Therapeutic efficacy of aerosolized liposome-encapsulated nubiotic against pulmonary *Pseudomonas aeruginosa* infection

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Respiratory tract infection caused by *Pseudomonas aeruginosa* is one of the major causes of mortality and morbidity in cystic fibrosis patients. However, physicians treating cystic fibrosis patients are confronting the growing problem of multiple-drug resistance in *P. aeruginosa*. Nubiotics are a novel class of nucleotide-based drugs that have the potential to improve clinical outcome against pulmonary infection by multidrug resistant *P. aeruginosa*. In this study, therapeutic efficacy of liposome-encapsulated nubiotic (Nu-3, a protonated deoxyribonucleotide-based antibiotic) was evaluated using a rat model of pulmonary *P. aeruginosa* infection. Nu-3 was loaded into small unilamellar vesicles using a remote loading procedure. Groups of Sprague–Dewley rats were infected intratracheally with cystic fibrosis strains of *P. aeruginosa*, which were enmeshed in agar beads (2–10 × 10^6 enmeshed bacteria/animal). At 2 weeks postinfection, the infected rats were treated with a single-exposure dose of liposome-encapsulated Nu-3 or phosphate-buffered saline, given either by intratracheal administration (150 µl of 2600 A/ml) or by aerosol inhalation using nebulization (50-min exposure, nebulized volume 16 ml of 1300 A/ml). At 4 days post-treatment, the rats were euthanized and the lungs were aseptically harvested, homogenized and cultured for *P. aeruginosa*. Intratracheal administration of liposome-encapsulated Nu-3 showed a modest approximately 1–2 $\log_{10}$ reduction of colony-forming units in the treated group compared with untreated controls. Treatment with a single aerosol exposure of liposome-encapsulated Nu-3 resulted in complete eradication of *P. aeruginosa* from the lung homogenates of 67% of the treated rats, while the other 33% showed significant colony-forming unit reductions compared with the phosphate-buffered saline-treated group ($p < 0.05$). In a parallel study, aerosolized liposome-encapsulated Nu-3 resulted in complete eradication in 90% of the mice infected with a ciprofloxacin-resistant strain of *P. aeruginosa* ($p < 0.05$). These results suggest that aerosolized liposome-encapsulated Nu-3 may represent a promising therapy for chronic pulmonary *P. aeruginosa* infection.

Keywords: aerosol, cystic fibrosis, nubiotic, *Pseudomonas aeruginosa*, therapy

Global prevalence of bacterial resistance to antibiotics is growing at an alarming rate [1]. To reduce the increasing human mortality and morbidity associated with the infectious diseases by drug-resistant bacterial pathogens, there is a compelling need to develop new therapeutic agents that are effective against drug-resistant mutants [1]. Nubiotics are a novel class of proprietary antimicrobial drugs. Structurally, nubiotics are synthetic oligonucleotides and nucleotides with nuclease-resistant backbones, and are fully protonated for enhanced ability to be taken up by bacterial cells [101]. Nubiotics have been shown to be bactericidal against a wide range of Gram-positive, Gram-negative and acid-fast bacteria [2]. With broad-spectrum activity and potential activity against drug-resistant bacteria, nubiotics may therefore represent an exciting and novel class of drugs that may have an important role in the treatment of clinical infections by drug-resistant bacteria [102]. An example of a potent nubiotic is Nu-3 (butyl-phosphate-5′-thymidine-3′-phosphate-butyl), a fully protonated deoxynucleotide whose chemical structure is shown in **Figure 1**. Nu-3 has been shown in previous studies to be efficacious in the treatment of both local and systemic forms of burn-wound infection caused by *Pseudomonas aeruginosa* in mice [3]. The objective of this study is to evaluate the *in vivo* efficacy of NU-3, encapsulated in liposomes, for the experimental treatment of chronic pulmonary infection by *P. aeruginosa*. Liposome-encapsulated Nu-3 was used in this study because it was found to have higher antibacterial efficacy than free, unencapsulated Nu-3 against a topical burn-wound infection caused by *P. aeruginosa* [3].

