Large Porous Particles for Sustained Protection from Carbachol-Induced Bronchoconstriction in Guinea Pigs

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Purpose. To determine whether a new formulated albuterol aerosol could sustain inhibition to bronchoconstriction for approximately one day in guinea pigs challenged with carbachol.

Methods. Large and porous particles, comprising a combination of endogenous or FDA-approved excipients and albuterol sulfate, were prepared by spray drying using a NIRO portable spray drier. The anesthetized animals inhaled 5 mg of large porous or small nonporous particles by forced ventilation via cannulae inserted in the lumen of their exposed tracheae. At regular intervals over a period of 36 hours after drug delivery, airway resistance was determined in response to carbachol challenge dose.

Results. Whereas inhalation of small nonporous albuterol particles protected from the carbachol-induced bronchoconstriction for up to 5 hours, inhalation of large porous albuterol particles produced a significant inhibition of carbachol-induced bronchoconstriction for at least 16 hours.

Conclusions. The absence of substantial side effects, verified over a period of 24 hours by evaluating cardio-respiratory parameters as well as pulmonary inflammation, supports the utility of large porous albuterol particles for sustained therapies in asthma and other types of lung disease.

KEY WORDS: large and light particles; albuterol; inhalation; prolonged bronchodilation; guinea pigs.

INTRODUCTION

Asthma is a chronic inflammatory disorder characterized by an increase in the responsiveness of the airways to a variety of stimuli, such as pharmacological agents, air pollutants, cold air or exercise. This state of inflammation-induced hyperresponsiveness is usually associated with airflow obstruction (narrowing of airways), but it is often reversible by treatment with bronchodilators. Inhaled β_2-adrenoreceptor agonists such as albuterol (short-acting) and salmeterol (long-acting for nocturnal asthma) are the mainstay bronchodilator drugs for the treatment of asthma of all grades of severity (1). Although most β_2-agonists are potent inducers of airway smooth muscle relaxation and are effective inhibitors of mediator release by mast cells (2), their bronchodilator effect has been limited by their relatively short duration of action. This has been demonstrated by the lack of benefit in blocking the late asthmatic response, 6–8 hours after allergen challenge, or on the subsequently enhanced non-specific bronchial reactivity (3,4). It is therefore desirable to develop longer-acting bronchodilator formulations to improve the treatment of asthma and other chronic lung diseases.

Lipophilic carrier particles can be used to sustain the action of inhaled, encapsulated, bronchodilators in lung airways. By making these particles large and porous, particle agglomeration can be lowered and efficacy improved (5,6). Relatively large size also potentially permits longer particle life in the lungs by minimizing phagocytic losses (5,13). These attributes make large porous particles attractive as sustained-release carriers of bronchodilators for treatment of asthma.

In this study, large porous particles have been formulated using nonpolymeric endogenous excipients for inhalation of albuterol, a relatively β_2-specific-adrenergic amine and a short-acting bronchodilator agonist. The size and porosity of the particles along with the therapeutic loading levels have been optimized to achieve pharmacokinetic control of the drug in a sustained-release manner. *In vivo* airway resistance (in response to carbachol challenge) was used to evaluate the sustained protection from carbachol-induced bronchoconstriction in guinea pigs with the controlled-release albuterol from inhaled porous particles.

METHODS

Animals and Chemicals

Male Hartley guinea pigs, weighing 600–800 g, were purchased from Hiltop Laboratory (Scottdale, PA). The animals were housed in individual cages and provided food and water ad libitum. After quarantine, the guinea pigs were acclimated in an environmentally controlled room (temperature: 22 ± 3°C; humidity: 48 ± 5% illumination time: 7 a.m. to 7 p.m.) at the Laboratory Animal Resource facility of Penn State University for at least 1 week prior to use in experiments. The research adhered to the "Principles of Laboratory Animal Care (NIH publication # 85-23, revised 1985)", and the experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Penn State University.

The following materials were obtained from the specified sources: ketamine hydrochloride and xylazine (Phoenix Pharmaceutical, St. Joseph, MO); pentobarbital sodium (Abbott, North Chicago, IL); succinylcholine chloride, carbamyle chloride (carbachol), phosphate-buffered saline (PBS), ethanol, human serum albumin, lactose, dipalmitoyl phosphatidylcholine (DPPC), and albuterol sulfate (Sigma Chemical, St. Louis, MO).

