Pharmacokinetics and Efficacies of Liposomal and Conventional Formulations of Tobramycin after Intratracheal Administration in Rats with Pulmonary *Burkholderia cepacia* Infection

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The objective of the present study was to determine the pharmacokinetics and efficacies of liposomal and conventional formulations of tobramycin against *Burkholderia cepacia* in a model of chronic lung infection. Male Sprague-Dawley rats were inoculated intratracheally with $10^6$ CFU of a very resistant strain of *B. cepacia* (strain BC 1368; MIC, 128 µg/ml) to establish lung infection. A 1,200-µg dose of tobamycin was administered intratracheally as a liposomal formulation and as a conventional formulation. Rats were anesthetized and exsanguinated by cardiac puncture at different times up to 24 h to assess pulmonary tobamycin concentrations and the number of residual CFU. Pharmacokinetic parameters were calculated by using a two-compartment model with NONMEM. The mean half-life of the β phase ($t_\text{1/2}_{\beta}$) and the pulmonary exposure (the area under the concentration-time curve [AUC]) of liposomal tobamycin were 19.7 h (coefficient of variation [CV], 24.2%) and 6,811 µg · h/lungs (CV, 19.7%), respectively. The pharmacokinetics of conventional tobramycin were statistically different, with a $t_\text{1/2}_{\beta}$ and AUC of 12.9 h (CV, 31.4%) and 821 µg · h/lungs (CV, 15.0%), respectively. Pearson chi-square analyses were performed on residual CFU data distributed in the following categories: $<10^3$, $10^3$ to $10^5$, and $>10^5$. Differences in CFU data between formulations showed a statistical trend ($P < 0.10$) when data from all time points were used, and statistically significant differences were found after 12 h ($P < 0.05$), with greater eradication achieved with the liposomal formulation. In conclusion, intratracheal administration of tobramycin in liposomes was associated with marked changes in the pharmacokinetics of the drug in the lung and an apparent trend for a prolonged efficacy against *B. cepacia*. These results support the hypothesis that inhalation of liposomal tobramycin may improve the management of chronic pulmonary infections caused by resistant bacteria in patients with cystic fibrosis.

*Burkholderia cepacia* is recognized as a pathogen of increasing importance, particularly in immunocompromised hosts (13) and cystic fibrosis patients (11, 15). Treatment of *B. cepacia* infections is difficult because of its high-level resistance to multiple antibiotics. Efficient resistance mechanisms such as a decreased outer membrane permeability (16, 18) and a very active antibiotic efflux pump (4) render the treatment of *B. cepacia* pulmonary infections a challenge.

Local administration of antibiotics has the advantage of delivering drug at the site of infection, reducing in certain cases unnecessary systemic exposure. Despite the use of potent antibiotics in aerosolized solutions or suspensions, total eradication of microorganisms is rarely achieved in cystic fibrosis patients (12, 15, 19, 21). Many researchers have demonstrated that the disposition of gentamicin, amikacin, or tobramycin markedly changes when these antibiotics are administered in liposomal forms (5, 6, 8, 9, 17). Moreover, encapsulation of drugs in liposomes has often resulted in improved overall therapeutic efficacy following administration by multiple routes (1, 5, 14, 20).

It was demonstrated that the use of a liposomal formulation of tobramycin was associated with a significant eradication of mucoid *Pseudomonas aeruginosa* and a better safety profile in a small group of animals whose lungs were infected (1). In vitro studies have also shown that liposomal tobramycin significantly reduces the growth of *B. cepacia*, *Escherichia coli*, *Stenotrophomonas maltophilia*, and *Staphylococcus aureus* (2). On the basis of the promising results of these in vivo and in vitro studies, we aimed to determine the in vivo pharmacokinetics and efficacies of liposomal and conventional formulations of tobramycin against *B. cepacia* in a model of chronic lung infection in rats.

