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Pulmonary delivery of amikacin liposomes and acute liposome toxicity in the sheep

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Summary

Although liposome-encapsulated antibiotics designed for intrapulmonary delivery by instillation or aerosolization have been proposed, little is known about the pharmacokinetic profile and toxicity of liposomal drug formulations when delivered to the lung. A technique for large-scale preparation of sterile amikacin liposomes including preparation of a lyophilized amikacin/phospholipid coprecipitate and a highly efficient hollow-fiber dialysis method for rapid removal of unencapsulated drug is described. Doses of 5 and 15 mg/kg amikacin solution or 15 and 45 mg/kg amikacin-containing liposomes consisting of soy phosphatidylcholine/phosphatidylglycerol (SPC/PG 7:3, molar ratio) or SPC/PG with cholesterol (SPC/PG/CH 4:3:3) were administered intratracheally into intubated, awake sheep. For the 15 mg/kg amikacin solution, the terminal half-life time ($t_{1/2}$) was 117 min with maximum plasma levels (c_{pmax}) of 8.3 μg/ml after 2 h and a bioavailability of 38%. The $t_{1/2}$ for both doses of amikacin-SPC/PG liposomes was greater than 3 h. Bioavailability varied from 35 to 58%, with a c_{pmax} of 5.5 μg/ml (15 mg/kg) and 23.6 μg/ml (45 mg/kg) after 1.5 h. The $t_{1/2}$ of amikacin-SPC/PG/CH liposomes was greater than 10 h with a c_{pmax} of 3.3 μg/ml after 3 h and a bioavailability of 46%. The dosage form was found to be the overall rate-limiting factor for amikacin pharmacokinetics. For assessment of pulmonary function and blood gases, awake sheep inhaled plain liposomes consisting of 15 and 150 mg/ml SPC or hydrogenated SPC (HSPC) for 30 min via a Collison nebulizer. Dynamic compliance (C_{dyn}), lung resistance (R_L), p_aO_2 and p_aCO_2 were analyzed for 6 h post-inhalation (acute effects 0–2 h; delayed effects 2–6 h). All parameters remained within physiologically normal ranges over the entire observation period. It was concluded that liposomes delivered by the pulmonary route act as local sustained release reservoir, and are safe and nonirritating to the lung.

Introduction

Incorporation of antibiotics within liposomes has been shown to enhance bactericidal activity when compared to free drug. This is particularly true for facultative intracellular organisms which

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infect monocytes and macrophages such as *Leishmania* (Alving et al., 1978; New et al., 1978, 1981), *Listeria* (Bakker-Woudenberg et al., 1985), *Brucella* (Dees et al., 1985; Fountain et al., 1985) *Salmonella* (Fierer et al., 1990), and *Mycobacterium avium-intracellulare* (Bermudez et al., 1987, 1990; Düzgünes et al., 1988; Cynamon et al., 1989; Klemens, 1990) (for reviews see Swenson et al. (1988) and Couvreur et al. (1991)).

Aerosolization of such antibiotic-carrying liposomes would provide a means of targeting these drugs to infected alveolar macrophages, for instance, in the case of pulmonary *M. avium-intracellulare* infections. This common but difficult to treat pulmonary infection can be especially devastating to immunosuppressed individuals, particularly AIDS patients (Iseman et al., 1985).

Pulmonary delivery of liposomes via inhalation appears feasible (for reviews see Mihalko et al. (1988) and Kellaway and Farr (1990)). Advantages of pulmonary application, i.e., improved retention at the target site and, concomitantly, reduction of side effects, have been demonstrated for the anticancer agent cytosine arabinoside (Juliano and McCullough, 1980), oxygen scavengers including superoxide dismutase (Padmanabhan et al., 1985) and glutathione (Jurima-Romet and Shek, 1991), antiviral (Gilbert et al., 1988; Wyde et al., 1988), antiallergic (Taylor et al., 1989) and bronchodilating agents (McCalden et al., 1989).

We have recently studied the physicochemical characteristics of liposome aerosols with respect to lipid composition (Niven and Schreier, 1990),

