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Enhanced activity of liposomal polymyxin B against *Pseudomonas aeruginosa* in a rat model of lung infection

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Abstract

The bactericidal effectiveness of liposomal polymyxin B against *Pseudomonas aeruginosa* was investigated in an animal model of pulmonary infection. Polymyxin B was incorporated into liposomes composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and cholesterol (Chol) (2:1). Lung infection was induced in rats following intratracheal instillation of 10^7 colony-forming units (CFU) of *P. aeruginosa* (ATCC 27853) embedded in agar beads. Starting on day 3 post-infection, animals were treated daily, for 3 consecutive days, with saline, empty liposomes, free polymyxin B, or liposomal polymyxin B (2 mg polymyxin B/kg body weight) by intratracheal instillation; animals were killed 24 hr after the third drug instillation. Treatment of infected animals with liposomal polymyxin B significantly reduced the pulmonary bacterial counts (3.7 ± 0.4 log CFU/paired lungs) as compared with that of free polymyxin B (5.1 ± 0.2 log CFU/paired lungs). Treatment of infected animals with empty liposomes gave pulmonary bacterial counts similar to those obtained from the saline-treated group. Pulmonary infection with *P. aeruginosa* also resulted in lung injury as evidenced by increases in wet lung weight and decreases in angiotensin converting enzyme activity as well as increases in myeloperoxidase activity, an index of the inflammatory response. Treatment with free polymyxin B ameliorated the lung injuries induced by the microorganism, a protective effect that was more pronounced in the liposomal polymyxin B-treated group. The levels of polymyxin B in the lungs of the infected animals treated with the liposomal suspension were significantly higher (42.8 ± 6.2 μg/paired lungs) compared with those treated with the free drug (8.2 ± 0.4 μg/paired lungs). These data suggest that direct delivery of liposomal polymyxin B to the lung can be effective in the treatment of pulmonary infection with *P. aeruginosa* by enhancing retention of the antibiotic in the lung.

Keywords: *Pseudomonas aeruginosa*; Polymyxin B; Liposomes; Lung infection; Inflammation; Lung injury

1. Introduction

Pseudomonas aeruginosa is a Gram-negative opportunist pathogen that can cause serious nosocomial infection [1] and has been the cause of serious illness in various debilitated patients, especially those with burn wounds, battlefield injuries, organ transplant, and respiratory diseases including cystic fibrosis [2–4]. The mortality rate from *P. aeruginosa* sepsis is high and exceeds the rates from all other Gram-negative agents [5].

In patients with pulmonary infections, particularly those with cystic fibrosis, the pharmacokinetics of the administered antibiotics are usually altered, thus necessitating the prolonged administration of excessive dosages [6], which, in turn, can lead to the development of adverse side-effects and antibacterial resistance. More recent studies, however, have shown that the encapsulation of antimicrobial agents within liposomes increases their intracellular delivery to specific target cells and subsequently increases their antimicrobial effects [7–9].

Polymyxin B is a polycationic peptide antibiotic known to have potent bactericidal activity against a broad range of Gram-negative bacteria [10] with no clinically significant activity against Gram-positive organisms or fungi [11]. Polymyxin B exerts its bactericidal action by interacting with acidic phospholipids and LPSs of bacterial membranes, thus disrupting the structure and function of the outer cell

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Abbreviations: ACE, angiotensin converting enzyme; CFU, colony-forming units; Chol, cholesterol; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; LPS, lipopolysaccharide; MIC, minimum inhibitory concentration; MPO, myeloperoxidase; PLA_2, phospholipase A_2.

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wall and cytoplasmic membranes of bacteria [12,13]. This results in perturbation of the osmotic equilibrium of the bacterial cell, leading to cell death [13–15]. However, due to its potential toxicity, polymyxin B is used almost exclusively in the treatment of Gram-negative bacilli infections that are resistant to other preferred antimicrobial agents or in patients intolerant to the preferred antimicrobials [11,13]. Thus, we attempted to deliver polymyxin B incorporated in liposomes directly to the lungs, with the aim of attaining high pulmonary levels of the drug with minimal toxic effects.