**MATERIALS AND METHODS**

Liposomal and conventional formulations of tobramycin. The novel liposomal formulation of tobramycin (Theralipids Inc., Montreal, Quebec, Canada) was prepared with synthetic phospholipids (Avanti Polar Lipids Inc., Birmingham, Ala.) in a 10:1 molar ratio of a noncharged dipalmitoylphosphatidylcholine phospholipid and a negatively charged dimyristoylphosphatidylglycerol phospholipid. The formulation was manufactured in the following manner. Briefly, appropriate amounts of lipid mixture were dissolved in chloroform in a round-bottom flask and dried to a lipid film by rotoevaporation (Buchi Rotavapor-R-144) at 65°C under vacuum. The lipid film was then rehydrated with 0.01 N phosphate-buffered saline (PBS) and lyophilized (FTS-Kinetix, BioPharm Division, New Berlinville, Pa.) in vials at 4°C. The vials were kept at $-70°C$ until use. After rehydration with a concentrated solution of tobramycin (Tobi; PathoGenesis Canada Ltd.), the liposomes were filtered in an extruder (Lipex Biomembranes, Inc., Vancouver, British Columbia, Canada) with polycarbonate membranes to sterilize and standardize the sizes of the liposomes between 230 and 400 nm. Control liposomes were similarly prepared, but PBS was used instead of the antibiotic. A formulation of tobramycin developed for inhalation therapy in cystic fibrosis patients (Tobi; PathoGenesis Canada Ltd.) was used as the conventional formulation for this study.
Animal housing. A total of 78 adult male Sprague-Dawley rats (39 animals per formulation; weight, between 175 and 225 g; Charles River, Saint-Constant, Quebec, Canada) were housed in groups of three and allowed free access to food and water for 1 week before any experiment was undertaken. Environmental conditions were monitored during the acclimation period (1 week) and the conduct of the study. Rats were fed a standard certified commercial laboratory diet, with reverse osmosis UV-treated water available ad libitum after dosing and after a fasting period of 12 h. All experiments were conducted in accordance with guidelines from the Canadian Council on Animal Care and Use of Laboratory Animals.

Bacterial strain. A clinical isolate of B. cepacia (strain BC 1368) was used in this study. BC 1368 is a stable strain isolated from the sputum of a patient with cystic fibrosis (Toronto Hospital for Sick Children, Toronto, Ontario, Canada). This strain of B. cepacia (genovar III, randomly amplified polymorphic DNA analysis group 002) is the one most commonly found in cystic fibrosis patients in the United Kingdom and Canada. The MIC of tobramycin for BC 1368 was 128 μg/ml. For all experiments, bacteria were cultured for 18 h in proteose peptone (Difco Laboratories, Detroit, Mich.) broth before inoculation.

Experimental infection and antibiotic treatment. Rats were anesthetized with a mixture of 70 mg of ketamine hydrochloride per kg of body weight and 7 mg of xylazine per kg by intramuscular injection before infection. Chronic pulmonary infection was performed by a method described elsewhere (1). Briefly, anesthetized rats were placed in the supine position, and the upper jaw was attached to the operating table with a rubber band. A plunger was driven through the incisors to remove the incisors. Rats were inoculated with agar beads containing 10^6 CFU of B. cepacia in 100 μl at the bifurcation of the trachea with a 1-ml tuberculin syringe followed by a bolus of air to ensure complete delivery. Three to 5 days later, rats were anesthetized and the infection was verified in all animals by swabbing of the throat. The throat swab samples were plated on a B. cepacia (Pseudomonas cepacia) selective medium (C-390) for CFU determination. Six days later, the rats were anesthetized by the same procedure and a 100-μl solution containing 1,200 μg of the liposomal or conventional formulation of tobramycin was administered intratracheally with a 200-μl calibrated pipette, and air was immediately instilled after drug administration to ensure complete delivery of the drug.

Sample collection and bacterial growth. Following drug administration, the rats were anesthetized and exsanguinated by cardiac puncture at the following time points: 0.5, 1, 2, 3, 5, 7, 9, 11, 13, 15, 16, 18, and 24 h (three rats/time point). Control rats were killed at 0.5 and 24 h after intratracheal administration of liposomes loaded with PBS. Entire lungs were aseptically weighed and immediately homogenized in 2 ml of cold PBS for 30 s with a Polytron homogenizer. After a sample was homogenized, the homogenizer was rinsed with ethanol, flamed, and finally rinsed again with cold sterile PBS before the next sample was homogenized. A 100-μl volume of homogenized lung tissue samples was immediately used to prepare serial dilutions in cold PBS. These manipulations resulted in a 20-, 200-, or 2,000-fold dilution of lung tissue samples in cold PBS to prevent the killing of the bacteria in homogenized tissue. Diluted samples were plated on Protease Peptone No. 2 agar plates (Difco Laboratories), and the plates were incubated for 20 to 30 h at 37°C (5% CO₂). The numbers of CFU were counted at the dilution at which a maximum of 300 CFU was found. Pending the analytical assay of tobramycin, samples of homogenized tissues were stored at −70°C in a methanol solution to extract tobramycin from the phospholipids and to precipitate the lung tissue.

