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Regional lung deposition of nebulized liposome-encapsulated ciprofloxacin

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Abstract

Liposome-encapsulated ciprofloxacin (22.2 mg ciprofloxacin/ml) was nebulized in 25 nebulizers (five nebulizers from each of five nebulizer types). The aerosol was inhaled by a breath simulator (square wave pattern, 0.75 l tidal volume, 0.3 l/s inhalation flow rate) and inhaled droplet sizes were characterized using in-line phase Doppler anemometry. Filter collection combined with centrifugation, UV spectrophotometry and measurement of original entrapment efficiency using C^{14}-radiolabelled ciprofloxacin was used to determine % encapsulation in the inhaled aerosol. Cascade impaction combined with chemical assay showed that encapsulated and free ciprofloxacin were homogeneously distributed among the inhaled droplet sizes. Combination of the above measurements with a mathematical lung deposition model allowed prediction of the amounts of encapsulated ciprofloxacin depositing in the tracheo-bronchial, alveolar and extrathoracic regions of the respiratory tract. The nebulizer types (Pari LC STAR, Pari LC +, Medix Sonix 2000, Hudson T-Updraft II, DeVilbiss Permaneb) differed by up to a factor of 30 in the amount of encapsulated ciprofloxacin depositing in the different regions of the lung (e.g. lung deposition of entrapped ciprofloxacin varied from 0.7% to 18.1% of total ciprofloxacin dose placed in the nebulizer). Much of this dramatic difference was due to the differing amounts of liposomal disruption, which varied among the different nebulizer types from no measurable disruption to nearly complete disruption.

Keywords: Liposomes; Encapsulation; Ciprofloxacin

1. Introduction

The concept of encapsulating therapeutic agents inside liposomal vesicles to enhance their effectiveness in the treatment of respiratory disease by aerosol inhalation has been studied by various

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authors (cf. Taylor and Farr, 1993a,b). Although nebulization is often the easiest method of delivery from a formulation point of view, several factors make such delivery an uncertain proposition. Two such factors include disruption of liposomes by nebulization (Niven and Schreier, 1990), as well as uncertainty in the amount of encapsulated drug that different nebulizer models will deliver to the different regions of the lung with a given formulation. Both of these factors are formulation specific, making it difficult to predict the behaviour of a new formulation from previous results. Our interest here is in addressing these two factors for a liposomal formulation of ciprofloxacin.

Ciprofloxacin is a potent and broad-spectrum antibacterial compound. Recent studies have shown that liposome-encapsulated ciprofloxacin is significantly more effective than free (unencapsulated) ciprofloxacin in the prevention and treatment of intracellular bacterial infections of the respiratory tract in mice (Di Ninno et al., 1993; Wong et al., 1995; Conley et al., 1997). Clinical trials are needed to determine whether such encouraging results are observed in humans. However, for such trials to proceed, an effective means of delivering liposome-encapsulated ciprofloxacin is needed. To this end, in the present work we estimate the amount of encapsulated ciprofloxacin that can be expected to deposit in the tracheobronchial, alveolar and extrathoracic regions of a Weibel model of the human lung when a liposomal formulation of ciprofloxacin is delivered by aerosol inhalation using five nebulizer models known by the authors to perform well in previous studies with nebulized salbutamol sulphate.

A second purpose of the present work is to describe a procedure for predicting regional lung deposition of nebulized liposomal formulations by combining bench-top measurements with a mathematical lung deposition model. To the authors' knowledge, only one other published archival publication has examined this issue (Waldrep et al., 1994), but their lack of measurement of liposome disruption, use of a one-way coupled hygrosopic model, and inattention to the undersizing of droplets that occurs when nebulized aerosols are diluted with ambient air prior to cascade impaction reduce the utility of their procedures. We address these deficiencies in the present work, and provide an in vitro procedure for predicting regional lung deposition of nebulized liposome-encapsulated compounds.

