Inhibition of Tracheal Vascular Extravasation by Liposome-Encapsulated Albuterol in Rats

Weiming Zhang,[1] Luke Guo,[2] Jay A. Nadel,[3] and Demetrios Papahadjopoulos[4,5]

Received September 29, 1997; accepted December 18, 1997

Purpose. To develop a liposome-based system for systemic delivery of anti-inflammatory drugs to airways and other inflamed tissues.

Methods. Postcapillary venular gap junctions open during airway inflammation and allow fluid accumulation and permit molecules (e.g. complement, kininogen) to enter tissues, initiating inflammatory cascades. Beta-adrenergic agonists prevent inflammatory plasma extravasation, but because of their deleterious side effects, they are not used intravenously. When sterically stabilized "stealth" liposomes are injected iv, they remain in the circulation for long periods. Inflammatory mediators [e.g., substance P(SP)] open postcapillary venular gaps and allow liposomes and their contents to be deposited selectively in the inflamed tissue.

Results. We hypothesized that liposomes encapsulating a beta-adrenergic agonist, such as albuterol, would deposit selectively in inflamed airway tissue, where the drug would slowly leak out of the liposomes, resulting in closure of the gaps, thus preventing subsequent inflammatory extravasation. To test this hypothesis, we delivered albuterol-loaded liposomes iv in rats. Then we injected SP to open the venular gaps and allow accumulation of the drug-loaded liposomes in airway tissue. We examined whether this treatment resulted in inhibition of subsequent plasma extravasation induced by SP. The results indicate that liposome-encapsulated albuterol inhibits subsequent extravasation, presumably by leaking out of liposomes in airway tissue. This inhibition occurs for prolonged periods of time and with limited side effects compared to the effect of free albuterol.

Conclusions. We conclude that liposomes loaded with appropriate drugs, by migrating to inflamed tissue and subsequently inhibiting inflammatory cascades, may be of therapeutic value in inflammatory diseases.

KEY WORDS: beta-adrenergic agonist; endothelial gap junctions; antiinflammatory drug; liposome drug delivery.

INTRODUCTION

It has been established that sterically stabilized "stealth" liposomes circulate in blood for a long period of time with minimal localization in normal tissues except liver and spleen (1). However, when vascular permeability is increased by inflammatory disease or by delivering mediators that increase vascular permeability [e.g., substance P (SP)], stealth liposomes can cross the endothelial barrier and become localized in the inflamed tissue (2).

In experimental animals, increased vascular permeability leads to extravasation of plasma proteins, which can be inhibited by the administration of beta-adrenergic agonists (3–5). In the present study, we encapsulated the beta-adrenergic agonist albuterol into stealth liposomes and injected it intravenously. Because albuterol leaks out of liposomes relatively slowly, few side effects should occur due to free drug released into the circulation. The vascular permeability in the trachea was then increased by injecting SP iv, which allows the drug-loaded liposomes to "home" selectively into the tracheal tissue. This is possible because the tracheal endothelium contains SP receptors which, when stimulated, open endothelial gaps in tracheal vessels, allowing the extravasation of the albuterol-loaded liposomes.

Our basic premise was that albuterol would diffuse at a reasonable rate out of the liposomes in the tracheal tissue, resulting in relatively high concentrations of free albuterol in the inflamed tissue. We hypothesized that the released albuterol would close the endothelial gaps and prevent subsequent extravasation when a second iv injection of SP is made. The results of these studies showed that albuterol-loaded liposomes can inhibit subsequent tracheal vascular extravasation for prolonged periods of time and with minimal side effects compared to those of free albuterol.

MATERIALS AND METHODS

Materials

Hydrogenated soy phosphatidylcholine (HSPC) was purchased from Lipoid K.G., Lugwigshafen, Germany. Polyethylene glycol (molecular weight, 1900) derivative of distearoylphosphatidylethanolamine, sodium salt (mPEG-1900-DSPE) was synthesized as described (6) and purchased from Sygena, Inc., Liestal, Switzerland. Cholesterol was purchased from Croda, Inc., New York, NY; Albuterol (Salbutamol, Hemisulfate salt) from Sigma Chemical Company, St. Louis, MO; Substance P (SP), purity 98% (HPLC), from Bachem Bioscience Inc., Philadelphia, PA; Evans blue from Polysciences, Inc., Warrington, PA; Pentobarbital (nembutal sodium) from Abbott laboratories, North Chicago, IL. Other solvents and chemicals were of analytical grade.