Lung exacerbations associated with chronic infection by *P. aeruginosa* are the major cause of morbidity and mortality in people with cystic fibrosis (CF). Most patients with CF die

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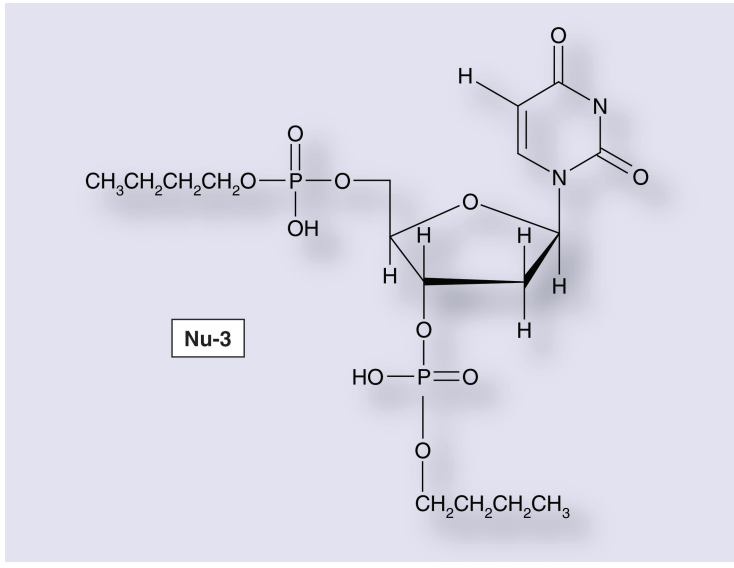
Figure 1. Chemical structure of nubiotic Nu-3 (butyl-phosphate-5´-thymidine-3´-phosphate-butyl).



of progressive pulmonary disease, which is often complicated by recurrent infections with *P. aeruginosa.* CF is traditionally treated with intravenously or orally administered antibiotics to reduce the severity and duration of pulmonary exacerbation and to minimize lung damage by reducing bacterial density in the lungs. Despite the use of aggressive and prolonged antibiotic therapy, infections by *P. aeruginosa* in CF patients are rarely eradicated [4]. A major challenge in the antibiotic chemotherapy of *P. aeruginosa* infection in CF patients is the increasing resistance to a number of antipseudomonal antibiotics, including tobramycin, tetracycline and ciprofloxacin [5]. The emergence of β-lactam-resistant *P. aeruginosa* strains in CF clinics has becoming more widespread [6]. As a result, there is a compelling need to develop new antimicrobial agents that can be effectively used for treating CF pulmonary exacerbations and reducing CF-related mortality and morbidity.

Liposomes are evaluated as a drug-delivery system in this study for the sustained release of nubiotics at the sites of infection in the lungs, and may serve to protect the nubiotics against possible nuclease degradation. Liposomes have been shown to target therapeutic doses of antibiotics to the lungs, thereby avoiding rapid pulmonary drug clearance [7,8]. Drugs encapsulated in liposomes can also be delivered to the lungs by aerosol inhalation [9]. Aerosol inhalation represents an effective and needle-free approach to deliver antibiotics to the lungs, and it has been demonstrated to improve clinical outcome for CF patients [10]. Aerosol inhalation provides direct drug delivery to the site of infection and reduces potential drug toxicity in many cases [11]. Local drug toxicity in the lungs may be potentially minimized by gradual release of the drug from the liposomes. In this study, an established rat model of chronic *P. aeruginosa* infection using agar bead-enmeshed bacteria was used to assess antipseudomonal activity of liposome-encapsulated Nu-3. Liposome-encapsulated Nu-3 was also evaluated for its therapeutic efficacy against pulmonary infection caused by a ciprofloxacin-resistant strain of *P. aeruginosa*.

Materials & methods

Animals

Male Sprague–Dawley rats were purchased from Charles River Canada Ltd (St Constant, QC, Canada). At the onset of the study, their body weights ranged from 250–350 g. BALB/c mice (females, 19–21 g) used in this study were obtained from the mouse breeding colony from Defence R&D Canada – Suffield (DRDC Suffield), with original breeding pairs purchased from Charles River Canada Ltd. The use of animals described in this study was reviewed and approved by the Animal Care Committee of DRDC Suffield. The care and handling of animals described in this study followed guidelines set out by the Canadian Council on Animal Care.

