3'	Position	Peptide	17976	17978	19606	M9	AYE	AB0057
acpP (6002279)	08-0162	GTGGCGTTTGA	-16 to -6	5'-$(RFF)_3R$	*	32	32	32	32	32
acpP (6002279)	08-0161	GTGGCGTTTGA	-16 to -6	5'-$(RXR)_4$	*	32	16	16	64	64
acpP (6002279)	08-0888	GTGGCGTTTGA	-16 to -6	3'-$(RXR)_4$	*	32	16	16	64	64
acpP (6002279)	08-0163	ATTCTCCTCAT	+1 to +11	5'-$(RXR)_4$	0.1	8	8	2	4	4
acpP (6002279)	11-0736	ATTCTCCTCAT	+1 to +11	5'-$(RFF)_3R$	0.5	8	8	8	4	4
acpP (6002279)	11-0126	CATTGCTTGTG	-8 to +3	3'-$(RXR)_4$	0.25	64	32	8	64	32
ftsZ (6003231)	06-0203	TCAAATGAGGC	+4 to +14	5'-$(RFF)_3R$	1	16	32	16	64	32
rpsJ (6000543)	11-0124	TAGACATACCA	-4 to +7	3'-$(RXR)_4$	2	>256	64	32	64	256
rpsJ (6000543)	11-0125	TACCAGTAAAC	-10 to +1	3'-$(RXR)_4$	64	>256	>128	>128	>128	>256
Scrambled controls										
$(RXR)_4$-Scr	06-0078	TCTCAGATGGT		5'-$(RXR)_4$	32	>128	128	64	256	128
Scr-$(RXR)_4$	06-0949	TCTCAGATGGT		3'-$(RXR)_4$	32	>128	>128	64	256	>128
$(RFF)_3R$-Scr	05-0655	TCTCAGATGGT		5'-$(RFF)_3R$	16	>64	64	64	64	>128

Standard antibiotic	MIC (μg/ml)					
Ampicillin	80	20	>256	>80	>256	>256
Aztreonam	8	32	32	64	>256	256
Cefotaxime	1	16	32	64	>256	256
Ceftazidime	2	16	16	32	256	>256
Imipenem	2	0.1	2	0.1	2	64
Meropenem	1	1	8	4	4	1

Gentamicin	0.06	1	32	>512	>512	512
Kanamycin	0.25	2	32	>32	>256	>128
Tetracycline	2	8	4	64	16	64
Tigecycline	1	1	2	2	2	2
Colistin	1	1	2	0.5	2	1
Polymyxin B	0.25	0.5	2	2	2	0.5

[1]Gene ID numbers for *A. baumannii* AYE. *acpP* encodes acyl carrier protein; *ftsZ* encodes cell division protein FtsZ; *rpsJ* encodes a ribosomal protein.

*Base sequence mismatch with *A. lwoffii.*