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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.

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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.

2.2. Preclinical toxicological study

Though arginine has been given through inhalation route earlier, no arginine respiratory formulation is available in combination with ethanol. Keeping this in mind, an acute animal toxicity study was performed for assessing the local effect of arginine respiratory formulation on lungs. The study was carried out in two animal species as per Schedule 'Y' guidelines of the Indian Council for Medical Research (ICMR). The animal experiments were approved by Institute's Animal Ethical Committee (INM/IAEC/2007/IX). Sprague Dawley rats (2–3 months; 200–250 g) and New Zealand white rabbits (1.5–2 years; 2–2.25 kg) from Institute's Experimental Animal Facility were used for the study. The animals were given free access to standard laboratory animal feed (Golden Feed Laboratory, Delhi, India) and water *ad libitum*. As per the experimental guidelines, three incremental doses (1.25%, 2.5% and 5%) of the test formulation, including the intended therapeutic dose were inhaled to different groups of animals ($n = 6$) using an animal inhalation chamber. Animals were nebulized twice in a day for seven weeks and monitored daily with respect to general health, food intake, water consumption, and for any morphological changes in cardio-respiratory system (short/fast breathing, restlessness, sluggishness). At the end of the study, animals were sacrificed and any histopathological changes in lungs and other vital organs of test animals were compared with those of controls.

2.3. In vitro estimation of respiratory fraction

In vitro estimation of respiratory fraction was carried out using Anderson Cascade Impactor (ACI) (Copley Scientific, Nottingham, UK), which consisted of an initiation port (IP), pre-separator (PS), seven stages and a final collection filter. Based on the results of formulation optimization study mentioned above, 3 mL of selected test arginine formulation was nebulized by jet nebulizer (American Bantex Corp., Alphaneb Plus nebulizer, NJ, USA) through a spacer that was in turn connected to initiation port of the ACI. Nebulization was performed for 10 min and the wash solutions from initiation port, pre-separator deposit, and various stages of ACI were collected and quantified for drug content by high-performance liquid chromatography (Quaternary HPLC system, Shimadzu, Japan). Respirable fraction was calculated as the ratio of percentage of total drug deposited in the lower stages of the ACI (stage 1 to filter) to total theoretical dose, describing the percentage of aerosolized drug deposited deeply into the lungs.

2.4. Clinical study

The study protocol was approved by the Institutional Human Ethics Committee, duly constituted for the purpose (INM/TS/IEC/013/07). The clinical study was performed in the following sequential manner: (a) safety study and evaluation of pulmonary drug deposition pattern of the developed formulation using gamma scintigraphy in healthy volunteers ($n = 6$; mean age 26.6 ± 4.7 years), (b) limited efficacy study in 15 chronic obstructive airway disease patients ($n = 15$; mean age 51.5 ± 8.2 years) followed by gamma scintigraphy study as mentioned above. The patients belonged to the outpatient department of Rajanbabu Institute of Pulmonary Medicine and Tuberculosis, Delhi with documented diagnosis of COPD. Patients having prior hypersensitivity to arginine or critically sick patients or those having mixed disease pattern, or those likely to develop severe grade of dyspnea, children and pregnant females were excluded from the study. All participants gave their written informed consent for participation in the study.

2.4.1. Gamma scintigraphic study

For in vivo pulmonary evaluation to ascertain total and regional lung deposition of nebulized arginine by gamma scintigraphic method, the drug was radiolabeled with Tc-99m using stannous based reduction protocol reported previously (Gulati et al., 2005). Radiolabeling efficiency and stability of Tc-99m radiolabeled arginine (99mTc-arginine) were determined by standard procedures involving instant thin layer chromatography (ITLC) and a gamma counter (Capintec, USA) (Sultana et al., 2011; Singh et al., 2007). Nebulization rate of un-labeled and Tc-99m radiolabeled arginine (analyzed by HPLC and radiometry respectively) was correlated to establish bio-equivalence between the two methods.

Gamma scintigraphy study was carried out using a dual head gamma camera system (Symbia T2, Siemens, Erlangen, Germany). Based on the results of formulation optimization study mentioned in previous section, 3 mL of selected test arginine formulation containing tracer quantity of 99mTc-arginine was nebulized through a spacer to the volunteers. Following inhalation, two dimensional scintigraphy images of the chest region were acquired within 5 min of inhalation covering oropharynx to stomach. All images were recorded on a computer system assisted with the software (Syngo, Siemens, Erlangen, Germany). Additional images of the chest region were taken at 30 min and 60 min to observe the movement of the drug. Regions of interest were drawn around the oropharynx, esophagus, stomach and whole lung for obtaining count statics. The counts within these regions were corrected for background radioactivity, radioactive decay and tissue attenuation of gamma rays (Pitcairn and Newman, 1997). The lung region was further sub-divided into central, intermediate and peripheral sections, representing mainly the respiratory tree, mixed and alveolar region respectively (Newman et al., 1989). Lung images were also visually compared to observe movement of the deposited radiolabeled drug particles with time from one compartment to another.