Bacteria

Two CF strains of *P. aeruginosa* were used in this study. P® P4438 and PAO were ciprofloxacin-resistant and -susceptible strains, with MIC_{90} determined by the broth dilution method to be 9 and 0.25 μg/ml, respectively. These strains were generously supplied by Harvey Rabin of the Cystic Fibrosis Clinic, Foothills Hospital, University of Calgary (Alberta, Canada).

Chemicals

Egg phosphatidylcholine (EPC) and cholesterol used for the liposome encapsulation of Nu-3 in this study were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Nu-3 was synthesized and purified by Oligos Etc. Inc. (Wilsonville, OR, USA) [2].

Agar bead-enmeshed bacteria

For the treatment study in rats, *P. aeruginosa* (PAO and P P4438 strains) enmeshed in agar beads were prepared according to the procedures described previously [12]. Sham agar beads (without bacteria) were prepared in the same way

except that bacterial culture was not added. For the study in mice, the agar beads were prepared using a modification of the procedure [12] as follows: *P. aeruginosa* (PAO and P P4438 strains) was cultured in 25 ml of Luria Base broth supplemented with 2% glucose for 16–18 h in a 100-ml black capped bottle. The turbidity was determined using a Shimadzu UV 160U spectrophotometer set at a wavelength of 600 nm. The culture was diluted to a concentration of 0.68–0.81 OD_{600}/ml (~7–8 × 10^9 colony-forming units [CFU]/ml). A 1-ml volume of the bacterial suspension was then added to 19 ml 2% noble agar. The 2% noble agar (Sigma) was warmed to 50°C in a hot-water bath prior to the addition of the bacterial suspension. The agar–bacteria mixture was then mixed vigorously by vortexing for approximately 20 s. The suspension was then drawn into a 1-ml syringe and allowed to solidify at room temperature. A G27 -1/2 gauge needle was then attached to the syringe and the agar was extruded through the needle into a 50-ml Falcon tube containing 5 ml of phosphate-buffered saline (PBS). The extruded agar was then drawn into a 10-ml syringe and extruded through the needle, and the extrusion was repeated twice.

Liposome-encapsulated Nu-3

The liposome preparations used for the encapsulation of nubiotic in this study were prepared using two procedures: remote-loaded vesicles (RLVs) and dehydration rehydration vesicles (DRVs). RLVs used for the encapsulation of Nu-3 were prepared using a modification of the procedure of Oh and colleagues [7]. Briefly, unilamellar vesicles were obtained after extrusion through two 0.2 µm polycarbonate filters. Nu-3 was loaded using a 400 mM ammonium sulphate gradient. Lipid components consisted of a 55:45 molar ratio of phosphatidylcholine/cholesterol (CHOL). The drug:lipid ratio was 6.65 mg:30 µmoles. DRVs for the encapsulation of Nu-3 were prepared using a modification of the procedure described by Wong and colleagues [13] and based on the process first described by Kirby and Gregoriadis [14]. This method was chosen because it is a simple and mild procedure. The lipid mixture contained EPC, CHOL and dioleolylphosphatidylserine (DOPS), in the molar ratio of 7:2:1, respectively. The drug:lipid ratio was 166 µg Nu-3:1 µmol lipid. In a typical preparation, 241 µM of total lipids were dissolved in 20 ml of chloroform in a large 1000-ml round-bottom flask. All of the chloroform was evaporated away in a rotoevaporator, leaving a uniform layer of lipid film on the bottom half of the round-bottom flask. The lipid film was further dried in a vacuum oven at 85 kPa for 1 h at 45°C. The lipid film was rehydrated with 0.9% saline, yielding multilamellar vesicles (MLVs), and then aliquoted into glass vials. The lipid solution in each vial was then frozen in liquid nitrogen. The samples were lyophilized for 18 h to form a white powder of lipid. A glass vial containing 0.5 ml of lyophilized lipid suspension was hydrated with 118 µl of Nu-3 (6600 A/ml). The sample was heated at 45°C for 15 min in a heating block and then allowed to stand at 22°C for 1 h. A 4-ml volume of 0.9% NaCl (pH 7) was added to the thick suspension and free Nu-3 was removed by ultracentrifugation at 125,000 × g for 2 h.