2. Materials and methods

Liposome-encapsulated ciprofloxacin was prepared using a modification of the remote loading procedure of Oh et al. (1995). Unilamellar vesicles were obtained after extrusion through a 0.2 mm pore size filter. Ciprofloxacin (Bayer Canada, Etobicoke, Ontario, Canada) was loaded using a 600-mM ammonium sulphate gradient. Lipid components consisted of a 55:45 molar ratio of phospahtidylcholine/cholesterol. A concentration of 22.2 mg liposome-encapsulated-ciprofloxacin/ml in normal saline was used for the nebulization in this study. Entrapment efficiency of ciprofloxacin in liposomes was determined by co-entrapping ^{14}C-labelled ciprofloxacin (Bayer) with unlabelled ciprofloxacin. Following ultracentrifugation, percentage entrapment of ciprofloxacin was determined by measuring radioactivity using a liquid scintillation counter (model 1409, Wallac, Turku, Finland) in the liposome pellet and comparing with the total radioactivity of labelled ciprofloxacin added. Using this approach, an entrapment efficiency of $90 \pm 2\%$ was observed.

Liposomal ciprofloxacin was nebulized in five nebulizer types, including four jet nebulizers: the Pari LC STAR and Pari LC + (Pari, Starnberg, Germany), T-Updraft II (model 1732, Hudson, Temecula, CA), Permaneb (model 700, Devilbiss/Sunrise Medical, Somerset, PA); and one ultrasonic nebulizer: the Sonix 2000 (model 86111, Medix, Catthorpe, UK). The two Pari nebulizers and the Permaneb are 'vented' nebulizers (also called 'breath-enhanced' or 'active venturi' nebulizers), meaning that all inhaled air travels through the droplet production region of the nebulizer. The T-Udraft II is a conventional T-mouthpiece nebulizer in which only the air from the compressor travels through the droplet production region, while the additional ambient air needed to make up the total inhalation flow rate

is supplied through a T-junction in the mouthpiece. All jet nebulizers were driven by a single Pulmo-Aide compressor (model 5610C, Devilbiss/Sunrise Medical). Nebulization was stopped when a pause of more than 15 s occurred without production of aerosol droplets. During nebulization, the compressor and each nebulizer were enclosed in air of relative humidity $50 \pm 5\%$ RH (measured with a hygrometer; Fisher Scientific, ON) and temperature of 24 ± 1°C using the procedure described in Prokop et al. (1995a). Flow rates to the nebulizers were measured using a dry gas meter (DTM-115, Singer, American Motor Division).

A volume fill of 2.5 ml of liposomal ciprofloxacin taken directly from refrigeration (3.5 ± 0.5°C) was nebulized in five units of each nebulizer type. A square wave tidal breathing pattern consisting of equal inhalation and exhalation times, no inspiratory pause, a tidal volume of 0.75 l, and inhalation flow rate of 0.3 l/s was supplied by an in-house breathing machine, consisting of a piston connected to a computer controlled stepper motor. Aerosol inhaled from each nebulizer by the breathing machine was collected on an absolute filter (# 303, Marquest Medical Products, Englewood CO). The presence of the connection volume (95 ml) between the nebulizer and the filter was corrected for by scaling the amount collected on the filter by the ratio of tidal volume/(tidal volume − connection volume). Sizing of the droplets inhaled by the breathing machine was done during tidal breathing using phase Doppler anemometry (Dantec, Mahwah, NJ) in an in-line, optically clear sizing region, as described by Prokop et al. (1995b). Particle number density was obtained from mass conservation with the measured particle size distribution (Stapleton et al., 1994).

Total ciprofloxacin inhaled on the filter was determined by washing with methanol and assaying with UV spectrophotometry (model 8452A, Hewlett-Packard, Mississauga, ON). The fraction of the total inhaled ciprofloxacin remaining encapsulated was determined in a manner that follows previous authors (see e.g. Taylor et al., 1990), by performing a second set of experiments in which the filters were instead washed with normal saline. A portion of the sample was then centrifuged at $12000 \times g$ for 1 h and the supernatant assayed for free ciprofloxacin, while a second portion of the same sample was dissolved in methanol and assayed for total ciprofloxacin. Samples of the original unnebulized preparation were submitted to the same procedures. Validation of these methods was performed.