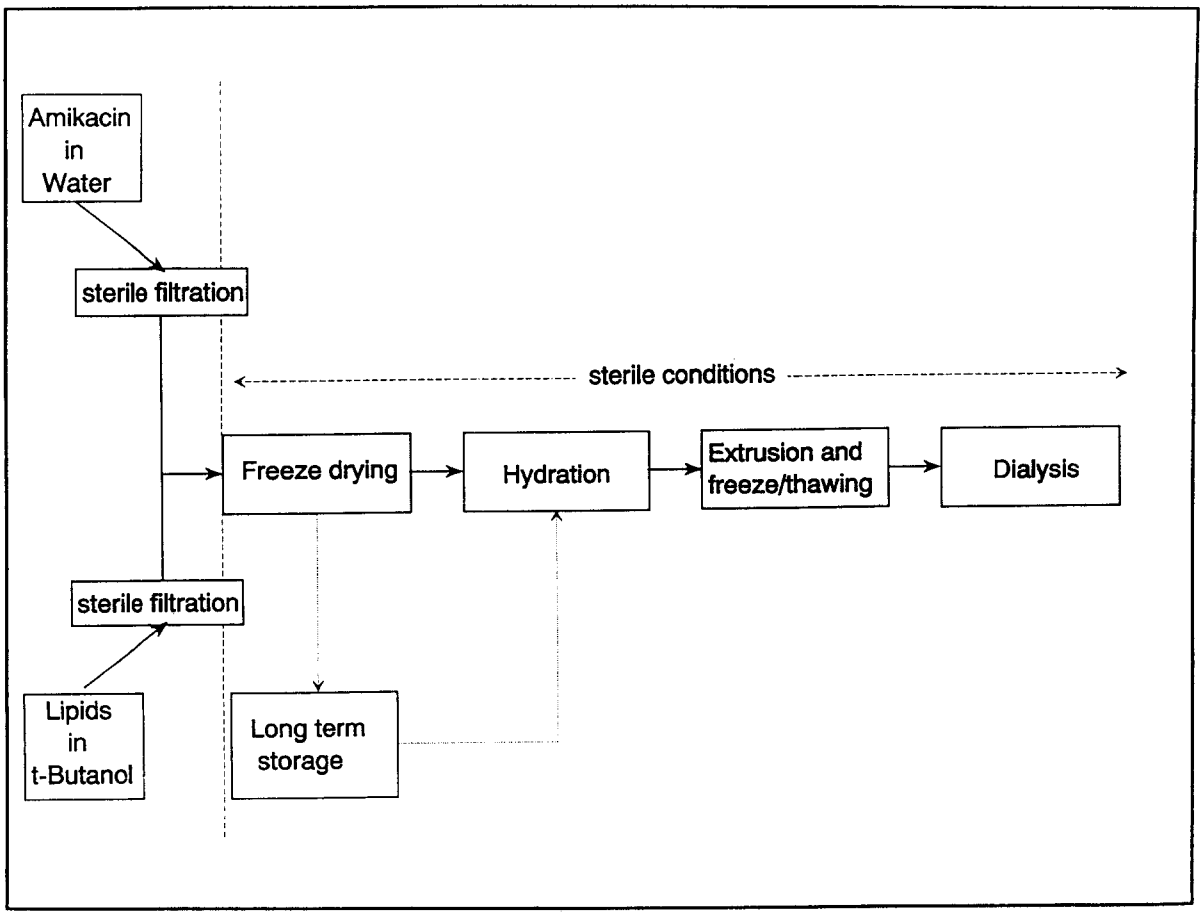

Fig. 1. Flow chart of the aseptic preparation method of liposomes. For details see Materials and Methods.

size (Niven et al., 1991), and nebulizer operating conditions (Niven et al., 1992). We have also characterized amikacin liposomes and their stability during aerosolization and when incubated in lung lavage fluid (Wichert et al., 1992). From a toxicologic point of view, we have assessed the toxicity of liposomes in alveolar macrophage culture (Gonzalez-Rothi et al., 1991), pulmonary toxicity in vivo following chronic administration in mice (Myers et al., 1990), and the acute effects of aerosolized liposomes on lung function in human volunteers (Thomas et al., 1991).

As a continuation of our studies with amikacin liposome aerosols, we report here the pharmacokinetics and bioavailability of liposome-encapsulated amikacin following pulmonary instillation, as well as a study of potential acute toxic effects of aerosolized blank liposomes on lung function (dynamic compliance C_{dyn}, resistance R_L) and gas exchange (p_aO_2 and p_aCO_2) in the uninfected, awake sheep. Furthermore, we describe a technique for large-scale preparation of sterile amikacin liposomes which includes preparation of a lyophilized amikacin/phospholipid coprecipitate and a highly efficient hollow-fiber dialysis method for rapid removal of unencapsulated drug.

Materials and Methods

Chemicals

Amikacin sulfate was a gift from the Bristol-Myers Squibb Co., Pharmaceutical Research Institute, Princeton, NJ (referred to hereafter as 'amikacin'). Soy phosphatidylcholine (Phospholipon 90; SPC) and hydrogenated soy phosphatidylcholine (Phospholipon 90H; HSPC) were purchased from the American Lecithin Co., Atlanta, GA, and phosphatidylglycerol (PG) from Avanti Polar Lipids, Alabaster, AL. Cholesterol (CH) was from Sigma Chemical Co., St. Louis, MO.