Pulmonary infection in rats & mice

Intratracheal administration was utilized for infecting both rats and mice. Groups of animals were anesthetized with ketamine/xylazine (90 mg ketamine/10 mg xylazine/kg of body weight for rats, and 50 mg ketamine/50 mg xylazine for mice, intramuscularly). The procedures for the inoculation of the agar bead-enmeshed *P. aeruginosa* intratracheally have been previously described [12]. The desired concentration of *P. aeruginosa* in the agar beads to establish a chronic, nonlethal, pulmonary infection of rats was determined to be 1 × 10^7 CFU/ml for rats (volume of inoculum used was 0.1 ml) and 3.1 × 10^5 CFU/ml in mice (volume of inoculum used was 0.05 ml). At 28-days postinfection, control infected animals were euthanized and the lungs were aseptically harvested, homogenized and plated for bacterial counts.

Nubiotic therapy of pulmonary P. aeruginosa *infection*

For the treatment of infected rats using the intratracheal administration of liposome-encapsulated Nu-3, nubiotic treatment was given at 2-weeks postinfection. Rats were first anesthetized with ketamine/xylazine by intramuscular injection as described previously. Once anesthetized, the rats were then treated with a single dose of liposome-encapsulated Nu-3 (RLV, DRV) at a concentration of 2600 A/ml (150 µl per rat) using intratracheal instillation to the right lung. Untreated infected control rats were given an equal volume of PBS. Following intratracheal administration, postsurgical care was maintained to ensure full recovery. At 4-days post-treatment, the rats were euthanized by ketamine/xylazine

overdose, and the lungs were harvested and homogenized and the bacterial loads were determined as described below.

Generation & characterization of liposome-containing aerosols

Liposome-encapsulated Nu-3 RLVs were aerosolized using a jet-nebulizer Pari LC STAR® (Pari, Starnberg, Germany). RLV liposome-encapsulated Nu-3 (1300 A/ml) at a volume of 4 ml was added in the nebulizer reservoir. Aerosols were generated using dry compressed air at 40 lbs/inch^2 with a flow rate of 4 l/min. Aerosol particles generated were monitored and characterized at various stages of the aerosol run using a laser aerodynamic particle sizer (APS) (model 3320, TSI Inc., St Paul, MN, USA) and an APS Advanced Software version 2.9 (TSI). The aerosol particles containing the liposome-encapsulated drugs were characterized for the mass mean aerodynamic diameters (MMADs), geometric standard deviations (GSDs) and peak particle counts (PPCs).

Treatment of pulmonary infection by aerosol delivery

For the treatment studies in rats and mice using aerosolized RLV liposome-encapsulated Nu-3, treatment was administered at week 2 post-infection. Animals infected with enmeshed *P. aeruginosa* were placed in a 24-port nose-only aerosol exposure chamber (In-Tox Products, Albuquerque, NM, USA), using holding tubes designed for rats and mice of various sizes. The animals were then exposed for 70 min to aerosols of liposome-encapsulated Nu-3. These aerosol particles were generated using the Pari LC STAR nebulizer and were characterized using the APS system as described earlier. At 4-days post-treatment, the rats were euthanized by ketamine/xylazine overdose, and the lungs were harvested and homogenized and the bacterial loads were determined as described below.

Bacterial determination of lung homogenates

To determine the bacterial load in the lungs of control and treated animals, the lungs were aseptically harvested. The left and right lungs were harvested separately for the rats and together for the mice. Each lung was then homogenized in sterile PBS (5 ml for rats and 2 ml for mice) with a hand-held tissue grinder and then placed on ice. A total of 100 µl aliquots of the suspension were then tenfold serially diluted in sterile PBS, and 100 µl of the aliquots were plated for growth on trypticase soya agar (TSA) plates. The inoculated plates were incubated at 37°C for 1–2 days, and the number of *P. aeruginosa* was determined. Three plates were used for each dilution for the CFU determinations, and the CFUs were means of the three plates.