Liposomes are artificially prepared phospholipid vesicles, characterized by the presence of hydrophobic bilayers alternating with aqueous compartments. Hydrophilic molecules can be encapsulated in the aqueous spaces, and lipophilic molecules can be incorporated into the lipid bilayers. Liposomes provide a relatively safe delivery system because they are biocompatible, biodegradeable, and relatively non-toxic. Considerable attention has focused on the use of liposomes to deliver drugs and molecules of potential therapeutic interest to specific sites of action, and in our case to the lungs via the airways [16,17]. Liposomes are well suited as vehicles for delivering antimicrobial agents because they usually provide a sustained drug release effect, minimize drug toxicity, and increase overall drug efficacy [16–19].

The present study was designed to evaluate: (i) the entrapment efficiency of a DPPC/Chol liposomal formulation, (ii) the *in vitro* antimicrobial activities of the liposomal polymyxin B formulation against several Gram-negative bacterial strains, and (iii) the therapeutic effectiveness of the liposomal polymyxin B formulation administered intratracheally in a rat model of chronic lung infection with *P. aeruginosa*.

2. Materials and methods

2.1. Chemicals

Polymyxin B was purchased from the Sigma Chemical Co. DPPC and Chol were obtained from Avanti Polar Lipids, Inc. All other chemicals were purchased from the Sigma Chemical Co. or BDH.

2.2. Bacterial strains

Bordetella bronchiseptica (ATCC 4617 and 10580), *Pseudomonas aeruginosa* (ATCC 25619 and 27853), and *Escherichia coli* (ATCC 25922) were purchased from PML Microbiologicals. A clinical strain of *P. aeruginosa* (W 22539) was obtained from the Clinical Microbiology Laboratory of the Wellesley Hospital. These organisms were stored at −70° in trypticase soy broth (PML Microbiologicals) supplemented with 10% (v/v) glycerol. For experimentation, these strains were inoculated onto blood agar plates (PML Microbiologicals) and incubated for 18 hr at 37°.

2.3. Preparation of liposomal polymyxin B formulations

Polymyxin B liposomes were prepared from a lipid mixture (90 μmol) of DPPC and Chol in a molar ratio of 2:1 (DPPC:Chol). In brief, the chloroform used to dissolve the lipids was removed under vacuum at 51° using a rotary evaporator (Buchi-Rotavapor R110, Brinkmann). To the thin dry lipid film, 6 mL of an aqueous solution of polymyxin B at a concentration of 10 mg/mL was added. The lipid suspensions, were extruded twice through double-stacked 100-nm pore-size polycarbonate membranes (Nucleopore Corp.) in an extruder (Lipex Biomembranes Inc.). Unencapsulated drug was removed by centrifugation (100,000 *g* for 1 hr at 4°), and the liposomal pellet was resuspended in saline. For all experiments, the size of the extruded liposomes was determined with the use of a Coulter N4SD particle-size analyzer (Coulter Electronics of Canada) and was found to have a mean diameter of 165 ± 20 nm. The entrapment efficiency was found to be 5.6 μmol polymyxin B/μmol lipid.

2.4. Liposomal entrapment efficiency of polymyxin B

The entrapment efficiency of liposomes was calculated as the percentage of polymyxin B incorporated into liposomes relative to the initial total amount of drug in solution.

2.5. Microbiological assay for the measurement of polymyxin B

The concentrations of polymyxin B were measured by a microbiological assay employing *B. bronchiseptica* (ATCC 4617) as an indicator organism. This organism was grown overnight in trypticase soy broth at 37°, and the bacterial suspension was adjusted with saline to contain an equivalence of a McFarland standard of 0.5 to ensure dense confluent growth on the plates. A total of 2 mL of diluted culture was added to 175 mL of autoclaved molten antibiotic medium No. 9 (PML Microbiologicals) that had been cooled to 56° and mixed gently by inversion to distribute the inoculum. The seeded medium was then poured into glass plates (260 × 200 mm) on a horizontal level surface and left to solidify for 30 min at room temperature. The wells (4 mm) in the plates were made by a puncher device in the agar and filled with 25-μL samples or standards. The glass plates were covered and incubated overnight (24 hr) at 37°. Duplicate zone inhibition diameters were averaged and compared with a series of standards. Standard curves were made with known quantities of free polymyxin B added to saline or normal pooled rat sera, depending on the sample to be assayed. The concentrations of unknown samples were obtained by extrapolation from the zones of inhibition of standards by

linear regression analysis. The range of linearity for polymyxin B was from 0.78 to 100 μg/mL with a correlation coefficient of at least 0.99. The minimum detection limit of the assay was 0.78 μg/mL.