Preparation of liposomes

Amikacin liposomes were prepared by a procedure that was suitable for sterile production of volumes of 100–200 ml liposomes as necessary for the instillation experiments. The two lipid compositions used were SPC/PG 7:3 (molar ratio) and SPC/PG/CH 4:3:3 (molar ratio). The method consists of the following steps which are also shown in the flow diagram in Fig. 1:

(1) Freeze-drying 20 ml samples ($\approx$ 5 cm vial filling height) of an amikacin/phospholipid mixture in water/*tert*-butanol (3:5 v/v) (filtered through 0.2 μm depth filter): samples are frozen for 4 h at −40°C, dried for 24 h at −25°C, followed by 10 h of secondary drying at +10°C. Freeze-drying is performed with an Edwards Supermodulyo freeze-dryer consisting of a separate condenser and a three-shelf chamber unit whose temperature is controlled between −40 and +60°C (±1°C) by silicone M5 fluid circulation. Sample stoppering is under vacuum via a mechanical screw thread for in-chamber vial closure.
(2) Reconstitution of the drug/lipid coprecipitate with sterile water for injection to give a liposome dispersion with a concentration of 200 mg/ml amikacin and 100 mg/ml total lipid: the water is injected via the rubber closure under laminar flow. Samples are agitated gently for a few seconds. Redispersion of the lyophilized cake is practically instantaneous.
(3) Extrusion of the crude dispersion through a 0.4 μm polycarbonate membrane (Nuclepore, Pleasanton, CA) (Olson et al., 1979) using the Extruder (Lipex Biomembranes, Vancouver, B.C.): five consecutive freeze-thawing cycles (−80/+40°C) (Mayer et al., 1985) are followed by five additional extrusions through 0.2 μm polycarbonate membranes.
(4) Dialysis of unincorporated amikacin using a custom-made hollow-fiber dialyzer similar to the system described by Schwendener (1986) for 1 h against 10 l of PBS.

Quantitative determination of amikacin and calculation of the corresponding encapsulation efficiency was performed as described elsewhere (Wichert et al., 1991, 1992). Total phospholipid concentration was determined according to the method of Stewart (1980). Due to the high pressure ($\geq$ 100 lb/inch^2) that can be applied with

the extruder, lipid recovery was generally above 90%. Liposome size and size distribution were determined by laser light scattering using a Nicomp Model 370 laser particle sizer (Particle Sizing Systems, Santa Barbara, CA).

All solvents were sterilized and all preparative steps were performed in a laminar flow hood (BioGuard, NuAire) using sterilized glassware, extrusion and dialysis equipment. All samples were tested for sterility by plating on sheep blood agar and incubation at 37°C overnight prior to experimentation.

Blank liposomes for pulmonary toxicity studies were prepared by conventional lipid film dispersion under aseptic conditions in a laminar flow hood. Lipids were dissolved in $CHCl_3$ and the solvent was removed under vacuum on a Büchi rotoevaporator. The lipid film was hydrated with PBS by mechanical agitation on a wrist-action shaker for 2 h at 65°C. The four liposome preparations were: (1) SPC 15 mg/ml (low dose) (LDSPC); (2) SPC 150 mg/ml (high dose) (HDSPC); (3) HSPC 15 mg/ml (LDHSPC); (4) HSPC 150 mg/ml (HDHSPC). Typically, 50 ml samples were prepared containing 2.5 g of the respective phospholipids (50 mg/ml). Samples were tested for sterility prior to experimentation as above.

Surgical preparation of sheep

Healthy, adult mixed-breed sheep were obtained from commercial suppliers, and housed for the duration of the experiments in NIH- and USDA-approved facilities. Surgery was performed under aseptic conditions using general anesthesia with inhaled methoxyflurane (Metofane, Pittman Moore) and 100% O_2. Mechanical ventilation of the animals was achieved using an endotracheal tube and a volume ventilator. Through a small thoracotomy, a balloon-tipped Silastic catheter was inserted into the right pleural space for later measurement of pulmonary mechanics as described in detail elsewhere (Sauder et al., 1986). A 5–8 cm length of the carotid artery was externalized and an enclosing sheath of skin fashioned for later sampling of arterial blood gases. Animals were allowed to recover from surgery for 3–5 days.

Liposome aerosol generation

Liposome aerosols were generated by a Collison nebulizer as described by Niven and Schreier (1990), with a gas flow of 4 l/min of 100% O_2. Aerosols were collected in a large reservoir bag from which the intubated sheep breathed. The reservoir was connected to a non-rebreather valve attached to the end of the endotracheal tube. Sheep were breathing spontaneously while inhaling one of the four liposome aerosols or saline as control for 30 min through the endotracheal tube.