Animals

Pathogen-free male rats of the F344 strain, 200-220 g body wt., were obtained from Simonsen Laboratories (Gilroy, CA). Experimental procedures followed in this study were approved by the Committee on Animal Research of the University of California San Francisco.

Preparation of Liposomes

Sterically stabilized liposomes composed of HSPC/cholesterol/mPEG-DSPE (weight ratio, 3:1:1) were prepared by thin

[1] Cardiovascular Research Institute, University of California at San Francisco, San Francisco, California 94143-0130.
[2] SEQUUS Pharmaceuticals, Inc., Menlo Park, California 94025.
[3] Department of Medicine, University of California at San Francisco, San Francisco, California 94143-0130.
[4] Department of Cellular and Molecular Pharmacology and Cancer Research Institute, University of California at San Francisco, San Francisco, California 94143-0130.
[5] To whom correspondence should be addressed at California Pacific Medical Research Institute, Liposome Research Laboratory, 2200 Webster St., 2nd Floor, San Francisco, California 94115.

0724-8741/98/0300-0455$15.00/0

film hydration of mixed lipids with a solution of 250 mM ammonium sulfate (pH ~5.5) at 60°C with rotation. The multilamellar vesicles (100 μmol total lipid/ml) thus formed were extruded through nucleopore polycarbonate filters (7), eight times each through 0.4, 0.1, and 0.05 μm pore size in turn. Extrusion was performed with a stainless steel extrusion cell from Lipex Biomembranes (Vancouver, Canada) under nitrogen pressure of 50 – 650 Psi. The extrusion system was maintained at 60°C. The liposome suspension was dialyzed against 10% sucrose, 5 mM sodium chloride for 3 days at 2 – 8°C, changing buffer once per day. The mean particle size of the liposomes was determined by dynamic light scattering using a sub-micron particle analyzer (Coulter model N4MD, Coulter Corporation, Miami, FL). The particle size (diameter) of liposomes was 100 – 130 nm.

Liposome-encapsulated albuterol contained the same lipid components and weight ratio as described above, and preparation of liposomes was also the same. Albuterol was dissolved in 10% sucrose solution at a concentration of 10 mg/ml. An equal volume of liposome and albuterol solutions were mixed, and final concentrations of total lipid and albuterol were ~50 μmol/ml and 5 mg/ml, respectively. The mixed liposome-albuterol solution was incubated at 60°C for 1 h, followed by rapid cooling on ice. To remove free albuterol, the liposome-albuterol solution was dialyzed against a buffer of 10% sucrose, 10 mM histidine, pH 6.5. Dialysis buffer was changed once per day for a period of 3 days. Albuterol-containing liposomes were filtered through a 0.45 μm Gelman acrodisc filter, then through a 0.2 μm filter into glass vials, sealed, and stored at 4°C. To examine drug loading efficiency, 400 μl of the sample after cooling on ice was loaded onto a Sephadex G-50 column of 15 cm x 0.1 cm, and then eluted with buffer containing 150 mM NaCl, 50 mM sodium acetate, 0.02% azide, pH 4.5. Thirty fractions of 1 ml were collected, diluted to 1:11 in 99% MEOH and 1% 1 N HCl. The UV absorbance of the diluted fractions was measured in a spectrophotometer at 281 nm. Albuterol-loaded liposomes appeared in fractions 4–8, and free albuterol appeared in fractions 11–20. The loading efficiency was ~60% of the original amount of drug added. The final preparation of albuterol-loaded liposomes contained ~36 μmol phospholipid/ml and ~1.9 mg albuterol/ml, where more than 99% of the drug is encapsulated. Phospholipid concentration was determined by phosphate assay as before (1) and albuterol by spectrophotometry at 28 nm.

Assessment of Plasma Extravasation

In this study, we examined the effects of albuterol, liposomes, and albuterol-loaded liposomes on substance P (SP)-induced plasma extravasation in rat trachea. Evans blue was used as a marker to assess plasma extravasation. All injections were i.v. (femoral vein).