Statistical analysis

Statistical analysis of the bacterial counts between control and test groups was carried out using the Mann–Whitney unpaired nonparametric two-tailed test. Differences were considered statistically significant at p-value less than 0.05.

Results

Establishment of a chronic pulmonary P. aeruginosa *infection*

Following intratracheal instillation of *P. aeruginosa* enmeshed in agar beads, the animals were monitored daily for up to 4 weeks. The animals recovered from the intratracheal administration of the agar beads without apparent side effects. There were no visible clinical signs from the intratracheal administration of *P. aeruginosa*, and the animals appeared to be healthy and active. In order to determine whether a chronic pulmonary infection was established in these animals over a period of 3 weeks, lungs were harvested from randomly selected rats from various cages, and the lung homogenates were plated on TSA plates to establish bacterial loads. Lungs from animals instilled with sham agar beads (containing no bacteria) were used as negative controls. Bacterial cultures from lungs harvested from rats at week 1, 2 and 3 postinfection indicated positive recovery of *P. aeruginosa* in the right lung, where the agar beads were instilled (**Table 1**). The CFU counts of the right-lung homogenates were maintained throughout the studies, but the total CFUs vary somewhat from 1×10^6 to 1×10^7.

Efficacy evaluation of liposome-encapsulated nubiotic

The two formulations of liposome-encapsulated Nu-3 (RLV and DRV) were evaluated for their efficacy in the treatment of pulmonary *P. aeruginosa* infection. RLV were small, unilamellar vesicles with an average homogenous vesicle diameter of 100 nm and DRV were large, multilamellar vesicles with heterogenous size distribution of 200 nm to 5 µm. In both cases, the concentration of liposome-encapsulated Nu-3

Table 1. Progression of respiratory *Pseudomonas aeruginosa* infection in rats.

Group	Animal number	CFU/right lung	CFU/left lung
7 days postinfection	1	3×10^6	NG
	2	1.2×10^7	1.3×10^3
14 days postinfection	1	1.6×10^6	6×10^2
	2	6.8×10^6	1.8×10^3
21 days postinfection	1	1.0×10^6	NG
	2	7.8×10^6	2.4×10^3

CFU: Colony-forming units; NG: No growth.

administered to the rats was 2600 A/ml (150 µl/rat). At day 4 post-treatment, the animals were euthanized, and the bacteria counts in the lungs were enumerated by plating on agar plates (**Table 2**). The CFUs in the PBS control group were lower compared with those seen in **Table 1**. This difference may reflect day-to-day experimental variability and/or batch-to-batch difference in the bacterial loads in the agar beads used in the two experiments. Both RLV and DRV caused a modest reduction of at least 1-$\log_{10}$ reduction in the bacterial CFU in the right lung (**Table 2**) compared with the PBS control group (RLV vs PBS control group: $p < 0.05$; DRV group vs PBS control group: $p > 0.05$). RLV was determined to be therapeutically more efficacious than DRV in that it resulted in complete eradication of *P. aeruginosa* from the lungs of the two treated rats, while the other two rats in the group showed greater bacterial reductions. Owing to the statistically significant difference in the CFUs between the RLV and the PBS control group compared with the DRV group, RLV was chosen to be the liposome formulation of choice for the aerosol inhalation study.

Table 2. Intratracheal treatment of pulmonary *Pseudomonas aeruginosa* infection using liposome-encapsulated Nu-3.

Group	Animal number	CFU/right lung	CFU/left lung
PBS	1	7.8×10^3	1.5×10^3
	2	1.6×10^4	NG
	3	2.0×10^5	NG
	4	3.1×10^4	NG
	5	1.2×10^3	NG
DRV	1	4.8×10^3	ND
	2	5.4×10^3	NG
	3	8.4×10^3	1.2×10^4
	4	1.6×10^3	NG
	5	3.0×10^3	NG
RLV	1	NG	NG
	2	1.2×10^3	NG
	3	7.2×10^4	NG
	4	NG	NG
	5	6×10^2	ND
	6	6×10^3	NG

CFU: Colony-forming units; DRV: Dehydration rehydration vesicle; NG: No growth; PBS: Phosphate-buffered saline; RLV: Remote-loaded vesicle.