Examination of the possibility that different aerosol droplet sizes contained different concentrations of encapsulated vs. free ciprofloxacin was done in another set of experiments in which methylene blue (36.26 mg/ml, certified reagent, dye content 82%, Acros Organics, Edmonton, AB) was added to the liposomal ciprofloxacin formulation as a tracer for the water with 6.43 mg/ml NaCl added to make the solution isotonic. The nebulized aerosol was collected in an Anderson cascade impactor (Anderson Mark II, Graseby Anderson, Smyrna, GA). Each impactor plate was then washed with normal saline. A portion of each extract was then dissolved in methanol and simultaneously assayed by UV spectrophotometry for total ciprofloxacin and methylene blue content, while a second portion of the same sample was centrifuged at $12000 \times g$ for 1 h and the supernatant assayed simultaneously for ciprofloxacin and methylene blue.

To predict the regional deposition of the inhaled droplets in the lung, a mathematical deposition model similar to that described in Stapleton and Finlay (1997), Finlay et al. (1996), Finlay and Stapleton (1995) and Stapleton et al. (1994) was used. This model uses the information obtained from the experimental procedure described above to specify the properties of the aerosol as it is inhaled. This aerosol is then tracked in a Lagrangian manner through a Weibel A lung geometry scaled to a functional residual capacity (FRC) of 3000 cm^3. The Weibel A lung is a symmetrically branching idealized lung geometry (Weibel, 1963), with generations 0–16 representing the tracheobronchial region and generations 17–23 representing the alveolar region. Deposition models based on other idealized lung geometries give results that are on average near that obtained with the Weibel A geometry (Yu and Diu, 1982; Martonen, 1983). Within each generation of the

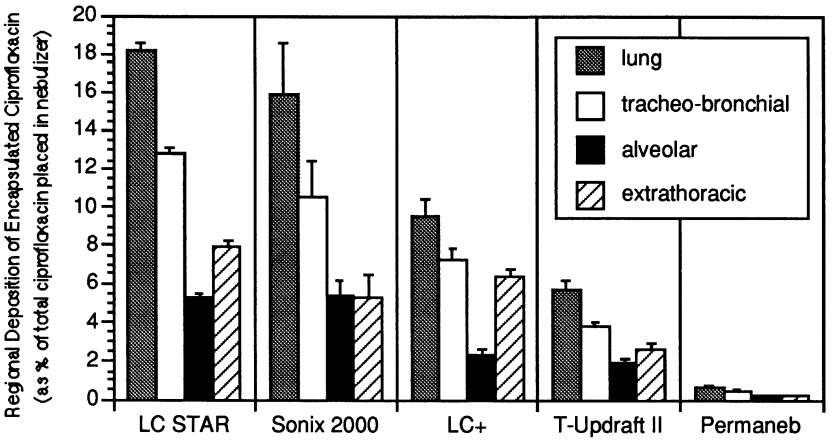

Fig. 1. The amount of encapsulated ciprofloxacin predicted to deposit in the different regions of a Weibel lung (as a % of the total ciprofloxacin initially placed in the nebulizer) is shown for the five nebulizer types tested. Error bars indicate standard error ($n = 5$). Lung deposition is simply the sum of alveolar and tracheo-bronchial deposition.

Weibel A lung, sedimentation probabilities are obtained from theoretical equations for deposition in laminar flow through inclined tubes (Wang, 1975), diffusion probabilities are obtained from the equations of Gormley and Kennedy (1949), while inertial impaction approximates the experimental data of Chan and Lippmann (1980). Extrathoracic deposition was determined using the equations of Rudolf et al. (1990). Mouth breathing through the nebulizer mouthpiece was assumed. The reader is referred to Stapleton et al. (1994) for further details.

