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 β2-adrenoceptor 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 β2-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 β2-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 (Scottsdale, PA). The animals were housed in individual cages and provided food and water ad libitum. After quarantine, the guinea pigs were acclimatized 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.
(4%). Figures 1A&B indicate the large size and irregular shape of the particles. They possess a mean geometric diameter of 10 \( \mu m \pm 4 \mu m \) (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 \( \mu m \pm 2 \mu m \) 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.

**In Vitro Aerosol Characterization**

To assess aerosolization performance, particles were loaded inside N² 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 (\( \leq 4.7 \mu m \)) 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 intraperitoneal injection of 5 mg/kg succinylcholine to eliminate spontaneous respiration. Airflow rate was measured using a