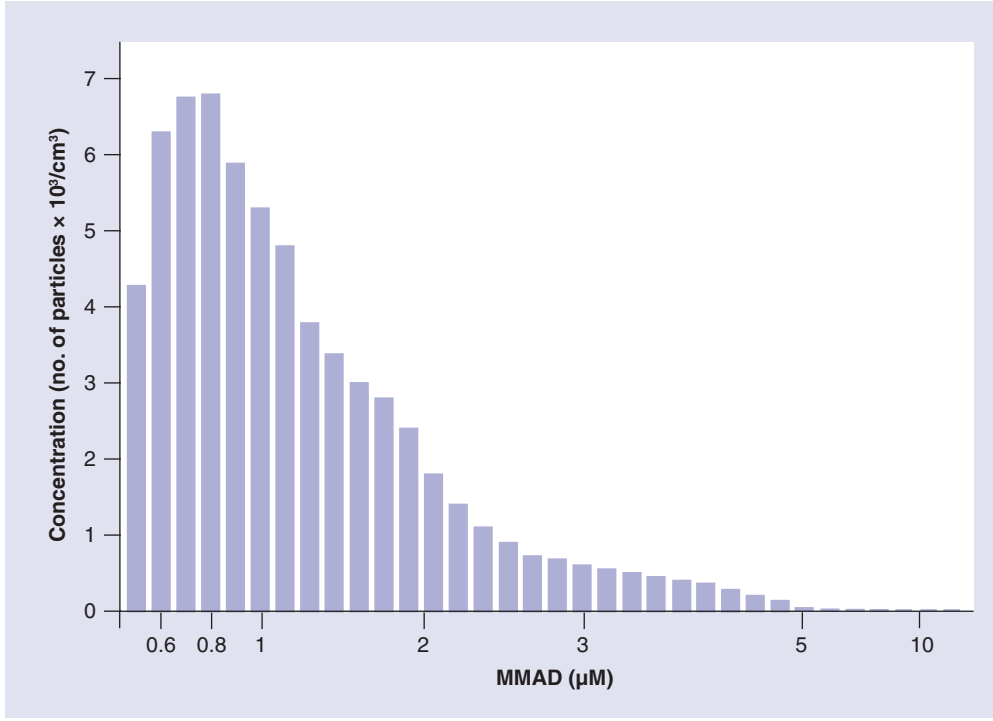
Generation of aerosols containing liposome-encapsulated Nu-3

In order to evaluate aerosol inhalation for the pulmonary delivery of liposomal Nu-3, it was essential that the aerosol particles generated by the nebulization were within the respirable size range and that the liposomes were not disrupted. Aerodynamic particle sizing of the liposome-encapsulated Nu-3 aerosols using APS analyses indicated that the MMADs of the aerosols were 3.8 ± 0.2 µm, with GSDs of 2.2 µm. Approximately 90% of the aerosol volume was in particle sizes smaller than 2 µm. A typical aerosol particle size distribution of the aerosols containing liposome-encapsulated Nu-3 is shown in **Figure 2**.

Aerosol therapy of pulmonary P. aeruginosa *infection*

The therapeutic efficacy of aerosolized liposome-encapsulated Nu-3 against pulmonary *P. aeruginosa* infection was evaluated as follows. Rats and mice intratracheally infected with agar-enmeshed bacteria were treated at 2-weeks postinfection, and were treated with a single exposure of aerosolized liposome-encapsulated Nu-3 or PBS for 70 min. At day 4 post-treatment, the animals were euthanized and their lungs were aseptically removed, homogenized and plated on TSA plates. Following 24-h incubation at 37°C, the CFUs were then determined and the total bacterial loads in the lungs were expressed as total CFU/total lungs (**Table 3**). In

Figure 2. Particle size distribution of aerosols generated by the Pari LC STAR® nebulizer.