2.6. Measurement of antimicrobial activity

The MICs of free or liposomal polymyxin B were determined by the agar dilution method [20]. Bacterial inocula were prepared from an overnight culture in trypticase soy broth and adjusted to yield 10^4–10^5 CFU/spot. The adjusted inocula were then delivered onto Mueller Hinton agar (PML Microbiologicals) plates containing 2-fold serial dilutions of antibiotics, using a Steer's replicator (Cmi). Free or liposomal polymyxin B, free polymyxin B plus empty liposomes, or empty liposomes alone were tested simultaneously against all bacterial strains. The lowest concentration of antibiotic that prevented the appearance of a visible growth within the inoculation area after 18 hr at 37° was defined as the MIC.

2.7. Rat model for lung infection and antibiotic treatment

Male Sprague–Dawley rats weighing between 225 and 250 g (Charles River, Saint-Constant) were housed in a 12-hr dark–light cycle animal facility with controlled temperature and humidity. The rats were fed Purina rat chow pellets and water *ad lib*. All animals were treated and cared for in accordance with the guidelines recommended by the Canadian Council on Animal Care.

Infection in the lungs of rats was established by intratracheal administration of *P. aeruginosa* (ATCC 27853) embedded in agar beads. In brief, a suspension of the bacterial strain incorporated into agar beads containing 10^7 CFU was administered in a volume of 100 μL by the intratracheal route to rats previously anesthetized with ketamine hydrochloride (Rogar/STB Inc.) and xylazine (BAYVET Division, Chemagro Limited). Three days after the inoculation of bacteria, infected animals (after being randomly distributed into the different groups) received one dose of 100 μL daily, for 3 consecutive days, of either saline, empty liposomes, free polymyxin B (500 μg), empty liposomes (90 μmol) plus free polymyxin B (500 μg), or liposome-entrapped polymyxin B (500 μg in 90 μmol lipid) by intratracheal instillation using the methodology described for the infection. The anesthetized rats were killed 24 hr after the last dose. The entire lungs and both kidneys were removed aseptically, weighed, and homogenized in cold PBS (20%, w/v) for 30 sec with a Polytron homogenizer. The homogenizer was rinsed and flamed between samples. Serial 10-fold dilutions in PBS of lung homogenates were prepared, and 100 μL of each dilution was spread on sheep blood agar (PML Microbiologicals). CFU were counted after 24-hr incubations at 37°. To confirm the identity of the counted colonies, *P. aeruginosa* colonies were tested on Cetrimide agar.

2.8. Biochemical assays

Lung ACE activity, an indirect marker of pulmonary endothelial cell damage, was carried out as described by Suntres and Shek [21]. PLA_2 is a marker of pro-inflammatory activity, and its concentration in plasma was estimated by using a specific enzyme-linked immunosorbent assay kit (Boehringer Mannheim Canada) according to the directions of the manufacturer [22]. The measurement of MPO activity, as an indicator of neutrophil infiltration, in sonicated whole lung homogenates was estimated by using a specific enzyme-linked immunosorbent assay kit (R&D Systems) according to the directions of the manufacturer.

2.9. Data analysis

The results from a representative experiment (N = 3 experiments) are expressed as means ± SEM (N = 5–6 animals per group). Comparisons among groups were evaluated by ANOVA with a Newman–Keuls test of multiple comparisons [23]. The level of significance was accepted at $P < 0.05$.

3. Results

3.1. Liposomal entrapment efficiency of polymyxin B

The entrapment efficiency of polymyxin B in extruded liposomes composed of DPPC:Chol (2:1) was found to be 3.7 ± 0.5%, and the loading capacity was estimated to be 5.6 μmol polymyxin B/μmol DPPC.