Formulation of the Inhaled Therapeutic

We made large porous particles by spray drying (using a Niro Atomizer Portable Spray Drier, Columbia, MD) a cosolvent aqueous solution (85% ethanol) containing a combination of human serum albumin (18% in weight), lactose (18%), dipalmitoyl phosphatidylcholine (DPPC, 60%), and albuterol sulfate

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(4%). Figures 1A&B indicate the large size and irregular shape of the particles. They possess a mean geometric diameter of 10 μm ± 4 μm (as measured with a Coulter Multisizer) and a bulk (tapped) density of 0.06 g/cm^3. For comparison purposes, we also prepared by spray drying fast-release albuterol particles comprising 4% albuterol and 96% lactose. Unlike the large porous particles, these particles appeared to be spherical in shape with smooth and nonporous surface (Fig. 1C). They possess a mean geometric diameter of 3 μm ± 2 μm and a bulk (tapped) density of 0.45 g/cm^3.

Our purpose in preparing large porous particles of this composition were four-fold: 1) because of the high proportion of DPPC, the particles tend to remain insoluble in water for long periods of time, potentially permitting sustained release of hydrophilic albuterol over a day or more in physiological environments; 2) because of their low mass density and large size (9,10), the particles can easily penetrate into the peripheral airways, and potentially escape the lungs' natural phagocytic clearance until the inhaled particles deliver their albuterol payload; 3) because they comprise materials that are either endogenous to the lungs or FDA-approved for inhalation, the particles are likely to be safe for human use; and 4) because spray drying permits relatively easy manufacturing scale-up, the fabrication of the particles is suited for commercialization.

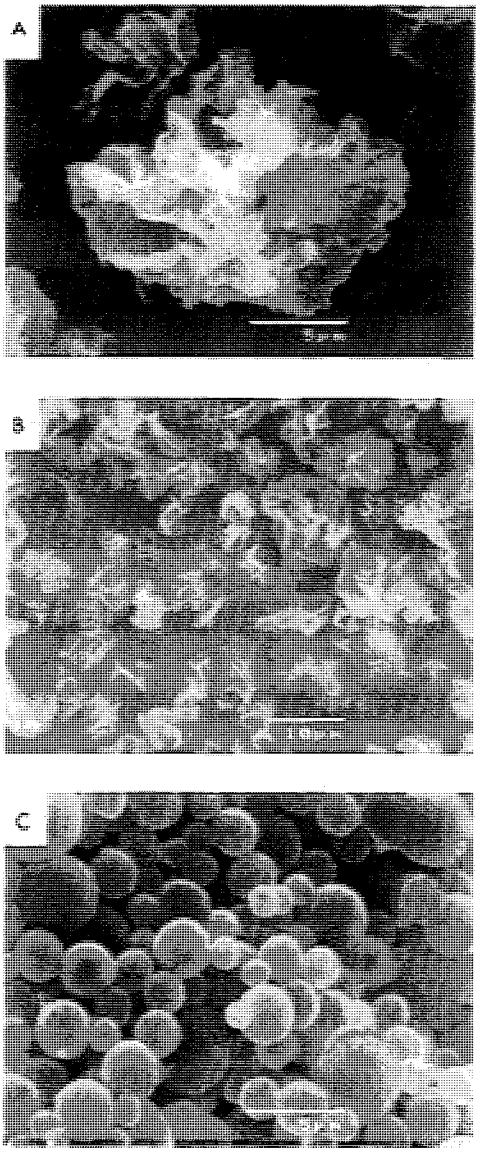

Fig. 1. Scanning electron microscope (SEM) image of the large porous (A and B) and small nonporous (C) particles.

In Vitro Aerosol Characterization

To assess aerosolization performance, particles were loaded inside N°2 hard gelatin capsules (Eli Lilly, Indianapolis, IN) to about half capsule volume with 12 mg powder, and the capsules were individually loaded in a Spinhaler dry powder inhaler (DPI). Particles were aerosolized into an Andersen Cascade Impactor (Mark II, Graseby Anderson Division, Atlanta, GA) from the Spinhaler DPI for 10 seconds at 28.3 liter/min, the flow rate for which the Andersen Impactor is calibrated (though it does not permit the most effective operation of the Spinhaler (7)). Following deposition on the stages of the impactor, particles were collected by glass fiber filters placed on each stage. The total particles masses were measured stage-wise, and both fine particle fraction (≤4.7 μm) and emitted dose determined based on the initial total mass of particles inside the Spinhaler. Alternatively, aerodynamic particle size was measured by aerosolizing the powder and measuring time-of-flight using an Aerosizer (AeroBreather, Amherst Process, Inc, MA).