Liposome-encapsulated Albuterol vs. Liposomes Alone

Seven groups of rats were used, 4 rats in each group. In group 1, the first injection was 0.9% saline. SP (3 nmol/kg body wt) was injected 3 min later. Evans blue (30 mg/kg) was injected 24 min after SP. Finally, the second dose of SP (3 nmol/kg) was injected 3 min after Evans blue. The animals were euthanized, and perfusion was performed with PBS buffer (pH 7.4) after another 8 min as follows: The chest was opened, a cannula was inserted into the ascending aorta through the left ventricle, the left atrium was incised, and perfusion was carried out for ~40 sec. Then the right atrium was incised, and perfusion was continued for another ~80 sec. Groups 2, 3, and 4 were the same as group 1, but saline was replaced with albuterol-containing liposomes. The doses of liposomal-albuterol for each group were 0.5, 1, and 2 mg/kg, respectively. Group 5 was also the same as group 1, except that liposomes alone (no albuterol) were used instead of saline. The amount of phospholipid given was ~36 μmol per kg of body wt (an amount equal to the phospholipid given in group 4. The difference between groups 1 and 6 was that the first injection of SP in group 1 was replaced with 0.9% saline in group 6. Group 7 was the same as group 6, but the albuterol-loaded liposomes (2 mg albuterol/kg) were used instead of the first saline.

Time Course of Action of Albuterol-loaded Liposomes vs. Free Albuterol

Injections of 0.9% saline, or albuterol (2 mg per kg of body wt) or albuterol-loaded liposomes (2 mg of liposomal albuterol/kg body wt) were followed by SP (3 nmol/kg) 3 min later. The second dose of SP (3 nmol/kg) was injected at various times: 0.5, 1, 2, and 4 h after saline, or albuterol or albuterol-loaded liposomes. Evans blue (30 mg/kg) was injected 3 min before the second dose of SP. The animals were perfused 8 min after the second injection of SP. Four rats each comprised of the groups of control and albuterol-loaded liposomes for each time point; in the free albuterol group only for 2 or 3 rats were studied for each time point, due to death of some rats after injection of albuterol prior to the completion of the experiments.

Measurement of Evans Blue Extravasation in Rat Trachea

After the perfusion, the trachea was cut below the larynx and above the bifurcation, and connective tissue was removed. Then, the anterior trachea was cut longitudinally, blotted with bibulous paper (Fisher Scientific Co. Pittsburgh, PA), and weighed. Evans blue in the trachea was extracted by immersion in 2 ml of formamide and shaking at room temperature overnight. The optical density of Evans blue in formamide was measured at a wavelength of 620 nm with a UV160U spectrophotometer (Shimadzu, Columbia, MD). The amount of Evans blue was determined by comparing the values to a standard curve of Evans blue and expressed in micrograms of Evans blue per g of trachea. This represents an assay for extravasated albumin, because Evans blue is known to bind to circulating albumin shortly after injection in blood.

Measurement of Arterial Blood Pressure

The effects of free albuterol and of albuterol-loaded liposomes on arterial blood pressure of rats were examined. The femoral vein was cannulated and connected to a pump. Intravenous injection of various drugs was carried out with the pump for 2 min: free albuterol, 2 mg/kg (N = 1 rat); albuterol-loaded liposomes, 0.5, 1, and 2 mg of albuterol/kg (N = 2 rats for each dose); liposomes alone: 36 μmol of phospholipid/kg (N = 1 rat). A femoral artery was cannulated and connected to a Statham transducer (model P23D, Statham, U.S.A.). Arterial blood pressure was recorded continuously for 30 min. In another experi-

ment, propranolol (1 mg/kg) was injected i.v. for 2 min, followed by injection of liposomal albuterol (2 mg/kg, i.v.) from 7 to 9 min, and arterial blood pressure was monitored continuously for 15 min (N = 1 rat).

Statistical Analysis

Values are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using the STATVIEW program, and the relationship between inhibition of extravasation and dose of albuterol-loaded liposomes was found to obey a quadratic regression.