3.2. Bacterial susceptibilities in vitro

The MICs of free polymyxin B and liposomal polymyxin B for different strains of *P. aeruginosa*, *B. bronchiseptica*, and *E. coli* are shown in Table 1. The MICs of

Table 1
In vitro activities of free or liposomal polymyxin B preparations against different strains of Gram-negative bacteria

	MIC (μg/mL)	
	Free polymyxin B	Liposomal polymyxin B
B. bronchiseptica (*ATCC 10580*)	4.0 ± 0.5	1.0 ± 0.2
P. aeruginosa (*ATCC 27853*)	16.0 ± 2.0	4.0 ± 1.0
P. aeruginosa (*W 22539*)	32.0 ± 4.0	8.0 ± 2.0
E. coli (*ATCC 25922*)	8.0 ± 2.0	2.0 ± 0.5

Minimum inhibitory concentrations (MICs) were determined by a standard agar dilution method. Two-fold dilutions for each drug concentration were prepared. The plates were incubated for 18 hr at 37° and then read. The MIC value was recorded to be the lowest concentration of the drug that prevented visible growth. The combination of plain liposomes and free polymyxin B had an antibacterial activity similar to that of free polymyxin B. Values represent the means ± SEM of 3 incubations.

Table 2
Antibacterial effect of liposomal polymyxin B on *P. aeruginosa* in an experimental model of chronic lung infection

Treatment	Bacterial count (log CFU/paired lungs)
Saline	6.0 ± 0.3
Empty liposomes	6.2 ± 0.2
Free polymyxin B	5.1 ± 0.2
Empty liposomes + free polymyxin B	5.7 ± 0.3
Liposomal polymyxin B	3.7 ± 0.4*

Three days after the infection of rats with *P. aeruginosa* (10^7 CFU/100 μL, i.t.), they were treated intratracheally, on a daily basis, with either saline, empty liposomes, free polymyxin B (500 μg), empty liposomes (90 μmol) + free polymyxin B, or liposomal polymyxin B (500 μg in 90 μmol lipid) for 3 consecutive days. The rats were killed 24 hr after receiving the last dose. Each value represents the mean ± SEM for 6 animals from a representative experiment.

* Significantly different ($P < 0.05$) from the value obtained from infected animals treated with free polymyxin B.

liposomal polymyxin B when compared to those of free polymyxin B were lower for all the bacterial strains examined.

3.3. Bactericidal effectiveness of free or liposomal polymyxin B in the lungs of infected rats

The results presented in Table 2 compare the *in vivo* bactericidal effect of polymyxin B encapsulated in liposomes with that of free polymyxin B. Treatment with liposomal polymyxin B was more effective in reducing the bacterial counts (3.7 ± 0.4 log CFU/paired lungs) in the lungs of infected animals than treatment with free polymyxin B (5.1 ± 0.2 log CFU/paired lungs). Treatment of infected animals with empty liposomes (6.2 ± 0.2 log CFU/paired lungs) did not have any significant bactericidal effect when compared with that of the saline-treated group (6.0 ± 0.3 log CFU/paired lungs), while treatment with empty liposomes plus free polymyxin B (5.7 ± 0.3 log CFU/paired lungs) had lung bacterial counts similar to that of the free polymyxin B-treated group.

3.4. Antibiotic levels in the lungs, kidneys, and serum of infected animals treated with free or liposomal polymyxin B

As shown in Table 3, the lung antibiotic level (42.8 ± 6.2 μg/paired lungs) 24 hr after intratracheal administration of the last dose of liposomal polymyxin B was about 5 times higher than that of animals administered free polymyxin B (8.2 ± 0.4 μg/paired lungs). No antibiotic was found in the kidneys or serum of the liposomal polymyxin B-treated group, while 3.4 ± 0.1 μg/organ weight and 1.0 ± 0.1 μg/mL were found in kidneys and serum, respectively, in the group of animals treated with free polymyxin B.

Table 3
Polymyxin B levels in the lungs, kidneys, and serum of infected animals treated with free polymyxin B or liposomal polymyxin B

Treatment	Polymyxin B concentration		
	Lungs (μg/paired lungs)	Kidneys (μg/kidneys)	Serum (μg/mL serum)
Free polymyxin B	82 ± 0.4	3.4 ± 0.1	1.0 ± 0.1
Liposomal polymyxin B	42.8 ± 6.2*	ND	ND

Rats were treated intratracheally with either free or liposomal polymyxin B and were killed 24 hr after receiving the last dose. Each value represents the mean ± SEM for 5–6 animals from a representative experiment. ND = not detected.

* Significantly different ($P < 0.05$) from the value obtained from infected animals treated with free polymyxin B.