Delivery of Albuterol Particles to Animal Lungs

To test the *in vivo* performance of the two types of particles, we developed a delivery system (Fig. 2) in which a small amount of dry powder can be efficiently aerosolized and inhaled through an exposed trachea of an anesthetized animal. In this study, guinea pigs were anesthetized using an intramuscular injection of 60 mg/kg ketamine hydrochloride mixed with 4 mg/kg xylazine. A small rigid tube (approximately 1 mm OD, much smaller than the tracheal ID) was loosely inserted, between two cartilaginous rings, into the lumen of the exposed trachea above the carina. This tube was then connected to a Harvard ventilator (Model 683; Holliston, MA) via inhalation and exhalation ports, while the animal continued to breathe spontaneously. A fixed quantity (typically 5.0 mg) of either porous or nonporous particles was placed in the inhalation port (for aerosolization) and insufflated into the lungs of the animals by forced ventilation at a 4 ml tidal volume and 100 strokes/min frequency. Following the short period of pulmonary delivery (typically 30 sec), the tube was removed from the trachea with no sign of injury. We have previously demonstrated (5) that this delivery system was efficient and reproducible, and minimizes the deposition losses of the inhaled particles in the tubing and trachea while maximizing their deposition in the respiratory airways.

Determination of Airway Resistance

At selected intervals over a period of 36 hours following inhalation of the particles, the recovered guinea pigs were reanesthetized and mechanically ventilated using the Harvard rodent respirator. The animals were then paralyzed with intraperitonial injection of 5 mg/kg succinylcholine to eliminate spontaneous respiration. Airflow rate was measured using a

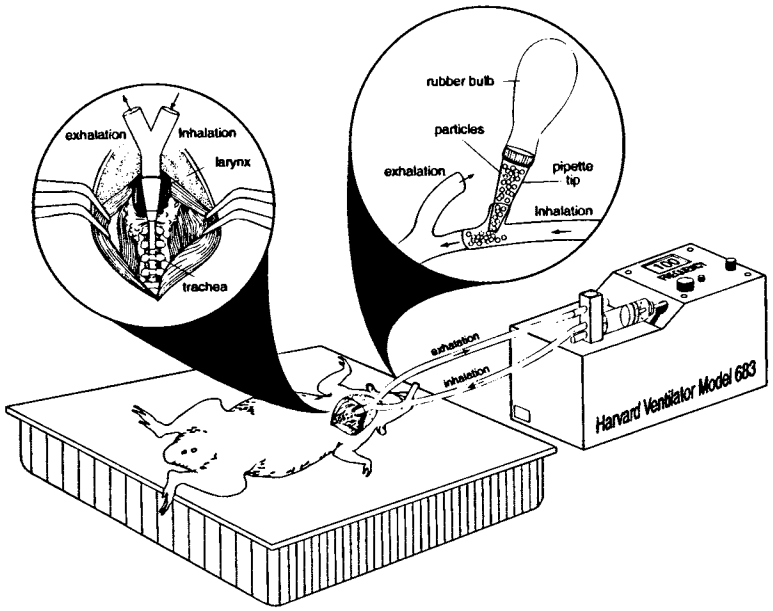

Fig. 2. Inhalation system for particle delivery to the respiratory tract of guinea pigs.

Hans Rudolph screen pneumotachograph (Series 8420; Kansas City, CT) coupled to a differential pressure transducer (Buxco Electronics; Sharon, CT). The pneumotachograph, which has a very small dead space volume (0.5 ml), was provided with an adjustable-temperature heat-controller system (Hans Rudolph) that prevented condensation of water vapor from exhaled breaths. Tracheal pressure was measured with a Sensym sensor that has a high-impedance and a low noise (Buxco Electronics, Model 650B SX01DN). Airflow resistance was determined from the recorded pressure and flow measurements using the Buxco data acquisition and analysis systems (Biosystem XA Software, Keithly DAS1201/1202).