RESULTS

Effect of Liposome-encapsulated Albuterol on Evans Blue Extravasation in Rat Trachea

Rats were pretreated by injection of different doses of liposomal albuterol (0.5–2 mg/kg, i.v.). This was followed by injection of substance P (SP, 3 nmol/kg, i.v.), to allow extravasation of the drug-loaded liposomes in the trachea (2). In this way, we were able to examine the effect of extravasated drug-loaded liposomes on the subsequent SP-mediated Evans blue extravasation. The results of this experiment (Figure 1A) indicate that pretreatment with albuterol-loaded liposomes inhibited subsequent SP-mediated extravasation in a dose-dependent manner. Liposomes containing 2 mg/kg of albuterol inhibited SP-induced extravasation by an average of 56% (Fig. 1A). Injection of liposomes alone (without encapsulated drug) did not affect the subsequent SP-induced extravasation (Fig. 1B). To determine whether leakage of albuterol from liposomes in blood was responsible for the subsequent inhibitory effect on extravasation, we performed studies where we injected liposomes loaded with albuterol (2 mg/kg) but where no SP was injected initially at 3 min after the drug-loaded liposomes. In this case, subsequent SP-induced extravasation was affected to a much lesser extent (Fig. 1C). From these studies we conclude that liposomes containing albuterol had to extravasate into airway tissue in order to have a large inhibitory effect on subsequent extravasation, although a certain amount of albuterol could have leaked from the circulating liposomes before extravasation.

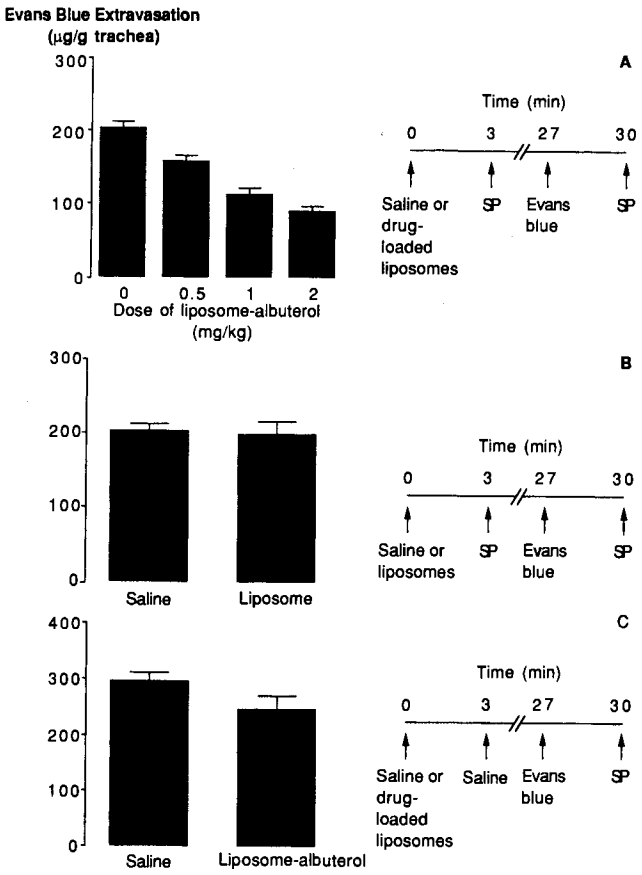

Fig. 1. Effect of albuterol-loaded liposomes on substance P (SP)-induced extravasation of Evans blue in rat trachea. Data reported as mean ± SEM (N = 4 rats per group). A. dose-dependent inhibition. Albuterol-loaded liposomes (0.5, 1, and 2 mg albuterol/kg body wt) or saline (0.9%) were injected i.v., followed by the first dose of SP (3 nmol/kg body wt) 3 min later. Evans blue was injected i.v. 24 min after SP, and then the second dose of SP (3.0 nmol/kg) was administered i.v. B. Effect of liposomes alone (~36 μmol total lipid/kg body wt) on inhibition of SP-induced extravasation. C. Effect of albuterol released into blood stream from albuterol-containing liposomes during circulation on inhibition of SP-induced extravasation. The experimental procedure was the same as described in A, except that the dose of liposomal albuterol was 2 mg/kg, and the first dose of SP was replaced by 0.9% saline.