Pharmacokinetic and bioavailability studies

For assessment of absolute bioavailability, amikacin was administered slowly over 1 min i.v. via a jugular vein. Intratracheal administration was performed by topically anesthetizing the nostrils of awake sheep with 2% lidocaine jelly prior to nasotracheal intubation. Drug was instilled into the distal trachea via a fine catheter inserted through the nasotracheal tube. Groups of three to four animals received a dose of 15 mg/kg amikacin solution by i.v. injection or instillation, or the same dose as liposome formulation. Each sheep served as its own control.

Blood samples (1 ml) were withdrawn from the external carotid artery using a heparinized fine (27 gauge) butterfly needle and a 1 ml syringe. Samples were drawn at −5, 5, 10, 15, 20, 30, 60, 120, 180, 240 min, and 24 h post-administration. Samples were centrifuged immediately, and serum was collected and stored at −20°C. Samples were analyzed for amikacin using a fluorescence polarization immunoassay (TDX, Abbott). The sensitivity of this assay is 0.6 μg/ml.

Serum creatinine was also monitored in order to detect potential renal toxicity. At least 7 days were allowed for recovery between experiments (wash-out period).

Pharmacokinetic analysis was performed using compartmental and non-compartmental analysis as follows:

Non-compartmental pharmacokinetic analysis

The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule. The terminal part of the AUC beyond the last measured data point (c_{px}) was estimated as c_{px}/β for the i.v. data, and c_{px}/k_e for the pul-

monary data, respectively. The total body clearance (CL) was calculated as dose divided by AUC. Mean residence time (MRT) was calculated as the area under the first moment curve (AUMC) divided by area under the curve (AUC). AUMC was determined using a plot of plasma concentration times time ($C_p \times t$) vs time and calculation of its area under the curve calculated by the trapezoidal rule. The terminal part of the AUMC beyond the last measured data point (c_{px}) at time t_x was estimated as $c_{px} \times (t_x/\beta) + (c_{px}/\beta^2)$ for the i.v. data, and $c_{px} \times (t_x/k_e) + (c_{px}/k_e^2)$ for the pulmonary data, respectively. The volume of distribution at steady state (Vd_{SS}) was determined as the product of total body clearance and mean residence time (CL × MRT). The bioavailability (f) of amikacin after pulmonary administration was calculated as $(AUC_{it}/AUC_{iv}) \times 100$. The mean absorption time (MAT) of amikacin after pulmonary administration was calculated as $MRT_{it} - MRT_{iv}$. The maximum plasma concentration (C_{max}) is the experimental value with the highest plasma concentration. The time of the maximum plasma concentration (t_{max}) is the experimental data point when C_{max} was measured.

Compartmental pharmacokinetic analysis

Compartmental pharmacokinetic analysis was performed using the nonlinear regression program RSTRIP (MicroMath, Salt Lake City, UT). Best results were obtained with a two-compartment body model for the i.v. data, whereas for the pulmonary data a one-compartment body model was satisfactory. Compartmental analysis was used for the estimation of the terminal half-life. The i.v. data were fitted to the biexponential equation

$$C_p = ae^{-\alpha t} + be^{-\beta t}$$

From the results the half-life ($t_{1/2}$) was calculated as $\ln 2/\beta$. Pulmonary data were fitted to the biexponential equation

$$C_p = b(e^{-ket} - e^{-kat})$$

From the results, the half-life ($t_{1/2}$) was calculated as $\ln 2/k_e$.

Acute toxicity studies: measurement of lung mechanics and blood gases

The technique used to measure pulmonary mechanics in awake sheep has been described in detail by Sauder et al. (1986). Briefly, sheep were nasotracheally intubated with a cuffed endotracheal tube, after the nostrils were numbed with 2% lidocaine jelly. A flexible fiberoptic bronchoscope was used to guide the tube through the nostril and vocal apparatus into the trachea. Animals were restrained in a standing position with a loose sling.

Transpleural pressure was measured with a ± 20 cmH_2O Validyne MP-45 differential pressure transducer (Validyne Engineering Corp., Northridge CA) and Validyne CD19A demodulator, with one side connected to the pleural pressure catheter, and the other to an airway-opening catheter inserted 2–3 cm beyond the cuffed end of the endotracheal tube (cuff inflated). Respiratory flow signals were generated by a no. 2 Fleisch heated pneumotachograph attached to the end of the endotracheal tube. The pressure differential across the pneumotachograph was measured and electronically integrated with a Validyne FV112 integrator to give a volume signal. To ensure a reproducible volume history, the sheep's lungs were inflated to a pressure of 30 cmH_2O 1 min before measurements were taken. Flow-volume and pressure-volume loops were displayed on an oscilloscope screen, and photographed with an oscilloscope camera. Measurements taken from the photographs were used to calculated values of dynamic compliance (C_{Dyn}) and resistance of the lung (R_L).