In tracking particles through the respiratory tract we have assumed hygroscopic effects are negligible. This is in contrast to previous predictions of regional lung deposition of nebulized liposomal formulations (Waldrep et al., 1994) where hygroscopic effects have been treated using a one-way coupled hygroscopic treatment (in which droplets evaporate or condense in response to their environment, but such evaporation or condensation does not affect the humidity and temperature of the air carrying the droplet). However, for the nebulizers used here, Finlay et al. (1997) have shown that hygroscopic size changes of droplets in the respiratory tract are negligible because of the large number of droplets inhaled per unit volume with these nebulizers, so that two-way coupled hygroscopic effects (in which droplet evaporation or condensation affects the humidity and temperature of the air carrying the droplets) essentially eliminate hygroscopic size changes of the aerosol droplets in their transit through the lung and make a one-way coupled hygrosopic treatment inaccurate.

Statistical tests were performed using ANOVA and Tukey HSD means comparisons with significance assumed to occur at a level of $p = 0.01$.

3. Results

The amounts of encapsulated ciprofloxacin predicted to deposit in the different regions of the respiratory tract are shown in Fig. 1. Differences in these amounts due to nebulizer type are statistically significant. Differences between the LC STAR and Sonix 2000 nebulizers do not reach statistical significance, but both these nebulizers deposit significantly more encapsulated ciprofloxacin in each region of the lung shown in Fig. 1 than the other nebulizer types.

Fig. 2 shows the amount of ciprofloxacin inhaled by the breathing machine in total and encapsulated in vesicles. Differences in total and encapsulated inhaled-ciprofloxacin due to nebulizer type are statistically significant.

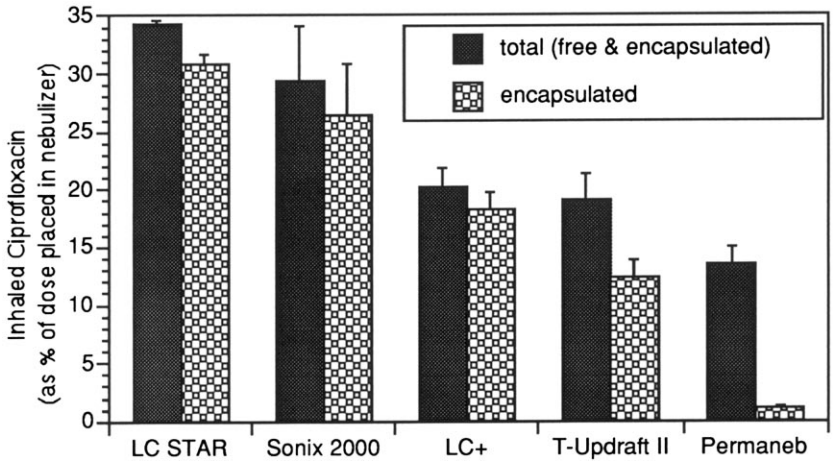

Fig. 2. The total amount of ciprofloxacin (both free and encapsulated) inhaled by the breathing machine as well as the amount inhaled encapsulated in vesicles is shown for the five nebulizer types tested. The amounts shown are given as % of the total amount of ciprofloxacin initially placed in the nebulizer. Error bars are standard error ($n = 5$).

Measurements of the free and encapsulated ciprofloxacin contained in the different particle size ranges collected on each Anderson cascade impactor plate show there was no significant difference ($n = 3$) between the cumulative size distribution of ciprofloxacin (either free or encapsulated) and that of the methylene blue. Regression analysis of the cumulative % mass distribution of encapsulated ciprofloxacin versus methylene blue yielded the same conclusion. On average, the % mass collected on each impactor plate differed by less than 1% between total ciprofloxacin, free ciprofloxacin, encapsulated ciprofloxacin or methylene blue, with the largest difference between any of these individual comparisons being 6% or less. These results indicate the encapsulated ciprofloxacin was homogeneously distributed among the droplets.