Duration of Action of Liposome-encapsulated Albuterol on Extravasation vs. Free Albuterol

To examine the duration of inhibition by albuterol-loaded liposomes, we first injected albuterol-loaded liposomes and then SP followed by injection of Evans blue, and a second dose of SP at varying times subsequently. Pretreatment with albuterol (2 mg/kg iv)-loaded liposomes inhibited SP-induced extravasation by approximately 56%. This inhibition of extravasation remained for the entire 4 h of the experiment (Fig. 2). When free albuterol (2 mg/kg) was injected i.v., SP-induced extravasation was similarly inhibited, but this inhibition lasted only a short time (Fig. 2). Furthermore, of the 12 rats given free albuterol, 5 died during the experiment, presumably due to hypotension (see below). From these studies, we conclude that liposome-encapsulated albuterol produces a greatly prolonged inhibitory effect on extravasation compared to free albuterol.

Effect of Liposome-encapsulated Albuterol on Arterial Blood Pressure vs. Free Albuterol

When we injected albuterol (2 mg/kg, i.v.)-loaded liposomes, arterial blood pressure decreased only moderately and recovered within 5 min (Fig. 3A). On the other hand, the same dose of free albuterol (2 mg/kg, i.v.) caused a profound and prolonged hypotension (Fig. 3A). The hypotensive effect of liposome-encapsulated albuterol was even smaller when lower doses of albuterol were used. Thus, the maximum decrease in blood pressure with 1 mg/kg was 20% and with 0.5 mg/kg was 15% (Fig. 3B). Liposomes alone did not affect blood pressure. The β-adrenergic antagonist, propranolol (1 mg/kg i.v.), completely inhibited liposomal albuterol-induced hypotension (data not shown). From these results we conclude that free albuterol causes profound hypotension, an effect that is prevented by encapsulation of the drug in liposomes.

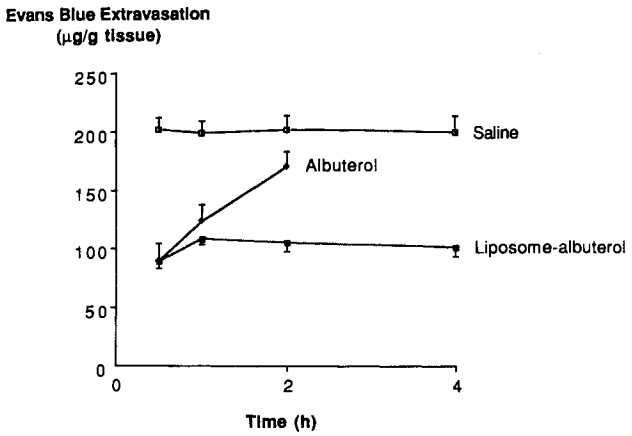

Fig. 2. Duration of inhibition of substance P-induced extravasation of Evans blue by albuterol-loaded liposomes vs. free albuterol in rat trachea. Albuterol (2 mg/kg body wt) or albuterol-loaded liposomes (2 mg albuterol/kg) or 0.9% saline was injected i.v., followed by SP (3 nmol/kg body wt) 3 min later. The second dose of SP (3 nmol/kg) was introduced i.v. at various times subsequently: 0.5, 1, 2, and 4 h after saline, albuterol or albuterol-containing liposomes. Evans blue (30 mg/kg) was administered 3 min before the second dose of SP. Data reported as mean ± SEM (N = 4 rats for the groups of control and albuterol-containing liposomes, and N = 3 or 2 rats for the groups of free albuterol, due to death of some rats).