Arterial blood pH, pO_2 and pCO_2 were measured using a Corning 617 blood gas machine (Corning Medical Instruments, New York, NY).

Control measurements of baseline lung function and blood gas values were made on at least 3 consecutive days prior to administration of liposomes. On the day of the experiment, baseline C_{Dyn} and R_L values were again measured at least three times prior to beginning aerosol exposure. A single measurement of blood gas values was also made. After the end of the 30 min aerosol exposure, C_{Dyn} and R_L were measured at 15 and 30 min intervals, and at 0.5 h intervals for the

next 5.5 h. Blood gases were measured at 15, 30, and 60 min intervals for the first hour after aerosolization and at 1 hour intervals for the next 5 h.

Pulmonary function data (C_{Dyn}, R_L, p_aO_2 and p_aCO_2) were plotted vs time from 0 to 2 h, and from 2 to 6 h, and the respective areas under the curve (AUC) were calculated. These AUCs are reported as percent response relative to baseline which corresponds to 100% response.

Results

Liposome preparation and characterization

The liposome preparation process employed is suitable for rapid production of 100 ml or larger volumes of liposomes from amikacin/phospholipid coprecipitates generated by lyophilization. The combination of extrusion and freeze-thawing cycles generated homogeneous preparations with a high encapsulation efficiency of 64 ± 17 mg/ml ($n = 5$), corresponding to a molar encapsulation efficiency of 0.86 mol amikacin per mol phospholipid (based on the molecular weight of SPC), and a reproducible size of 222 ± 40 nm ($n = 5$). The hollow-fiber dialysis technique facilitated efficient removal of unencapsulated material within 1 h and can also be employed to concentrate (or dilute) the preparation if desired (by applying or releasing back pressure). A comparison of the efficiency of removal with the hollow-fiber dialysis vs conventional bag dialysis is shown in Fig. 2.

Pharmacokinetic and biopharmaceutic analysis

The resulting plasma concentrations from a 15 mg/kg amikacin dose administered i.v., i.t. as solution or in the two liposome formulations are shown in Fig. 3. An overall compilation of the pharmacokinetic data is given in Table 1.

After i.v. administration amikacin was rapidly eliminated with a $t_{1/2}$ of approx. 70 min (Fig. 3). The total body clearance averaged 2.6 ml/min per kg which is close to the glomerular filtration rate of 2.4 ml/min per kg in sheep (Hill et al., 1988). The volume of distribution was 0.25 l/kg

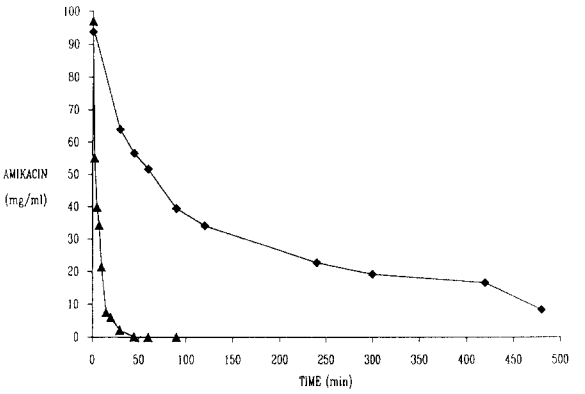

Fig. 2. Comparison of removal of unencapsulated amikacin from liposomes via conventional bag dialysis (♦) and hollow-fiber counter-flow dialysis (▲) For experimental details see Materials and Methods.

which is identical to the value reported in humans (Pechere and Dugal, 1979).

After intratracheal administration of a solution, amikacin was absorbed into the systemic circulation. The average $t_{1/2}$ was 117 min, and maximum levels of 8.3 μg/ml were reached after 2 h. The average bioavailability was 38% of the dose. These results indicate a slow absorption from the lungs with the absorption phase being

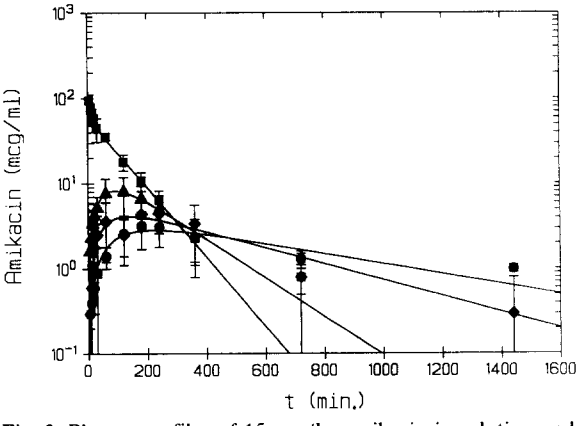

Fig. 3. Plasma profiles of 15 mg/kg amikacin in solution and as liposomal dosage form following intravenous (i.v.) injection and intratracheal (i.t.) instillation in sheep. I.V. solution (■); I.T. solution; (▲); I.T. liposomes (SPC/PG 7:3) (♦); I.T. liposomes (SPC/PG/CH 4:3:3) (●). Error bars indicate mean ± S.D. ($n = 4$).