Analytical assay. Tobramycin concentrations were determined in lungs tissues by a high-pressure liquid chromatography (HPLC) method described elsewhere (1, 17). Briefly, the HPLC system consisted of a system controller and chromatographic pump (Waters Alliance 2690), a UV detector set at 350 nm (Waters 996 photodiode array), and an autoinjector (no. C2237; Chromatographic Specialties Inc.) controlled with the appropriate software (Waters Millenium 32 Chromatography Manager). The separation was carried out on a Symmetry C18 column (150 by 4.6 mm, 5 μm; Waters no. WAT045905). The mobile phase consisted of 0.1 N acetic acid (90:10) solution pumped at a flow rate of 1.3 ml/min. The suitability of the chromatographic system was verified by the following parameters: capacity factor (k') was lower than 2, and the efficiency was 20,000 theoretical plates (N). The tailing factor (T) was lower than 2, and the capacity factor (k') was higher than 2. Reproducibility was assessed after six injections of a 50-μg/ml standard solution, and the resulting coefficient of variation (CV) was less than 2%. Linearity was assessed after single injections of each standard solution (6, 2.5, 12.5, 25, 50, 100, 200, 400, and 800 μg/ml) and the resulting coefficient of correlation (r²) of the response was higher than 0.995. The limit of quantitation was 6.25 μg/ml (corresponding to approximately 10^3 CFU/g/lungs), with a CV of 8.1%. This analytical method for the liposomal formulation gives pulmonary tobramycin concentrations that represent the summation of the concentration achieved with the encapsulated form and the concentration achieved with the free form.

Pharmacokinetic analysis. The pharmacokinetic parameters for tobramycin were calculated with NONMEM software (version 5) (3). Different compartmental models were investigated for the quality of fit, and the most appropriate one was selected on the basis of the law of parsimony and by minimizing the objective function. The most appropriate model describing the amounts of the liposomal and conventional formulations of tobramycin in the lungs was a two-compartment model with first-order absorption and elimination (ADVAN4). The bioavailability (Fₐ) of tobramycin in the lungs was identifiable since actual amounts were fitted. These were calculated by multiplying the concentration by the volume of each lung. On the other hand, volumes of distribution were not fitted because of this. The model was simply parameterized in terms of absorption (kₐ, transfer (kₐ, and kₑ), and elimination (kₑ) rate constants. The half-life at the α phase (t₁/₂α), the half-life at the β phase (t₁/₂β), and the pulmonary exposure (the area under the concentration-time curve [AUC]) were derived by using standard noncompartmental and compartmental equations (10) from the Bayesian estimates obtained in the POSTHOC analysis. The NONMEM first-order method was initially used during the model-building process, but the final population estimates were obtained by using the first-order conditional estimation method. Observations were fitted by using a weighting procedure of 1/S², where the variance (S²) was calculated by using a proportional and additive error model.

Pharmacodynamic analysis. The numbers of CFU were counted and presented as log₁₀ units. The residual pulmonary CFU of B. cepacia following the intratracheal administration of the liposomal and conventional formulations of tobramycin was plotted over 24 h. The number of residual pulmonary CFU of B. cepacia was distributed in the following categories: <3 log₁₀ residual CFU, 3 to 5 log₁₀ residual CFU, and ≥5 log₁₀ residual CFU.

Statistical analyses. Two-sample t tests were used to assess differences in population pharmacokinetic parameters between liposomal and conventional formulations of tobramycin. Differences in CFU data between the two formulations were assessed by Pearson chi-square tests on the number of observations per category (<10, 10 to 10⁴, and >10⁴ residual CFU). Corrections were applied by Fisher’s exact test when the number of observations was less than 5 in any given category. Trends for statistically significant differences were set at a P value <0.10, while statistically significant differences were set at a P value >0.05. Statistical analyses were performed by using SYSTAT (version 8.0).

RESULTS

The pharmacokinetics of the liposomal and conventional formulations of tobramycin in lungs were fitted by using a two-compartment model with additive and proportional errors. Individual observed and fitted amounts (in micrograms) of tobramycin in the lungs following intratracheal administration of the two formulations are presented in Fig. 1. The amounts of drug in the lungs of individual rats were simulated by using Bayesian estimates of F₂ₕ, kₐ, k₂ₕₐ, and k₂ₙ from the POSTHOC analysis.