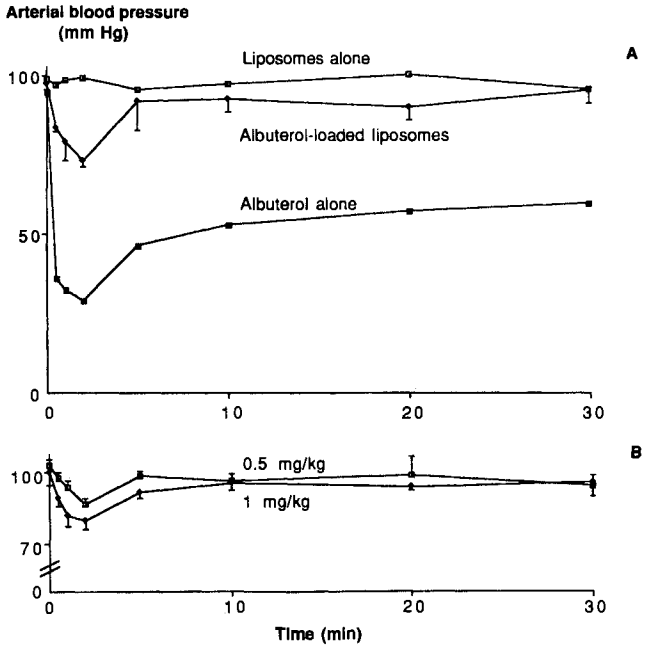

Fig. 3. Effect of albuterol-loaded liposomes and free albuterol on arterial blood pressure in rats. A. Injection of albuterol (2 mg/kg body wt, i.v.), albuterol-containing liposomes (2 mg albuterol/kg), and liposomes alone (~36 μmol total lipid/kg). B. Injection of albuterol-containing liposomes (0.5 and 1 mg albuterol/kg). Data reported as means ± SEM (N = 2 rats for the group of albuterol-loaded liposomes, N = 1 for liposomes alone and for free albuterol).

DISCUSSION

Airway inflammation is characterized by opening of the gap junctions in postcapillary venules. This results in the leakage of fluid and subsequent edema formation. In addition, the opened gaps allow large molecules such as kininogen and complement to escape from the circulation and to initiate inflammatory cascades. Sterically stabilized liposomes remain in blood circulation for long periods of time (1) . When vascular permeability is increased in airways by injection of SP, a neuropeptide recognized by receptors on the airway postcapillary venular endothelium (8,9), liposomes are able to accumulate selectively in airway tissue (2).

Sterically stabilized liposomes have been known to extravasate into tissues showing increased vascular permeability, such as tumors (1) and also areas of infection and inflammation (21–23). In such cases, a substantial amount of the initially injected dose has been found to accumulate in these tissues (1–5%/g tissue). Although the injection of SP increases the uptake of liposomes by trachea (and by inference bronchial airways) by a large factor (24–52 fold depending on the marker used, Ref. 2), we don't have a useful numerical comparison in terms of % injected dose.

In the present study, we injected liposome-encapsulated albuterol, followed by SP (both i.v.) to allow the drug-containing liposomes to deposit selectively in airway tissue. We hypothesized that the albuterol would diffuse out of the extravasated liposomes slowly, delivering relatively high concentrations of the drug in the tissue near the venules. This might inhibit subsequent extravasation with minimal side effects. Therefore, we devised experiments to compare the relative effects of albuterol-loaded liposomes with free albuterol in inhibiting plasma extravasation. The side effects of the two forms of delivery of albuterol on arterial blood pressure were also compared. The results indicated that albuterol-loaded liposomes and free albuterol both inhibited subsequent SP-induced extravasation. However, there were striking differences in the effects of the two methods of delivery: One, liposome-encapsulated albuterol inhibited subsequent extravasation for the entire four hour period of study, while free albuterol's action was much shorter. Two, when free albuterol was injected, there was a profound and prolonged decrease in arterial blood pressure (and 5 of the 12 animals died). When the same amount of albuterol was injected encapsulated in liposomes, only slight hypotension ensued and returned to control values within 5 min. It is suggested that the slight drop in blood pressure that occurred when albuterol-laden liposomes were injected was due to leakage of albuterol out of the liposomes before the start of the study or within the blood stream immediately following injection. The hypotension was prevented by pretreatment with a beta-adrenergic antagonist, propranolol, indicating that the hypotension was due to beta-adrenergic vasodilation.

We used albuterol as a model drug for the following reasons: (1) albuterol is a selective β_2-adrenergic agonist, with minimal stimulation of α and β_1 receptors (10,11). (2) albuterol has a more rapid onset of action than longer-acting β_2-adrenergic agonists such as salmeterol (12); (3) albuterol is water soluble and can be encapsulated efficiently into liposomes with the established method of ammonium sulfate gradient loading (13).

Substance P, a neuropeptide released from sensory nerves in the airway mucosa and other tissues, causes neurogenic inflammation. In this study, SP was injected twice intravenously. The first administration of SP was used to increase vascular permeability, thus allowing previously introduced albuterol-loaded liposomes to extravasate from blood and to deposit selectively in the airway tissue. The second injection of SP was used to trigger additional inflammation in which plasma leakage was evaluated by extravasation of Evans blue. This experimental set-up served as a model of an inflammatory disease and allowed us to examine inhibition of plasma extravasation by intravenously injected liposomal albuterol. The results discussed earlier showed that liposome-encapsulated albuterol can reduce SP-induced plasma extravasation in rat trachea in a dose-dependent manner. In this study, we did not examine the role of albuterol as a bronchodilator, but it is possible that targeted delivery of β-adrenergic agonists to airways will also promote bronchodilation.