3.5. Effect of treatment with free or liposomal polymyxin B on wet lung weights and ACE activity

Infection of lungs with *P. aeruginosa* resulted in significant increases (132% of value from uninfected animals) in wet lung weights, indicative of lung edema (Fig. 1). The increases of the lung weights were lower in the liposomal polymyxin B-treated group (34% of value from uninfected animals) than in rats treated with free polymyxin B (67% of value from uninfected animals). Treatment of animals with empty liposomes did not alter the P. *aeruginosa*-induced lung weight increases. The wet lung weight in animals treated with empty liposomes and free polymyxin B was reduced significantly, and it was found to be similar to that observed for free polymyxin B.

ACE, localized primarily in pulmonary capillary endothelial cells, has been used as a marker of lung injury [24]. Infection of lungs with P. *aeruginosa* resulted in a dramatic reduction (43%) in ACE activity (Fig. 1), suggesting that the capillary endothelial cells are adversely affected. Treatment of animals with saline or empty liposomes did not alter the P. *aeruginosa*-induced decreases in ACE activities significantly. On the other hand, treatment of animals with free polymyxin B or liposomal polymyxin B ameliorated the *P. aeruginosa* changes in ACE activities with the effect of the liposomal preparation being far superior (Fig. 1).

3.6. Effect of treatment with free or liposomal polymyxin B on MPO and PLA_2 activities

In the present study, infiltration and activation of neutrophils in the lungs of infected animals were assessed indirectly by measuring the activities of MPO and PLA_2 (Fig. 2). Infection of animals with *P. aeruginosa* resulted in significant increases in pulmonary MPO activity (3-fold), suggestive of neutrophil infiltration. Also, pulmonary infection was associated with increases in PLA_2 concentration (3.3-fold increase), suggestive of stimulation of the inflammatory cascade. Treatment of animals with either saline or empty liposomes did not alter significantly

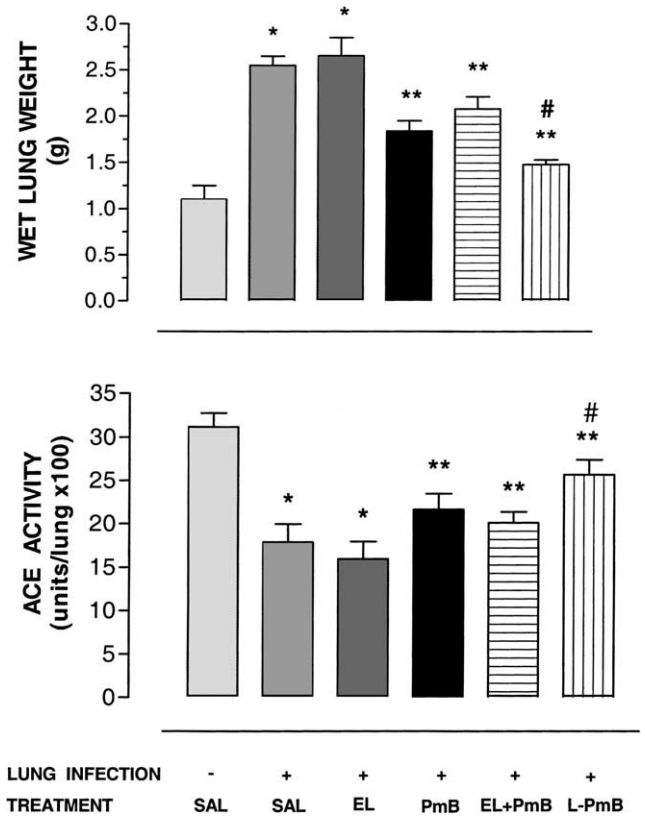

Fig. 1. Changes in wet lung weights (upper panel) and ACE activities (lower panel) in rats chronically infected with *P. aeruginosa* and treated intratracheally with free polymyxin B (PmB) or liposomal polymyxin B (L-PmB) or empty liposomes (EL). Treatment was initiated 3 days after the instillation of *P. aeruginosa* (10^7 CFU/animal) and was administered daily, for 3 consecutive days. Rats were killed 24 hr after the final antibiotic dosage. Values represent the means ± SEM from 5–6 animals per group from a representative experiment. Key: (∗) significantly different ($P < 0.05$) from the corresponding value obtained from non-infected animals treated with saline; (∗∗) significantly different ($P < 0.05$) from the corresponding value from infected animals treated with saline; and (#) significantly different ($P < 0.05$) from the corresponding value from infected animals treated with free polymyxin B.