Final population parameter estimates and the corresponding CVs for the liposomal and conventional formulations of tobramycin are presented in Table 1. A total of 36 rats were used in the conventional tobramycin formulation group since 2 animals died during surgical procedures (3 h postdosing) and the infection level could not be confirmed in 1 animal after throat swabbing. Pulmonary uptake of liposomal tobramycin was fast, with a mean kₐ of 2.30 h⁻¹ (CV, 30.8%). Pulmonary uptake of conventional tobramycin was even quicker, with a kₐ of 4.68 h⁻¹ (CV, 38.7%). The differences in the kₐ values between the liposomal and the conventional formulations were statistically different (P < 0.05). No significant differences in lung F₂ₕ values were observed. Encapsulation of tobramycin in liposomes prolonged its t₁/₂β significantly (P < 0.05), from 12.9 h (CV, 31.4%) to 19.7 h (CV, 24.2%). Consequently, the AUC for the liposomal formulation was significantly higher than that for the conventional formulation (P < 0.05). The residual...
variabilities of the population analyses of the liposomal and the conventional tobramycin formulations were 18.1 and 18.4%, respectively.

The intratracheal administration of liposomal PBS to control rats resulted in 5.17, 5.23, and 5.19 log_{10} CFU of *B. cepacia*, respectively, at 0.5 h and 5.28, 5.55, and 5.22 log_{10} CFU, respectively, at 24 h. The residual pulmonary CFU data obtained following intratracheal administration of the liposomal and conventional formulations of tobramycin are depicted in Fig. 2. Administration of the liposomal and conventional formulations of tobramycin resulted in rapid decreases in the numbers of pulmonary CFU of *B. cepacia*, with most of the observations falling in the range of 10³ to 10⁵ CFU. Compared to the conventional tobramycin formulation, the bactericidal activity of liposomal tobramycin seemed to persist for a longer period. Since the liposomal formulation displayed sustained amounts of tobramycin in the lungs and appeared to have a prolonged efficacy, the residual CFU data and the frequency of observations were also distributed before and after 12 h. Histograms of the residual CFU distribution are presented in Fig. 3. In order to assess differences in bactericidal activities, nonparametric statistical methods were used since the two formulations displayed highly variable efficacies. Three distinct responses were compared: when residual pulmonary CFU counts at any given time were less than 10³, between 10³ and 10⁵, and greater than 10⁵. The distribution of the data, the frequency of observations, and statistical comparisons (Pearson chi-square test) of the residual numbers of pulmonary CFU are presented in Table 2. Differences in the distributions of the residual CFU data between the two formulations showed a statistically significant trend (*P* < 0.10) when data from all time points (0 to 24 h) were used, with approximately 18 and 8% of the observations falling below 10³ CFU for the liposomal and conventional formulations, respectively. No significant differences were observed before 12 h (*P* = 0.783), while statistically significant

![FIG. 1. Observed and fitted pulmonary tobramycin amounts in male Sprague-Dawley rats following intratracheal administration of 1,200 µg of tobramycin in a liposomal (○) or conventional (●) formulation.](http://aac.asm.org/)

**TABLE 1.** Final population parameter estimates and their corresponding CVs following intratracheal administration of 1,200 µg of the liposomal or conventional formulation of tobramycin to male Sprague-Dawley rats.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>ka (h⁻¹)</th>
<th>FL (%)</th>
<th>k_{23} (h⁻¹)</th>
<th>k_{32} (h⁻¹)</th>
<th>k_{20} (h⁻¹)</th>
<th>t_{1/2a} (h)</th>
<th>t_{1/2b} (h)</th>
<th>AUC (µg·h/lungs)</th>
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<tr>
<td>Liposomal</td>
<td>2.30⁰ (30.8)ᵇ</td>
<td>0.85 (12.2)</td>
<td>1.19⁰ (38.5)</td>
<td>0.407 (40.2)</td>
<td>0.155⁰ (30.2)</td>
<td>0.40⁰ (11.0)</td>
<td>19.7⁰ (24.2)</td>
<td>6,811⁰ (19.7)</td>
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<td>(n = 39)</td>
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<tr>
<td>Conventional</td>
<td>4.68 (38.7)</td>
<td>0.77 (17.2)</td>
<td>5.58 (43.9)</td>
<td>0.330 (37.8)</td>
<td>1.10 (19.8)</td>
<td>0.12 (17.1)</td>
<td>12.9 (31.4)</td>
<td>821 (15.0)</td>
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<td>(n = 36)</td>
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ᵃ *P* < 0.05 compared with the conventional formulation.

ᵇ The values in parentheses are percent CV.
differences in the distributions of the CFU data were observed between the two formulations after 12 h ($P < 0.05$).

**DISCUSSION**

Aerosol delivery of antibiotics to the lower respiratory tract is becoming an increasingly important and rational approach for the treatment of various pulmonary infections since the respiratory tract is the most common route of entry and the primary site of infection for a number of airborne pathogens. The $F_L$ values of the liposomal and conventional formulations of tobramycin in the lung observed in this study were approximately 80%, supporting the efficiency of this route of administration for the treatment of lung infections.