TABLE 1

Summary of pharmacokinetic parameters

Route/dosage form	i.v./solution	i.t./solution	i.t./liposomes	i.t./cholesterol-containing liposomes
AUC (μg ml^{-1} min)	7015 ± 1752	2762 ± 514	2480 ± 1315	3209 ± 359
MRT (min)	97 ± 13	233 ± 18	422 ± 120	1032 ± 578
$t_{1/2}$ (min)	73 ± 10	117 ± 21	208 ± 4	648 ± 417
C_{max} (μg ml^{-1})	131.4 ± 25.1	8.3 ± 3.7	5.5 ± 0.2	3.3 ± 1.4
t_{max} (min)	0	120 ± 49	150 ± 127	195 ± 30
C_L (ml min^{-1} kg^{-1})	2.23 ± 0.48	–	–	–
Vd (l kg^{-1})	0.22 ± 0.06	–	–	–
f (%)	100	38.2 ± 3.1	35.3 ± 18.7	45.7 ± 5.1
MAT (min)	0	137 ± 33	325 ± 120	935 ± 578

Summary of pharmacokinetic parameters following intravenous (i.v.) and intratracheal (i.t.) administration of 15 mg/kg amikacin in solution or as liposomal dosage form in sheep. The liposomal formulation consisted of SPC/PG 7:3 (molar ratio) and SPC/PG/CH 4:3:3 (final column). For more experimental details see Material and Methods.