Airway resistance was measured before (base line) and after carbachol challenge (100 μg intramuscular injection) in order to evaluate the inhibition of bronchoconstriction by the inhaled albuterol particles (response). Airway resistance was monitored for 5–10 minutes post-carbachol challenge, after which the animals were removed from the ventilator and euthanized with a lethal dose of pentobarbital sodium. We performed airway resistance tests following carbachol challenge on a control group of guinea pigs (non-treated animals), and on thirteen different groups for three albuterol doses (10 μg, 100 μg, 200 μg). For the 200 μg dose of albuterol, the airway resistance tests were performed at the following selected time intervals: 30–60 min, 7–8 hr, 14–16 hr and 34–36 hr for the large porous particles, and 3 hr, 5 hr and 8 hr for the small nonporous particles. For the 10 μg and 100 μg doses of albuterol, airway resistance was measured only at one selected time (15–16 hr) following particle delivery. A total of 59 guinea pigs was used for these treatments, with each treatment group (n = 4) evaluated for airway resistance at one time period.

Monitoring of Respiratory Parameters and Heart Rate

To investigate whether long residence of the particles in the lungs affects spontaneous respiration and heart rate of the animals, we monitored the heart rates and ventilatory parameters of three groups of guinea pigs: the control group received no treatment; the placebo group inhaled large porous albuterol-free particles; and the treated group inhaled large porous albuterol particles. In all three groups, the heart rate and arterial oxygen saturation (SpO_2) were noninvasively monitored at selected intervals over 24 hours using an SDI pulse oximeter (Model #4402 Vet/Ox; Waukesha, WI) specifically made for veterinary applications. While the animals breathed spontaneously through an intratracheal cannula, several respiratory parameters from many consecutive breaths (5 min recordings) were collected, analyzed, and averaged using the Buxco software system (Biosystem XA). The following parameters were computed using these data: inspiratory and expiratory times (Ti and Te), tidal volume (VT), minute volume (MV), and breathing frequency (f).

Assessment of Pulmonary Inflammation

Pulmonary inflammation was assessed by analysis of the cellular and fluid components (8) of the bronchoalveolar lavage fluid (BALF). The airways and lungs were washed with phenol red-free Hank's balanced salt solution (HBSS) without Ca^{++} and Mg^{++}, in order to minimize cell clumping. BALF was obtained by slowly injecting 10 ml of HBSS into the trachea and then withdrawing the liquid from the lungs. The lavage procedure was repeated with four more 10-ml aliquots until a total volume of about 50 ml was collected. The recovered BALF was centrifuged and the supernatant removed, concentrated, if necessary, using membrane filters (Amicon, UM-2, Lexington, MA) and stored at −70 °C for later analysis. The parameters that can be examined are total proteins, indicative of transudation of serum proteins across the capillary barrier and lactate dehydrogenase, indicative of general cell injury. If increased levels of these enzymes were detected (indicating cellular injury), additional enzymes were examined for their activity, including acid phosphatase, a lysosomal enzyme which is released during phagocytosis and/or macrophage and neutrophil damage as well

Table I. Spontaneous Respiratory Parameters[a]

Group[b]	n	Wt (g)	Ti (sec)	Te (sec)	f (bpm)[c]	VT (ml)	MV (ml/min)
Control	4	773	0.589	0.406	61	2.84	166
	±25	±0.045	±0.038	±1	±0.09		±8
Placebo	4	833	0.671	0.413	56	3.36	187
	±7	±0.030	±0.030	±1	±0.18		±9
Albuterol	4	890	0.593	0.427	61	3.18	192
		±27	±0.030	±0.086	±2	±0.20	±14

Note: All these values are not significantly different from each other ($P > 0.4$).
[a] Values are mean ± SE of: Ti, inspiratory time; Te, expiratory time; f, respiratory frequency; VT, tidal volume; MV, minute volume.
[b] Control: animals received no treatments; Placebo: animals inhaled albuterol-free particles; Albuterol: animals inhaled particles containing albuterol.
[c] bpm: breaths per minute.

as alkaline phosphatase, an indicator of Type II epithelial cell damage (9,10). The pelletized cells were resuspended in HBSS and counted by hemacytometer. Cell viability was determined by trypan blue dye exclusion. Cytocentrifuge preparations were stained with Diff-Quik (Harleco, Gibbstown, NJ) for differentiation of white blood cell types. Examination of cell number and type is a good indicator of potential pulmonary damage (11). An influx of polymorphonuclear leukocytes is a sensitive indicator of inflammation and is expected early in the response. A later increase in pulmonary alveolar macrophages is characteristic of a persistent and long term inflammatory response (9).