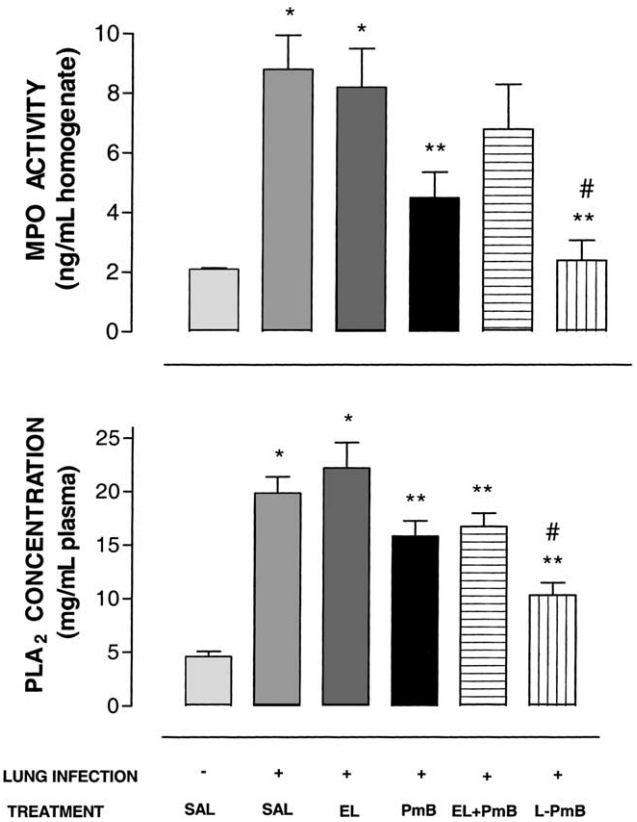

Fig. 2. Changes in pulmonary MPO concentration (upper panel) and PLA_2 concentration (lower panel) in rats chronically infected with *P. aeruginosa* and treated intratracheally with free polymyxin B (PmB), liposomal polymyxin B (L-PmB), or empty liposomes (EL). Treatment was initiated 3 days after the instillation of *P. aeruginosa* (10^7 CFU/animal) and was administered daily, for 3 consecutive days. The rats were killed 24 hr after the final antibiotic dosage. Values represent the means ± SEM from 5–6 animals per group from a representative experiment. Key: (∗) significantly different ($P < 0.05$) from the corresponding value obtained from non-infected animals treated with saline; (∗∗) significantly different ($P < 0.05$) from the corresponding value from infected animals treated with saline; and (#) significantly different ($P < 0.05$) from the corresponding value from infected animals treated with free polymyxin B.

the inflammatory responses to the pulmonary infection. In contrast, treatment of infected animals with free polymyxin B or liposomal polymyxin B reduced the P. *aeruginosa*-induced changes in MPO concentration significantly (48 and 73% reduction, respectively) and PLA_2 concentration (15 and 48% reduction, respectively).

4. Discussion

Polymyxin B is a cationic polypeptide antibiotic effective in the treatment of Gram-negative bacterial infections. Its clinical use, however, is limited due to its toxic effects, the most important being nephrotoxicity and neuromuscular blockade. Incorporation of antibiotics in liposomes is known to enhance their antibacterial activities while minimizing their toxic effects [7–9,18,25]. In the present study, we demonstrated that polymyxin B can be incorporated into liposomes for pulmonary delivery. DPPC was used to prepare liposomes for pulmonary delivery because it is the major lipid component of surfactant and is relatively non-toxic [26].

In this study, our results indicated that the encapsulation of polymyxin B in DPPC/Chol liposomes generally enhanced its *in vitro* antibacterial activity against several strains of Gram-negative bacteria; however, the precise mechanism(s) for this action cannot be delineated at the present time. The failure of a combination of free polymyxin B and empty liposomes to exert an antimicrobial effect better than that of free drug suggests that liposomal encapsulation is required for improved drug efficacy. It is possible that, as in the case of liposomal aminoglycosides, the enhanced antimicrobial activity exerted by liposomal antibiotics may be attributed to the fusional interaction between membrane phospholipids of liposomes and bacterial cells [27,28].