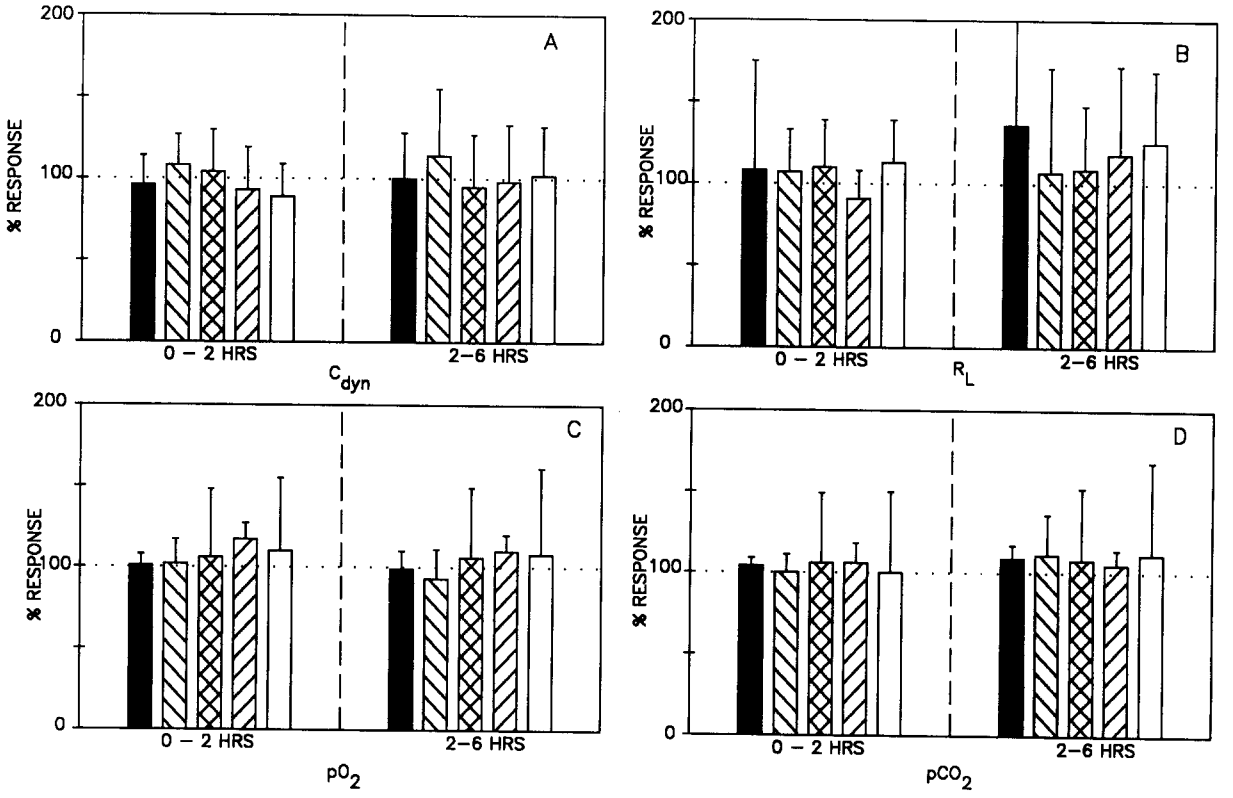

Fig. 4. Lung function (C_{Dyn}; R_L) and blood gas (p_aO_2; p_aCO_2) analysis after 30 min liposome and saline (control) aerosol administration in the awake sheep. Liposomes consisting of soy phosphatidylcholine (SPC) or hydrogenated SPC (HSPC) were administered as 'low dose' (LD = 15 mg total lipid/ml saline) or 'high dose' (HD = 150 mg total lipid/ml saline). The area under the response vs time curve (AUC) from 0 to 2 h and from 2 to 6 h is plotted relative to the baseline response (100%; dotted line). The 0–2 h response was considered the acute response, and the 2–6 h value the late response. Saline (control) (■); HD-SPC (▧); HD-HSPC (■); LD-SPC (▨); LD-HSPC (□) Error bars indicate mean ± S.D. (n = 4 for LDSPC, HDSPC, LDHSPC; n = 5 for HDHSPC; n = 8 for PBS).

the overall rate-limiting step of the pharmacokinetics ('flip-flop').

After intratracheal administration of liposomal amikacin, the terminal $t_{1/2}$ was more than 3 h for the 'phospholipid only' (SPC/PG) formulation, and over 10 h for the cholesterol-containing (SPC/PG/CH) formulation, indicating that, in contrast to the effects seen with amikacin solution, the dosage form is now the overall rate-limiting factor for the pharmacokinetics. Maximum achieved concentrations were 5.5 μg/ml after 2.5 h for SPC/PG, and 3.3 μg/ml after 3 h for SPC/PG/CH liposomes. Bioavailability varied between 35 and 46% of the administered dose.

Pulmonary function and blood gases following liposome aerosol inhalation

The effects of a 30 min liposome inhalation on pulmonary function and blood gases are shown in Fig. 4A–D. Due to the extensive preparation time involved in carrying out each aerosol trial, the sample sizes for each aerosol trial ($n = 4$ for LDSPC, HDSPC, and LDHSPC, $n = 5$ for HDHSPC, $n = 8$ for PBS) were too small to enable a rigorous statistical analysis of the data. However, previous studies with sheep (Stecenko et al., 1989) have shown that physiologically significant changes in dynamic compliance (C_{Dyn}) and lung resistance (R_L) are indicated by a decrease of C_{Dyn} to below 65%, and an increase of R_L by more than 100% from baseline values. Any change found in the first 2 h after aerosolization was arbitrarily defined as an early phase bronchoconstrictive response. Any significant change in pulmonary function between 2 and 6 h after aerosolization was considered a late phase response.

C_{Dyn} showed on average -11 to $+8$% deviation from baseline (100%) for the 0–2 h phase, and a -5 to $+14$% deviation from baseline for the late phase (2–6 h). Clearly, none of the changes in C_{Dyn} approached the reduction to 65% baseline which would be considered physiologically significant.

Similarly, R_L values varied from -9 to $+13$% around baseline during the 0–2 h measurement, and from $+9$ to $+36$% during the 2–6 h observation phase. Although this indicates somewhat more variability than was seen for C_{Dyn}, none of the responses approached a physiologically meaningful increase of 100%. Also, we have found in previous studies on sheep that baseline resistance increases slightly in the afternoon, even without aerosol challenge (unpublished data).

For p_aO_2, the variability of the 0–2 h measurements was $+1$ to $+17$% above baseline, and -7 to $+10$% for the 2–6 h measurements. None of the observed changes in dissolved oxygen tension was physiologically (or for that matter 'clinically') significant.

For p_aCO_2, values varied from 0 to $+6$% relative to baseline for the 0–2 h period, and from $+5$ to $+11$% for the 2–6 h period. None of these responses was physiologically significant.