Data Analysis

All the data for each treatment and at each selected time interval were presented by mean values plus or minus (±) the standard errors (SE) of the means. The mean value of bronchoconstriction data obtained on the control group (nontreated animals), in response to carbachol challenge, was considered as the maximum airway resistance. The inhibition of bronchoconstriction (or bronchodilation) by inhaled particles was then compared to the control value by plotting changes in airway resistance (determined from the difference between data points of base line and response to carbachol challenge) as a function of time, following inhalation, for a single albuterol dose (time dependence), and as a function of albuterol dose at a selected time (dose-response).

Statistical differences in the airway resistance, the respiratory parameters, and the heart rate, as well as the inflammatory responses between air-exposed, albuterol-free and albuterol particles-exposed animals were compared by using a two-way analysis of variance (ANOVA) followed by an unpaired Student's t-test. A two-tailed $P < 0.05$ was considered to be statistically significant.

RESULTS

In Vitro Aerosolization Results

The large porous particles exhibited better fine particle fraction (% of particle mass with MMAD less than 4.7 μm, relative to the initial particle mass) (48.5 ± 3.7%) than the small nonporous particles (15.9 ± 6.5%); the porous powder exhibited a 92.7 ± 7.2% emitted dose compared to a 73.5 ± 19.8% emitted dose for the nonporous powder. The nonporous particles appeared somewhat more aggregated than the porous powder based on the mass median aerodynamic diameter (MMAD) values. The MMAD of the large porous formulation according to Aerosizer measurement was 1.6 μm, a factor of 0.65 smaller than the theoretical estimate (2.45 μm). The MMAD of the small nonporous formulation according to Aerosizer measurement was 4.5 μm, a factor of 2.24 larger than the theoretical estimate (2.01 μm).

Airway Response to Inhaled Particles

Temporal changes in response to the three formulations of inhaled particles were evaluated by measuring airway resistance to airflow following carbachol challenge. Each treatment

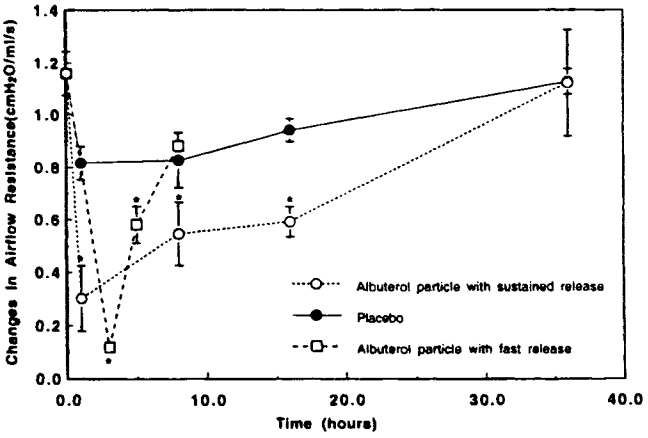

Fig. 3. Airway resistance as a function of time following inhalation of large porous (open circles), small nonporous (open squares) albuterol particles (inhaled albuterol dose = 200 μg), and albuterol-free particles (closed circles), after carbachol challenge. The data at time 0 (closed square) were obtained in the nontreated animals (control), and correspond to maximum airway resistance. All the data are expressed as the changes in resistance to airflow from base line values (before carbachol challenges) to response values (after carbachol challenges). Each data point represents the mean of independent experiments obtained on 4 animals, and the vertical bars represent standard errors of the means. * indicates a significant change in airflow resistance ($P < 0.05$) between Control or Placebo and Albuterol particles.