Aerosols containing the dehydration rehydration vesicle of liposome-encapsulated Nu-3 were generated by the nebulizer at an airflow rate of 4 l/min. The aerosol particles were analyzed with a laser aerodynamic particle sizing system (APS) using the APS advance software program.
MMAD: Mean mass aerodynamic diameters.

lungs from rats treated with aerosolized PBS, lungs from all five rats revealed high *P. aeruginosa* CFU numbers, ranging from 1.2×10^6 to 4.0×10^8 in the right lung. By contrast, the lungs from the aerosolized liposome-encapsulated Nu-3-treated rats showed either significantly reduced CFUs (two out of six rats) or showed undetected growth in the right lung (four out of six rats; $p < 0.05$).

The therapeutic efficacy of aerosolized liposome-encapsulated Nu-3 was also observed in mice infected with chronic infection with a ciprofloxacin-resistant strain of *P. aeruginosa* (**Table 4**). In this study, the overall bacterial loads in the lungs of the saline-treated infected mice were found to be 2–3 $\log_{10}$ lower than that of the infected rats described above. This is likely to be because the infectious dose used in mice was approximately 2 $\log_{10}$ lower than that used in rats. In the mice treated with liposome-encapsulated Nu-3 by aerosol inhalation, nine out of the ten mice showed complete eradication ($p < 0.05$ vs the saline group), while one treated mouse (number four) was a nonresponder. These results suggest that liposome-encapsulated Nu-3 delivered by aerosol inhalation is highly effective in the treatment of pulmonary *P. aeruginosa* infection.

Discussion

CF is a major pulmonary disease that can result in chronic pulmonary exacerbation, which requires prolonged antibiotic therapy. Although pulmonary infection is the most prominent feature of CF patients, therapy using antipseudomonal antibiotics, such as ceftazidime, tobramycin and ciprofloxacin, is generally administered intravenously or orally. Aerosol delivery of antimicrobial drugs to the lungs is becoming increasingly relevant in the management and therapy of CF [15]. Aerosol inhalation of antipseudomonal drugs offers many distinct therapeutic advantages compared with conventional intravenous administration of these drugs for CF therapy. It offers a safe, effective and needle-free approach for targeting high therapeutic levels of the drugs in the lungs without causing systemic burden in the healthy organs [16,17]. The

Table 3. Treatment of pulmonary infection by aerosol inhalation.

Group	Animal number	CFU/right lung	CFU/left lung
Aerosolized PBS	1	8.6×10^6	NG
	2	5.6×10^7	3.2×10^7
	3	6.0×10^5	NG
	4	4.0×10^8	NG
	5	1.2×10^6	4.0×10^6
Aerosolized RLV	1	4.0×10^6	NG
	2	NG	NG
	3	NG	NG
	4	NG	NG
	5	NG	NG
	6	6.0×10^5	2.4×10^4

CFU: Colony-forming unit; NG: No growth; PBS: Phosphate-buffered saline; RLV: Remote-loaded vesicle.

sustained-release characteristics of drugs from the liposomes have resulted in higher therapeutic efficacy of a number of antimicrobial drugs for a number of respiratory infections [7,8,13]. However, even with specific targeting of the drugs to the lungs with aerosol inhalation, the major therapeutic problem confronted in CF antibiotic therapy is antibiotic-resistance associated with clinical isolates of CF strains of *P. aeruginosa* [5,18,19]. Nubiotics may represent a promising and effective class of antipseudomonal drugs for CF management and treatment. This study suggests that nubiotics are effective against a ciprofloxacin-resistant strain of *P. aeruginosa,* and may therefore be useful against clinical isolates, which are resistant, or are developing resistance to conventional antibiotics, including ciprofloxacin.

To generate therapeutic aerosols of liposome-encapsulated Nu-3, a jet nebulizer called PARI LC Star was used in this study. This jet nebulizer was found to cause the least amount of liposome disruption in five clinical nebulizers, with less than 10% of the total liposomes disrupted during nebulization of an antibiotic ciprofloxacin encapsulated in liposomes [20]. Subsequent studies conducted in our laboratory indicate that this nebulizer does not appear to cause significant disruption to liposomes, regardless of whether the material encapsulated is RNA or DNA [Unpublished Data]. Nebulization of liposome-encapsulated Nu-3 using this aerosol delivery device, generated aerosol droplets with MMAD of 3.8 µm. It is generally accepted that a particle size range of less than 5 µm with GSD of less than 2 µm is ideal for pulmonary delivery [8,9]. It is suggested that aerosol particles of this size range are optimal because they have been predicted by a computer model of the lung to deliver the largest doses to peripheral lung sites.