Adverse effects include cardiovascular complications, hypotension and muscle tremor, which are caused by stimulation of β-adrenergic receptors (14). Because albuterol was encapsulated into liposomes, it was expected that adverse effects of the drug would be reduced. To test that, we evaluated blood pressure, comparing the effects of free albuterol and of liposomal albuterol. The data indicated that encapsulation of free β_2-adrenergic agonists into liposomes prevented severe, prolonged hypotension in rats. We believe that the slight drop in blood pressure that occurred when albuterol-loaded liposomes were injected was due to an initial leakage of albuterol out of the liposomes.

Presently, β-adrenergic agonists are used widely in the treatment of asthma and chronic obstructive pulmonary disease (COPD). These drugs promote bronchodilation (15–17) and have anti-inflammatory effects including reduction of microvascular leakage (3–5) and prevention of release of inflammatory mediators from mast cells (18,19). Beta-adrenergic agonists can be delivered by inhalation or by oral or parenteral routes. The inhalation route is preferred for treatment of bronchospasm because of the lower dosage needed than oral and fewer systemic adverse effects. However, in severe acute asthma, inflammatory cascades cause mechanical obstruction of the airways, a situation which is not amenable to present forms of therapy. In such cases, the intravenous route of administration of β-adrenergic agonists may be more effective (20), and the liposome-encapsulated form, which diminishes systemic effects should prove an effective strategy. Moreover, severe inflammations occur in many other organs in diseases including rheumatoid arthritis, colitis, rhinitis, etc. These diseases are severe enough to cause hospitalization. In these patients, intravenous therapy is acceptable, if the therapeutic strategy is successful. This strategy may certainly involve liposome encapsulation, and the same strategic advantage would hold for other types of drugs in addition to albuterol. Therefore, it may be useful to develop an intravenous "targeted" route with minimal side effects for β-adrenergic agonists and other drugs, to treat severe asthma, COPD, and other inflammatory diseases more effectively.

In conclusion, we have shown that delivery of liposomes loaded with beta-adrenergic agonists into airway tissue can effectively inhibit subsequent vascular extravasation, with minimal systemic side effects. This system opens the possibility for novel antiinflammatory therapy for severe asthma and other inflammatory diseases.

ACKNOWLEDGMENTS

This work was supported in part by National Institute of Health Program Project Grant HL24136 and Grant GM28117 and by the National Institutes of Health Grant DK47766. The authors acknowledge Josh Gittelman and Shandala Hayes for assistance with liposome preparation.