The *in vivo* bactericidal activity of polymyxin B was also improved significantly in infected lungs when the antibiotic was delivered as a liposomal formulation. Treatment of animals with liposomal polymyxin B or free polymyxin B resulted in significant reductions in the bacterial counts in lungs of animals infected with *P. aeruginosa*; the reduction in the bacterial count was more pronounced in the group of animals treated with the liposomal drug. An improved therapeutic index resulting from encapsulation of antimicrobial drugs within liposomes has been demonstrated against experimental infections caused by several microorganisms [7,18,29], and to a great extent, it has been attributed to the ability of liposomes to facilitate the transfer of antibiotics into bacteria [28].

The improved effectiveness of the liposomal antibiotic over the free drug may be due to the increased availability of polymyxin B at the site of infection, namely the lungs. The content of polymyxin B in the lungs of infected animals treated with the liposomal suspension was 42.8 ± 6.2 μg/paired lungs, while in those treated with the free drug it was 8.2 ± 0.4 μg/paired lungs. Also, the absence of measurable quantities of polymyxin B in kidneys and serum of animals treated with liposomal polymyxin B suggests that most of the liposomal polymyxin B did not escape into the general circulation. Moreover, since polymyxin B was measured by a bactericidal assay, the measurable antibiotic remaining in the lungs must still be pharmacologically active. Although the lipid component of the liposomal formulation did not exhibit any antibacterial activity, it perhaps facilitates uptake by phagocytic cells where the bacteria reside.

In addition to its superior antimicrobial activity, liposomal polymyxin B also appears to be capable of reducing the extent of lung injury in animals infected with *P. aeruginosa*. In this study, lung injury was evidenced by increased wet lung weights (indicative of edema) and decreased ACE activity (indicative of alveolar endothelial cell injury) [24] in infected animals. The increase in lung weight could be due to an increased leakage of the capillary-alveolar barrier induced by inflammatory cells in response to infection. Indeed, infection of lungs with *P. aeruginosa* resulted in significant increases in MPO and PLA_2 activities, suggestive of neutrophil infiltration and activation. The ability of liposomal polymyxin B to further reduce the number of viable bacteria would result in a lesser degree of inflammation.

Another explanation for the improved effectiveness of the liposomal polymyxin B in ameliorating lung injury may be attributed to the ability of the antibiotic to neutralize LPS. LPS, a component of Gram-negative bacteria, has been shown to induce neutrophil activation and adherence to microvascular endothelial cells, leading to neutrophil accumulation and endothelial cell injury, which results in leakage across the microvascular basement membrane [30]. It has been demonstrated that administration of polymyxin B prior to or concurrently with LPS administration alleviates the lung injury and edema due to its potent LPS-neutralizing properties [31,32]. In our study, the levels of polymyxin B remaining in the lungs of infected animals were significantly high, possibly allowing the antibiotic to sequester LPS released from dying bacteria.

Detectable levels of polymyxin B in the serum and kidneys of animals treated with polymyxin B are evidence to suggest that the antibiotic accumulates in the kidneys of animals. It is well known that the clinical use of polymyxin B is limited due to its nephrotoxic action. In the present study, the nephrotoxic action of polymyxin B was not assessed, but we failed to detect polymyxin B in the serum and kidneys of animals treated with the liposomal antibiotic. In light of these observations, it is conceivable that the administration of polymyxin B as a liposomal suspension would be less nephrotoxic, since the extent of nephrotoxicity depends on the accumulation of the drug in the kidneys.

The potential use of liposomes as a carrier system for drug delivery to the lungs has been reviewed by many investigators [16,17,19,33]. It has been demonstrated that liposomes, due to their slow and sustained release of entrapped drugs, may enhance the efficacy of drugs at the site of action. In addition, diffusion of antibiotic through bacterial external envelopes of a resistant strain of *P. aeruginosa* [28] has been promoted by its incorporation into liposomes. In the present study, the therapeutic effectiveness of liposomal polymyxin B, administered intratracheally to the lungs of rats infected with *P. aeruginosa*, was demonstrated. Thus, liposomal polymyxin B appears promising in the management of pseudomonal pulmonary infection.

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