Nebulization times, mass median diameter, geometric standard deviation and flow rates of the nebulizers tested are given for reference in Table 1. Differences due to nebulizer type in these quantities are statistically significant.

4. Discussion

Fig. 1 shows that the five nebulizer types differ dramatically in the amount of encapsulated ciprofloxacin predicted to deposit in the different regions of the respiratory tract. For example, the LC STAR gives lung, tracheo-bronchial, alveolar and extrathoracic dosages of encapsulated ciprofloxacin that are 28, 30, 25 and 34 times those of the Permaneb, respectively. Fig. 2 shows that much of this difference between nebulizers is due to the disruption of liposomes by some of the nebulizers. In particular, Fig. 2 shows that the LC STAR, LC+ and Sonix 2000 do not differ from the original, unnebulized formulation in the level of encapsulation of ciprofloxacin in the inhaled aerosol, whereas only 8% of the inhaled ciprofloxacin remains encapsulated with the Permaneb, and 65% with the T-Updraft II.

The amount of either free or total ciprofloxacin depositing in the different regions of the lung can be inferred from the data on encapsulated ciprofloxacin shown in Fig. 1, since our impactor measurements indicate that the free ciprofloxacin was homogeneously distributed among the inhaled droplets in the same manner as the encapsulated ciprofloxacin. Thus, multiplication of the values shown in Fig. 1 by the ratio, x, of free to encapsulated ciprofloxacin in the inhaled aerosol (inferred from Fig. 2) gives the regional dosages of free ciprofloxacin delivered by each nebulizer. This ratio is the same at $x = 0.11$ for the LC STAR, LC+ and Sonix 2000 nebulizers, but

Table 1
Nebulization time (defined as the time until a pause of 15 s occurred in droplet production), mass median diameter, and geometric standard deviation (measured by phase Doppler anemometry) of the aerosols produced by the five nebulizer types tested are shown with standard error ($n = 5$)

Nebulizer type	Nebulization time (min)	MMD (μm)	GSD	Flow rate (l/min)
LC STAR	7.8 ± 0.3	5.5 ± 0.1	1.65 ± 0.01	4.4 ± 0.2
LC+	10.4 ± 0.5	6.8 ± 0.2	1.78 ± 0.01	6.2 ± 0.3
Permaneb	15.6 ± 0.5	4.7 ± 0.3	1.86 ± 0.05	4.2 ± 0.2
T-Updraft II	19.6 ± 0.1	4.6 ± 0.4	2.3 ± 0.1	5.9 ± 0.3
Sonix 2000	12.3 ± 0.8	4.6 ± 0.2	1.71 ± 0.03	n/a

$x = 11.1$ for the Permaneb, and $x = 0.55$ for the T-Updraft II. Total depositing ciprofloxacin in each lung region is then obtained by multiplying the values in Fig. 1 by $(1 + x)$. Regional dosages of total ciprofloxacin differ by at most a factor of 3.1 between the different nebulizers, which is much less than the above mentioned differences in deposition of encapsulated ciprofloxacin because consideration of total ciprofloxacin does not include the large differences in disruption between the nebulizer types.

It is interesting to note that the ultrasonic nebulizers (Sonix 2000) did not cause disruption of the liposomes, despite the elevated temperature (37°C) that the liposomal formulation reached during nebulization with this nebulizer.

The differences in the amount of liposomal disruption we observed between different nebulizer types shows that it is important to measure the amount of encapsulated drug in the inhaled aerosol. Measurements of only the total amount of drug delivered (e.g. Waldrep et al., 1994) may be less meaningful if differences in liposome disruption occurs between nebulizers, as observed here. Additionally, the differences we observe in disruption between the different jet nebulizers show that caution is advised in making general statements regarding the ability of different nebulizers to nebulize a liposomal formulation, even if the nebulizers are all of the same general design, e.g. jet nebulizers.

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