at each time point was studied in a different animal in order to eliminate any time-dependent alteration in the physiological condition due to anesthesia, surgery and respiratory muscle paralysis. The results are plotted in Fig. 3 which shows the temporal changes in airway resistance due to carbachol-induced bronchoconstriction following particle delivery (this amplitude of airway response is the difference between resistances evaluated at pre- and post-carbachol challenge). The maximum response, shown in the plot at time 0, corresponds to carbachol-induced bronchoconstriction in the control group (nontreated animals). In the placebo group (animals inhaled albuterol-free particles), although the carbachol challenge produced less bronchoconstriction than in the control group over a period of 15 hours, the response is statistically significant only at 1 hour after particle delivery (Table II); 36 hours following inhalation of albuterol-free particles, the carbachol challenge induced similar change in airway resistance to that in the control group. It can be seen, however, that inhalation of large porous particles loaded with 200 μg dose of albuterol produced about 75%, 55% and 50% inhibitions of carbachol-induced bronchoconstriction at 1, 8 and 16 hours following drug delivery, respectively. These temporal reductions in airway resistance either from the control group value or from the the corresponding temporal values of placebo group were all statistically significant (Table II). This is an indication that the inhaled large porous particles encapsulating 200 μg dose of albuterol were capable of preventing bronchoconstriction for at least 16 hours. In the group of animals who inhaled small nonporous particles (comprising 200 μg dose of albuterol), the carbachol challenge produced significantly less bronchoconstriction at 3 and 5 hours after drug delivery than in the control group; the carbachol-induced bronchoconstriction was, however, similar to that in the placebo group and was not significantly different from the control group 8 hours following drug delivery, suggesting that the effective period for bronchodilation with inhaled small nonporous albuterol particles was less than 8 hours. Although no attempt was made to finely characterize the early time behavior of bronchodilation (i.e., within 3 h after inhalation), both the porous and nonporous aerosols seem to produce rapid onset of bronchodilation.

The evaluation of an inhibitive action of albuterol to carbachol-induced bronchoconstriction at several albuterol doses, 15 hours following inhalation of large porous particles, is illustrated in Fig. 4 which shows that the change in airway resistance in response to the carbachol challenge was almost identical at the three doses (10, 100 and 200 μg) of inhaled albuterol, corresponding to a significant inhibition (50–60%) of the carbachol-induced bronchoconstriction. These results indicate that a dose as low as 10 μg dose of albuterol encapsulated in the inhaled large porous particles offered statistically significant protection of animal airways from the carbachol challenge for at least 15 hours.

Effects on the Cardio-Respiratory Parameters

Common ventilatory parameters characterizing the respiratory physiological conditions of the animals during spontaneous breathing were evaluated 24 hours following inhalation of the large porous particles (albuterol or placebo aerosols) or assisted ventilation. These measured parameters, listed in Table I,

Table II. Comparison Between Airway Resistances[a]

Time (hr)	n	Control[b]	Placebo[c]	Alb-Sr[d]	Alb-Fr[e]
A. Base Line Values					
0	7	0.138 ± 0.014	—	—	—
1	4	—	0.165 ± 0.01	0.207 ± 0.039	—
3	4	—	—	—	0.152 ± 0.014
5	4	—	—	—	0.164 ± 0.011
8	4	—	0.148 ± 0.012	0.139 ± 0.0.005	0.138 ± 0.019
16	4	—	0.126 ± 0.005	0.122 ± 0.010	—
36	4	—	0.136 ± 0.015	0.105 ± 0.005	—
B. Responses to Carbachol Challenge					
0	7	1.30 ± 0.09	—	—	—
1	4	—	0.981 ± 0.071[f]	0.503 ± 0.129[g,h]	—
3	4	—	—	—	0.270 ± 0.012[g]
5	4	—	—	—	0.745 ± 0.062[g]
8	4	—	0.975 ± 0.113	0.585 ± 0.117[g,h]	1.180 ± 0.035
16	4	—	1.07 ± 0.054	0.714 ± 0.060[g,h]	—
36	4	—	1.26 ± 0.064	1.23 ± 0.253	—

[a] Values are mean ± SE in cm H_2O/ml/sec.
[b] Control: animals received no treatments.
[c] Placebo: animals inhaled albuterol-free particles.
[d] Alb-Sr: animals inhaled sustained-release albuterol particles.
[e] Alb-Fr: animals inhaled fast-release albuterol particles.
[f] Response in Placebo is significantly different from Control ($P < 0.05$).
[g] Responses in Alb-Sr or in Alb-Fr are significantly different from Control ($P < 0.01$).
[h] Responses in Alb-Sr are significantly different from Placebo ($P < 0.05$). The absence of symbols means that responses are not significantly different from Control or from Placebo ($P > 0.2$).