REFERENCES

1. D. Papahadjopoulos, T. M. Allen, A. Gabizon, E. Mayhew, K. Matthay, S. K. Huang, K. D. Lee, M. C. Woodle, D. D. Lasic, C. Redemann, and F. J. Martin. Sterically stabilized liposomes: Improvements in pharmacokinetics, and anti-tumor therapeutic efficacy. *Proc. Natl. Acad. Sci. (USA)* **88**:11460–11464 (1991).
2. J. Rosenecker, W. Zhang, K. Hong, J. Lausier, P. Geppetti, S. Yoshihara, D. Papahadjopoulos, and J. Nadel. Increased liposome extravasation in selected tissues: Effect of substance P. *Proc. Natl. Acad. Sci., USA* **93**:7236–7241 (1996).
3. S. R. O'Donnell and C. G. A. Persson. β2-Adrenoceptor mediated inhibition by terbutaline of histamine effects on vascular permeability. *Br. J. Pharmacol.* **62**:321–324 (1978).
4. J. L. Beets and W. Paul. Actions of locally administered adrenoceptor agonists on increased plasma protein extravasation and blood flow in guinea-pig skin. *Br. J. Pharmacol.* **80**:461–467 (1980).
5. I. Erjefalt and C. G. A. Persson. Anti-asthma drugs attenuate inflammatory leakage into airway lumen. *Acta Physiol. Scand.* **1986**:653–655 (1986).
6. T. M. Allen, C. Hansen, F. Martin, C. Redemann, and A. Yau-Yong. Liposomes containing synthetic lipid derivatives of poly (ethylene glycol) show prolonged circulation half-lives in vivo. *Biochim Biophys. Acta* **1066**:29–36 (1991).
7. F. Olson, C. A. Hunt, F. C. Szoka, W. J. Vail, and D. Papahadjopoulos. Preparation of liposomes of defined size distributing by extrusion through polycarbonate membranes. *Biochim. Biophys. Acta* **557**:9–23 (1979).
8. J. M. Lundberg and A. Saria. Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature* **302**:251–253 (1983).
9. A. Saria, J. M. Lundberg, G. Skofitsch, and F. Lembeck. Vascular protein linkage in various tissue induced by substance P, capsaicin, bradykinin, serotonin, histamine and by antigen challenge. *Naunyn-Schmiedebergs Arch. Pharmakol.* **324**:212–218 (1983).
10. E. R. McFadden. Beta 2 receptor agonists—Metabolism and pharmacology. *J. Allergy Clin. Immunol.* **68**:91–96 (1981).
11. H. S. Nelson. Beta adrenergic agonists. *Chest* **82**:33S–38S (1982).
12. K. Blake and A. K. Kamada. Asthma. In E. T. Herfindal and D. R. Gromley, *Textbook of therapeutics: drug and disease management.*, Williams & Wilkins, Baltimore, 1996, 651–684.
13. D. D. Lasic, P. M. Frederick, M. C. A. Stuart, Y. Barenholz, and T. J. McIntosh. Gelation of liposome interior: a novel method for drug encapsulation. *FEBS Lett* **312**:255–258 (1992).
14. K. M. Lulich, R. G. Goldie, G. Ryan, and J. W. Paterson. Adverse reactions to beta 2-agonist bronchodilators. *Med. Toxicol.* **1**:286–299 (1986).
15. H. S. Nelson. Adrenergic therapy of bronchial asthma. *J. Allergy Clin. Immunol.* **77**:771–785 (1986).
16. M. T. Newhouse and M. B. Dolovich. Control of asthma by aerosols. *N. Engl. J. med.* **315**:870–874 (1986).
17. N. Svedmyr and C. Lofdahl. Physiology and pharmacodynamics of beta-adrenergic agonists. In J. S. Jenne, and S. A. Murphy (eds.), *Drug therapy for asthma: research and clinical practice*, Marcel Dekker, New York, 1987, pp. 177–213.
18. P. H. Howarth, S. R. Durham, T. H. Lee, A. B. Kay, M. K. Church, and S. T. Holgate. Influence of albuterol, cromolyn sodium and ipratropium bromide on the airway and circulating mediator responses to allergen bronchial provocation in asthma. *Am. Rev. Respir. Dis.* **132**:986–992 (1985).
19. J. M. Hughes, J. P. Seale, and D. M. Temple. Effect of fenoterol on immunologcal release of leukotrienes and histamine from

human lung in vitro: selective antagonism by beta-adrenoceptor antagonists. *Eur. J. Pharmacol.* **95**:239–245 (1983).
20. B. Cheong, S. R. Reynolds, G. Rajan, and M. J. Ward. Intravenous beta agonist in severe acute asthma. *Br. Med. J.* **297**:448–450 (1988).
21. I. Bakker-Woudenberg, A. F. Lokerse, M. T. ten Kate, and G. Storm. Enhanced localization of liposomes with prolonged blood circulation time in infected lung tissue. *Biochem. Biophys. Acta* **1138**:318–326, 1992.
22. I. Bakker-Woudenberg, A. F. Lokerse, F. H Roerdink, D. Regts, and M. F. Michel. Free versus liposome-entrapped ampicillin in treatment of infection due to *Listeria monocytogenes* in normal and athymic (nude) mice. *J. Inf. Dis.* **151**:917–924 1985.
23. I. Bakker-Woudenberg, A. F. Lokerse, M. T. ten Kate, J. W. Mouton, and M. C. Woodle. Liposomes with prolonged blood circulation and selective localization in *Klebsiella pneumonia*-infected lung tissue. *J. Inf. Dis.* **168**:164–171, 1993.