Treatment of pulmonary *P. aeruginosa* infection using the rat model indicated that liposome-encapsulated Nu-3 is effective in the postexposure treatment of chronic infection by both antibiotic-susceptible and -resistant CF strains. It is postulated here that the enhanced efficacy with liposome delivery of Nu-3 may be associated with enhanced deposition and sustained release of the drug in the lungs; however, pharmacokinetics studies will be required to confirm this. Nu-3 and its related analogues have been shown to be bactericidal against a number of respiratory pathogens, including *Staphylococcus aureus* and *Escherichia coli*. It follows that aerosol delivery of liposome-encapsulated Nu-3 may therefore be therapeutically useful for a number of pulmonary infections, including those caused by respiratory pathogens that are resistant to conventional antibiotics.

Table 4. Aerosol treatment of *Pseudomonas aeruginosa* infection in mice.

Group	Animal number	CFUs/lung
Saline	1	5×10^5
	2	6×10^5
	3	3×10^5
	4	1×10^5
	5	4×10^4
	6	2×10^5
	7	2×10^5
	8	4×10^4
Aerosolized RLV	1	NG
	2	NG
	3	NG
	4	4×10^4
	5	NG
	6	NG
	7	NG
	8	NG
	9	NG
	10	NG

CFU: Colony-forming units; NG: No growth; RLV: Remote-loaded vesicle.

Future studies are needed to exploit indications against other infectious diseases involving the respiratory tract.

Conclusion

The results presented suggest that pulmonary delivery of liposome-encapsulated nubiotics, whether by intratracheal administration or aerosol inhalation, was effective in the treatment of *P. aeruginosa* infection. Since aerosol-inhalation delivery is a surgery-free and more practical and acceptable means of antibiotic treatment compared with intratracheal administration in clinical applications, it is considered to be route of administration of choice for nubiotic. Aerosol inhalation may present a valuable anti-pseudomonal antibiotic therapy in CF patients. This novel therapeutic agent can be extremely useful in the treatment of CF where *P. aeruginosa* has developed multidrug resistance to conventional antibiotics. The use of liposomes to deliver the high sustained doses of nubiotics to the primary sites of infection in the lungs without causing a systemic burden may render this approach more advantageous than intravenous or parenteral forms of antibiotic therapy for CF. The ability of a single aerosol exposure dose of liposome-encapsulated Nu-3 to eradicate *P. aeruginosa* from the lung in 67% of the treated rats is therapeutically important for CF therapy. This approach may translate into a shorter antibiotic-therapy regimen for CF patients and may reduce the development of drug resistance. In contrast to intravenous antibiotic therapy, which requires a hospital or clinical setting, aerosol inhalation can be self-administered at home and therefore may provide a convenient and cost-effective means of treatment.

Executive summary

- A novel nucleotide-based nubiotic encapsulated in liposomes was evaluated for its efficacy against pulmonary *Pseudomonas aeruginosa* infection in rats and mice.
- Nebulization of liposome-encapsulated nubiotic Nu-3 directly targets the drug to the lungs, the site of infection, thereby maximizing its effects.
- A single course of aerosolized treatment provided complete reduction in colony-forming units of *P. aeruginosa* in 67% of the lungs of treated rats.
- Treatment with aerosolized liposome-encapsulated Nu-3 was effective against a ciprofloxacin strain of *P. aeruginosa*.
- The results suggest that aerosolized liposome-encapsulated Nu-3 may represent a novel and promising therapy against chronic *P. aeruginosa* infection in cystic fibrosis patients.
- This novel drug formulation may merit consideration as one of the solutions to combat the growing drug resistance of *P. aeruginosa* to conventional antibiotics.

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Patents

101. Oligos Etc, Inc. US6211162 (2001).
102. Oligos Etc. Inc. US6211349 (2001).