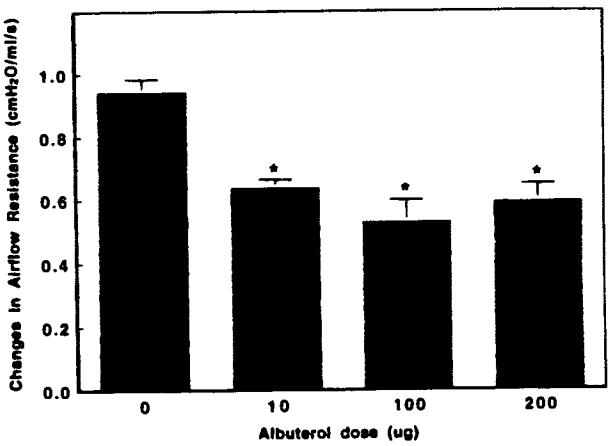

Fig. 4. Airway response to carbachol challenge 15 hours following inhalation of large porous particles encapsulated different albuterol doses. Each data point represents the mean of independent experiment obtained on 4 animals, and the bars represent standard errors of the means. * indicates a significant change in airflow resistance ($P < 0.05$) between Control and Albuterol particles.

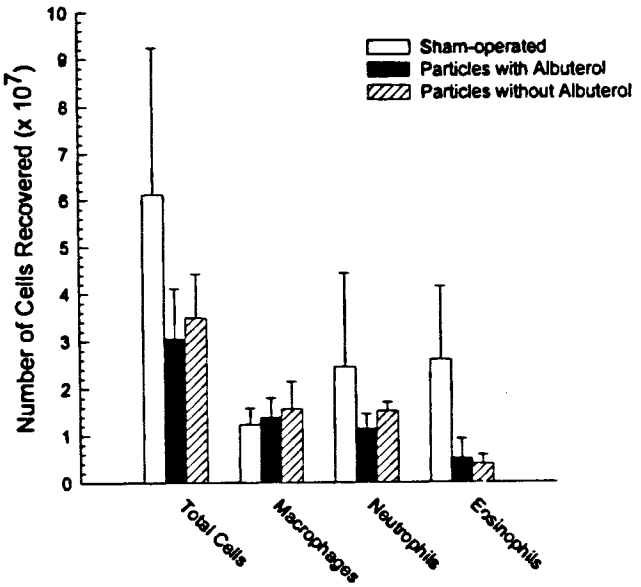

Fig. 5. Cell recovery from bronchoalveolar lavage fluid (Mean ± SE, n = 4) 24 hours after inhalation of large porous particles loaded with 200 μg albuterol (solid bars), 24 hours after inhalation of albuterol-free porous particles (hatched bars), and 24 after forced air respiration (open bars). There were no significant differences between cells recovered from BALF of the 3 groups of animals ($P > 0.1$).

include inspiratory and expiratory times, breathing frequency, tidal volume and minute ventilation. As shown, significant differences in these values were absent between groups, an indication that particle inhalation did not cause deterioration in the measured variables.

The systemic effects from particles inhalation were evaluated by measuring arterial oxygen saturation (SpO_2) and heart rate 3, 6, 9 and 24 hours after particles inspiration or assisted ventilation. The data indicate that there were no significant differences in the values of heart rates (156–203 for control; 162–197 for placebo; 158–205 for albuterol) or SpO_2 (87–92 for control; 90–92 for placebo; 90–94 for albuterol) between groups.

Evaluation of Lung Toxicity

Changes in cell populations in BALF following particle inhalation are shown in Fig. 5. As indicated, there was no significant difference in the cell recovery, whether macrophages, neutrophils or eosinophils. This was true both for the guinea pigs with assisted ventilation (control nontreated group) and those who inhaled larges porous albuterol particles (treated group) or albuterol-free particles (placebo group). It should be noted that the typical number of macrophages recovered from bronchoalveolar lavage fluid of a guinea pig is about 2×10^7 (12). Similarly, the data indicate no significant difference in total protein recovered from the lungs of the three groups of animals (27 mg for sham-operated; 21 mg for placebo; 21 mg for albuterol). These results indicate that particle inhalation did not produce either pulmonary inflammation or lung cellular injury.