Discussion

Pharmacokinetic and biopharmaceutic analysis

The pharmacokinetic and biopharmaceutic assessment of intratracheally instilled amikacin as solution or encapsulated within liposomes clearly demonstrated retention of a large fraction of the drug in the lungs, with about 40% of the administered dose absorbed systemically. Control of pulmonary release of the drug by the liposome formulation becomes most evident by a comparison of the mean residence times (MRT) which are a measure for how long an average drug molecule stays in the body. After intravenous administration the MRT was about 100 min. After intratracheal administration in solution or in SPC/PG liposome dispersion the MRT increased to approx. 4 h, equivalent to a mean absorption time of approx. 2 h. After administration of the cholesterol-containing liposomes the MRT was prolonged to 17 h, hence the mean absorption time was dramatically prolonged in comparison to non-cholesterol-containing liposomes. The data presented here correspond well with several published studies assessing the kinetics of pulmonary delivery of compounds via liposomes including the fluorescent marker 6-carboxyfluorescein (Woolfrey et al., 1988), the antiallergic agent

sodium cromoglycate (Taylor et al., 1989), and the bronchodilator metaproterenol sulfate (McCalden et al., 1989). In all reported cases, liposomes proved their potential to serve as a local pulmonary reservoir for therapeutic agents which, in the case of metaproterenol, could clearly be correlated with a reduction of the main side effect, i.e., tachycardia (Mihalko et al., 1988; McCalden et al., 1989).

As stated in the Introduction, the main goal of our work is to deliver antibiotic-containing liposomes intracellularly to *M. avium-intracellulare*-infected alveolar macrophages. For this purpose, the liposome should ideally retain its content quantitatively until uptake by the (infected) alveolar macrophage is accomplished. The plasma concentration measurements performed here are downstream relative to the targeted site of action and, therefore, provide an accurate measure for the systemic 'spillover' of treatment, while the data do not provide direct information as to the fraction of encapsulated drug available in the lung for uptake by alveolar macrophages. However, the pharmacokinetic data generated here correlate favorably with in vitro release data from liposomes in bronchoalveolar lavage fluid (Wichert et al., 1992). In vitro data indicated that SPC/PG liposomes released their content (the fluorescent marker 5,6-carboxyfluorescein) relatively rapidly over 24 h when exposed to lung lavage fluid, whereas cholesterol-containing liposomes retained their content essentially quantitatively over the same observation period. Hence, as a synopsis of these two studies, one may conclude that at least of the cholesterol-containing liposomes a large fraction is indeed retained in the lung intact and would therefore be available for uptake by alveolar macrophages. We have recently demonstrated the latter in vivo in mice, again using the fluorescent marker 5,6-carboxyfluorescein (Myers et al., 1992).

While preparations such as those used here are intended for aerosol use in a clinical setting, in the present study intratracheal instillation, as opposed to aerosolization, was selected for pharmacokinetic evaluation because a known exact dose needs to be administered to calculate relative bioavailabilities; with aerosols, no exact dose can be administered, thus aerosol deposition in animals would only allow approximation of the dose delivered. The question as to what fraction of an aerosolized dose is retained in and absorbed from the lung, respectively, remains to be answered in a follow-up study.

Pulmonary function and blood gas analysis

None of the four parameters of pulmonary function investigated, i.e., C_{Dyn}, R_L, p_aO_2 and p_aCO_2, were affected by a 30 min inhalation of two concentrations (15 and 150 mg/ml) of liposome aerosols consisting of either natural soy lecithin (SPC) or its semi-synthetic fully hydrogenated derivative (HSPC). The measurements used to assess alterations in lung function primarily evaluated airflow obstruction and impairment of gas exchange. Dynamic compliance (which decreases acutely with small airways obstruction) and arterial oxygenation (which also will decrease if pulmonary blood flow does not match alveolar ventilation) are particularly sensitive indicators of lung dysfunction. Lung function was followed for 6 h as bronchial challenge with putative inhalants can be associated with acute early phase airflow obstruction due to bronchoconstriction or a delayed late phase of airflow obstruction, occurring a few hours after challenge, which is due to airway inflammation secondary to mediator release in the airways. No evidence for any lung dysfunction was found due to liposome administration, even at the high dose of 150 mg/ml phospholipid.

This lack of any untoward effects of aerosolized liposomes on lung function is in accordance with our own prior findings in mice following a 4 week chronic liposome aerosol treatment (Myers et al., 1990, 1992) and in human volunteers following a 1 h inhalation of 15 and 150 mg/ml doses of SPC liposome aerosols (Thomas et al., 1991), and with studies by others in mice (Wyde et al., 1988) and human volunteers (Gilbert et al., 1988). These data again are in accordance with a large experimental and clinical body of evidence that pulmonary application of phospholipids for the treatment of respiratory distress syndrome in neonates is safe (for review see Jobe and Ikegami (1988); e.g., Table 5).

In summary, we conclude that the data presented here confirm that liposomes delivered by the pulmonary route act as a local sustained release reservoir, and we provide further evidence that liposome aerosols are safe and non-irritating to the lung.

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