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ORIGINAL ARTICLE

Formulation, preclinical and clinical evaluation of a new submicronic arginine respiratory fluid for treatment of chronic obstructive pulmonary disorder

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Abstract Inhalational drugs often suffer from low pulmonary deposition due to their micronized size. Aim of present study was development and evaluation of a novel submicronic L-arginine respiratory fluid formulation for treatment of cardiopulmonary complications associated with chronic obstructive pulmonary disorder (COPD). Objectives were (a) to develop and characterize submicronic L-arginine respiratory fluid formulation, (b) pre-clinical safety/toxicity study in 2-animal species, (c) in vitro and in vivo evaluation in terms of respiratory fraction, and (d) clinical study to assess safety/efficacy in healthy volunteers/COPD patients. Formulation was optimized on the basis of particle size of aerosolized medication with particle size in the range of 400–500 nm. Anderson cascade impaction (ACI) studies were performed to validate the advantage in terms of respirable fraction, which indicated a high respirable fraction (51.61 ± 3.28) for the developed formulation. In vivo pulmonary deposition pattern of optimized formulation was studied using gamma scintigraphy in human volunteers using ^{99m}Tc-arginine as radiotracer. It clearly demonstrated a significant pulmonary deposition of the submicronic formulation in various lung compartments. Efficacy of the developed formulation was further assessed in COPD patients (n = 15) by evaluating its effect on various cardiopulmonary parameters (spirometry, pulse-oxymetry, echocardiography and 6-min walk test). A marked improvement was seen in patients after inhalation of

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submicronic arginine in terms of their cardiopulmonary status. Results suggest that submicronic arginine respiratory fluid has the potential to be developed into an attractive therapeutic option for treating COPD associated cardiopulmonary complications.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a prominent but unrecognized cause of morbidity and mortality globally and is estimated to be the third leading cause of death worldwide by 2020 (Murray and Lopez, 1997; Pauwels and Rabe, 2004). COPD is characterized by airflow obstruction and various symptoms such as chronic cough, expectoration, exertional dyspnea and wheezing (Rennard, 1998). It is also associated with inflammatory process which affects the respiratory tract up to smaller peripheral airways, which are <2 mm in diameter (Berge et al., 2011). These smaller airways play a significant role in pathogenesis of the disease and subsequently during any therapeutic intervention (Burgel, 2011). Progression of disease leads to deterioration of pulmonary function and the risk of alveolar hypoxia increases causing vasoconstriction in the pulmonary circulation and a transient increase in pulmonary vascular resistance (PVR) (Elwing and Panos, 2008; Rabe et al., 2007). These changes are observed even in mild COPD (Santos et al., 2002). Inhaled short-acting ß2-agonists such as salbutamol sulfate and others are suggested for the acute relief of airway obstruction and exercise associated bronchospasm. These drugs are generally inhaled via nebulization or metered dose inhaler or dry powder inhalation. However, being micronized particles, a lot of drug is wasted in each of these processes because the preferred site of drug deposition is pharynx and stomach, followed by trachea-bronchial system, and not alveoli. This results in suboptimal delivery to the lungs thereby compromising the therapeutic outcome (Labiris and Dolovich, 2003; O'Callaghan and Barry, 1997).

Deposition of aerosolized drugs inside lungs depends upon many factors such as the size of the aerosol particles, breathing conditions, the geometry of airways, and the mucociliary clearance mechanisms (Tena and Clara, 2012). It is well recognized now that inhaled therapeutic agents should be able to reach up to the lower airways to obtain maximum therapeutic effect. This is possible by small sized aerosol particle, preferably submicron sized, which tend to travel farther and settle in the deeper compartments of lungs (Capstick and Clifton, 2012; Wedaa et al., 2004). Thus in the management of respiratory diseases like COPD, one of the recent strategies has been reduction of drug particles size to enhance penetrability so that the drug could be targeted to the desired area in pharmacological dose (Wedaa et al., 2004). This approach has been successfully used in our laboratory for the development of various novel inhalational formulations for different clinical indications (Ali et al., 2013, 2009; Bhavna et al., 2009; Kumar et al., 2011; Sultana et al., 2014, 2011).

Various preclinical studies have demonstrated the advantage of short or long-term nitric oxide (NO) inhalation in pulmonary hypertension and COPD models (Jiang et al., 2004, 2002; Yoshida et al., 1997; Katayama et al., 1994). Inhalation of NO mediated formulations such as L-arginine, sodium nitrite and others have been shown to have bronchodilator action (Dupuy et al., 1992; Högman et al., 1993; Kacmarek et al., 1996; Sapienza et al., 1998; Chambers and Ayres, 2001; Grasemann et al., 2013). Keeping these effects of NO donors, including L-arginine in mind, present study was designed so as to explore the advantage of nanosizing along with known bronchodilator effect of L-arginine for the treatment of patients of COPD, with particular reference to improvement in their cardiopulmonary parameters.

We here report the development, in vitro and in vivo characterization of a novel submicronic respiratory formulation of L-arginine hydrochloride. Aim was to prepare submicron sized arginine particles in the range of 400–500 nm and to evaluate the effect of short-term treatment on various cardiopulmonary parameters (spirometry, echocardiography, pulse oxymetry, and 6-min walk test) in COPD patients. Unit dose for nebulization was kept at 1.25% on the basis of preliminary proof-of-concept studies in human volunteers and previously reported work on L-arginine (Grasemann et al., 2013; Sapienza et al., 1998). Another highlight is the use of gamma scintigraphy to generate real time in vivo data with respect to total and regional drug deposition pattern in lungs, a technique which has previously been used in developing various other drug formulations from our laboratory (Ali et al., 2009; Bhavna et al., 2009; Rajpal et al., 2009, 2010).

2. Materials and methods

L-arginine hydrochloride and stannous chloride dihydrate ($SnCl_2{\cdot}H2O$) were procured from Sigma–Aldrich Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade purchased from Merck India Ltd. (Mumbai, India). Technetium-99m (Tc-99m) was supplied by BRIT, BARC (India).

2.1. Preparation of test formulation

Three milliliter solution of 1.25% L-arginine in normal saline or in different concentrations of ethanol–saline (10–50%) was taken as the potential test formulation. Ethanol was added to produce aerosol particles and to increase the rate of drug output (Sultana et al., 2011). In vitro nebulization rate and nebulization fraction of these prospective test formulations were determined by a standardized and previously reported method from our laboratory (Mittal et al., 2010). A laser particle size analyzer (Lasair II, Particle size measuring systems Inc, USA) was used to estimate the Mass Median Aerosol Diameter (MMAD) with respect to, (a) 1.25% L-arginine in saline, (b) preparations containing different concentrations of ethanol, and (c) chosen test formulation aerosols passed through a large-volume spacer. For all further experiments, the chosen test formulation was dispensed in sealed sterile vials after passing through 0.22 μm filter.