DISCUSSION

The results of this investigation complement those of previous studies (5,6), showing that large porous particles aerosolize efficiently and can provide long-lasting therapeutic effects in lungs of guinea pigs (as indicated by the sustained protection of albuterol from carbachol-induced bronchoconstriction). While previous investigations have demonstrated these effects for systemic administration, our results show here that very light ($\rho \sim 0.1$ g/cm^3) and large ($d \sim 10$ μm) albuterol particles can also produce sustained local protection from bronchoconstriction. A new finding of this study is that sustained release of a hydrophilic substance (albuterol) can be achieved from large porous particles by combining the drug with a high-percentage of DPPC. The combination of hydrophilic drug with endogenous soluble excipients provides an advantage over polymer-encapsulated systems since disappearance of inhaled particles coincides with the end of therapeutic release, adding to the advantage of not requiring exogenous polymeric excipient.

Based on our *in vitro* aerosolization results, the fine particle fraction was 49% for large porous particles and 16% for small nonporous particles. While these data were obtained *in vitro* using a DPI, our previous results (5), obtained on rats with the same inhalation system shown in Fig. 2, demonstrated that the nonporous particles deposited primarily in the trachea (~79% of all particle mass that entered the trachea), whereas only 46% of the large porous particle mass deposited in the trachea and 54% deposited in the bronchial tree and alveolar space. As described elsewhere, a reason for this efficient delivery to the lung is reduced powder aggregation (5,13). Increasing the size of aerosol particles results in a reduced fractional surface area (or likelihood) of particle-particle contact in a dry powder and thus reduced tendency to aggregate (5,13). This diminished aggregation means that less energy is required to aerosolize particles, or that particles are more efficiently aerosolized with a given energy of aerosolization.

While it is difficult to specify the contribution of the "deep-lung" fraction of deposited particles to bronchodilation

(given that β-receptors are widespread in the respiratory tract), it is possible that the long-lasting protection from bronchoconstriction by the inhaled porous albuterol particles is at least partly due to the more slowly cleared deep-lung particles because of the role of large particle size in escaping phagocytosis. We have previously tested this hypothesis (5,13) by lavaging the lungs of rats immediately after inhalation of the porous and nonporous particles as well as 48 hours after inhalation. Immediately after inhalation, 30% of macrophages contained small nonporous particles compared to 8% of macrophages containing large porous particles. After 48 hours, 18% of macrophages contained 3 or more small nonporous particles compared to 4% of macrophages containing 3 or more large porous particles (5).

The sustained protection from bronchoconstriction to carbachol challenge (a cholinergic neurotransmitter analog) for approximately 1 day following a single inhalation appears to be the longest reported duration of action of an inhaled β_2-agonist in animals. It exceeds the 1-hour salbutamol or 6-hour formoterol bronchodilation effects in guinea pigs as reported by Hammerbeck *et al.* (14), when these bronchodilators were formulated in chlorofluorocarbon and hydrofluoralkane metered dose inhaler devices. It also exceeds the 12-hour action of intratracheally instilled isoproterenol (another bronchodilator) encapsulated in poly(glycolide-co-lactide) microspheres reported by Lai *et al.* (15). The relatively low dose of albuterol (Fig. 4) required for sustained bronchodilation is consistent with the finding of Lai *et al.* (15) who obtained significant bronchodilation at relatively low dose (35-μg dose isoproterenol) by sustained release of the bronchodilator. Contrastingly, Hammerbeck *et al.* (14), using the same guinea pig model as used here, requiered 100 μg salbutamol dose for significant bronchodilation. Further literature evidence that slow release of bronchodilators in the lungs permits a lower net dose is discussed by Lai *et al.* (15).

In summary, the absence of cardio-respiratory effects and the lack of acute inflammatory responses following inhalation of large porous albuterol particles appears to indicate that albuterol can be delivered via large porous particles with relatively low risk. Inhalation of porous albuterol particles might be clinically beneficial to patients with asthma and other lung diseases by effectively preventing bronchoconstriction for long periods of time, diminishing the frequency of drug use, and minimizing side effects.

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