The pharmacokinetics of the two formulations of tobramycin in the lung were well described by a two-compartment model with first-order absorption and elimination. The quality of fit was very good and resulted in a residual variability of approximately 18% for the two formulations. This number represents the variability that is not explained by the model and includes the intraindividual variability, the experimental “noise,” and the error arising from the pharmacokinetic modeling itself. The encapsulation of tobramycin markedly changed its pulmonary pharmacokinetics, with a significant increase in $t_{1/2}$ from the lungs. As a consequence, the level of exposure to tobramycin in the liposomal formulation was significantly higher than the level of exposure to tobramycin in the conventional formulation. The results of pharmacokinetic studies for the liposomal formulation are in agreement with those of in vivo studies, which show that the rate of elimination of liposome-encapsulated drugs is slower than the rate of elimination of conventional formulations (5, 6, 14, 20).

The experimental model of chronic lung infection in male Sprague-Dawley rats was appropriate since the numbers of CFU of *B. cepacia* were maintained above $10^5$ until 24 h after intratracheal administration of liposomes loaded with PBS. Differences in the distributions of residual CFU data between the two formulations showed a statistical trend when data from all time points were considered. Due to the marked differences in pharmacokinetics between the two formulations, the bactericidal activities of the two formulations were compared before and after 12 h. No significant differences were observed before 12 h, while statistically significant differences in the distribution of the CFU data were observed after 12 h. The latter difference was due to the greater decrease in CFU achieved with the liposomal formulation, with 26.7% of the observations falling below $10^3$ CFU. This apparent delay in efficacy may be representative of the amount of tobramycin in the lung that was sustained over $\approx 100$ mg over the whole kinetic study. These important amounts of tobramycin in lung tissues might have resulted in an important carryover of the antibiotic to the subculture plates. Prior to the study, serial dilutions of lung

**FIG. 2.** Residual CFU of *B. cepacia* in lungs of male Sprague-Dawley rats following intratracheal administration of 1,200 $\mu$g of tobramycin in a liposomal (○) or conventional (●) formulation.
tissue samples spiked with tobramycin (up to 1,200 μg/both lungs) revealed that antibiotic carryover had no significant effects on the growth of \textit{P. aeruginosa}, a bacterium for which the tobramycin MIC is lower than that for \textit{B. cepacia}. On the basis of these observations and those from other investigators (7), we considered that serial dilutions of lung samples minimized the antibiotic carryover to the subculture plates and resulted in a negligible effect on the growth of \textit{B. cepacia}.

The pharmacodynamic observations of the present study are not consistent with those from in vitro experiments described in the literature, which demonstrated that the liposomal formulation of tobramycin has a markedly higher level of bactericidal activity against \textit{B. cepacia} than the conventional formulation (2). In our study, a trend for a prolonged therapeutic efficacy against \textit{B. cepacia} was observed for the liposomal formulation of tobramycin over 24 h, although the amounts of tobramycin in the lung were markedly higher than those achieved with the conventional formulation of tobramycin. One possible explanation for the discrepancy between the efficacy and pharmacokinetics of the liposomal formulation of tobramycin is that liposomes are likely to stay in lipophilic environments of the lungs, whereas the \textit{B. cepacia} bacteria are more likely to reside in the interstitial fluids of the lungs. Therefore, the amount of tobramycin in the homogenized tissue is representative of the total amount of tobramycin recovered and not necessarily the amount at the site of infection where bacteria are located. Nevertheless, the results of the present investigation are encouraging and support the need for further projects, since the trend for the greater bactericidal activity of the liposomal formulation of tobramycin was observed with a highly resistant strain of \textit{B. cepacia} (tobramycin MIC, 128 μg/ml).

In conclusion, the encapsulation of tobramycin in a liposomal formulation markedly changed its pulmonary pharmacokinetic profile, resulting in a slower distribution and a slower elimination. The net effect was a significantly higher level of pulmonary exposure of the liposomal formulation of tobramycin and an apparent trend for a prolonged efficacy against \textit{B. cepacia}. Further experiments with the liposomal formulation will need to be performed to determine the dose and frequency of administration that will result in optimal activity against \textit{B. cepacia} over a longer period. These results are promising and support the working hypothesis that the local administration of a liposomal tobramycin formulation may improve the management of chronic pulmonary infections caused by resistant bacteria in patients with cystic fibrosis.

### ACKNOWLEDGMENT

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### REFERENCES