2.4.2. Limited efficacy study in COPD patients

The study was carried out in 15 COPD patients. On the first visit their baseline parameters were ascertained, followed by nebulization of 3 mL of submicronic arginine respiratory formulation thrice a day for next four days. Spirometry (Portable Mir Spirometer; Spiro Lab III, Italy), pulse-oxymetry (Planet-40, Larsen and Toubro Limited, India), echocardiography (VIVID-7 model, GE Healthcare System, Germany) and 6-min walk test were performed on patients before and at the end of the study to assess the effect on their cardiopulmonary status.

2.5. Statistical analysis

Data were presented as mean ± S.D. Paired student's *t*-test was applied for the calculation of significance at $p < 0.05$ using GraphPad Instat version 3.00 for Windows XP, GraphPad Software, San Diego, CA.

3. Results and discussions

The present study describes optimization of a novel submicronic 1.25% arginine hydrochloride respiratory formulation; its in vitro and in vivo characterization; gamma scintigraphy based evaluation of drug's pulmonary deposition pattern; and clinical study to evaluate its effect on various cardiopulmonary parameters in COPD patients.

3.1. Optimization of test formulation

Table 1 depicts the correlation between nebulization patterns of ^{99m}Tc-labeled arginine and un-labeled arginine. Results show a good correlation between their nebulization patterns, corroborating the results previously reported from our laboratory (Mittal et al., 2010) and validating radiometry as a suitable technique for estimating amount of arginine nebulized or deposited in various compartments. For this, arginine hydrochloride was firstly radiolabeled with Tc-99m using already established stannous based reduction protocol (Gulati et al., 2005). The drug was successfully radiolabeled (^{99m}Tc-arginine) with a high radiolabeling efficiency of >96%. Serum stability studies showed 93% and 90% radiolabeling efficiency of arginine after 6 and 24 h respectively, indicating the stability of labeled drug.

Nebulization rates and nebulized fraction of potential arginine formulations are shown in Table 2. Results show that nebulization rate as well as the nebulized fraction for arginine respiratory fluid was maximum when 30% ethanol–saline was used in the formulation for nebulization. Particle size analysis done through laser particle size analyzer (Lasair II, Particle size measuring systems Inc, USA) revealed the following MMAD: (a) control formulation (1.25% arginine in normal saline): 7.9 ± 0.5 μm, (b) different arginine-ethanolic saline preparations containing 10%, 20%, 30%, 40% or 50% ethanol: 4.8 ± 0.6 μm, 3.6 ± 0.5 μm, 1.6 ± 0.3 μm, 1.5 ± 0.2 μm and 1.1 ± 0.2 μm respectively, and (c) chosen test formulation (1.25% arginine in 30% ethanol–saline) passing through spacer: 0.44 ± 0.1 μm.

Table 1 Bio-equivalence data between arginine and ^{99m}Tc-arginine nebulization rates at different intervals (n = 6).

Time	^{99m}Tc-arginine in nebulization chamber (%)	Arginine in nebulization chamber (%)
0 min	100	100
5 min	78.4 ± 4	79.5 ± 3.3
10 min	72.6 ± 2.8	71.8 ± 3.0
20 min (after drying)	65.8 ± 2.6	65.1 ± 2.5

Correlation value (r) = 0.902.

On the basis of the results obtained, which corroborated with previous reports of use of 30% ethanol–saline in respiratory fluids (Sultana et al., 2011), 1.25% arginine in 30% ethanol–saline to be nebulized through a spacer was chosen as the final formulation.

Large spacers attached in series to any standard nebulizer assembly are known to impart a few advantages over conventional nebulization, like generation of smaller sized particles for inhalation and less wastage of drug aerosols to surrounding environment (Mittal et al., 2010). It is well accepted that reduction in drug aerosol size leads to deeper penetration of the medication into the lungs (Finlay et al., 1997; Hadinoto et al., 2007; Hoet et al., 2004). However, submicronic aerosols tend to be exhaled back, resulting in sub-optimal use of nano-inhalation therapy in clinical settings. This problem was circumvented by using ethanol, which has been used earlier for producing vaporized drug particles for inhalation and retaining them within the lung microvasculature (Sultana et al., 2011). Gamma scintigraphy done with ^{99m}Tc radiolabeled arginine-ethanol saline formulation showed significant delivery and deposition of the submicronic drug in alveolar spaces. Clinical results of this study support the in vitro and scintigraphy observations.

3.2. Preclinical toxicity study

Experimental animals exposed to inhalation of arginine respiratory fluid in three incremental doses (1.25%, 2.5% and 5%) did not exhibit any biochemical or behavioral changes. Histopathology of vital organs revealed no gross changes with respect to their weight or general morphology. Fig. 1 shows microphotographs of the lungs in control and test animals exposed to highest arginine concentration (5%). No local pulmonary toxicity was seen except for some congested microcirculation within the lung microvasculature.

3.3. In vitro estimation of respiratory fraction

Fig. 2 represents the ACI results (% drug deposition pattern) of novel submicronic 1.25% arginine in 30% ethanol–saline respiratory formulation. The formulation had a high respirable fraction of 51.61 ± 3.28. The respirable fraction was calculated as the ratio of total drug deposited in lower stages of the ACI (stage 1 to filter) to total theoretical dose, and it is a

Table 2 Effect of varying concentration of ethanol–saline on nebulization rate/min and nebulized fraction using ^{99m}Tc-arginine (n = 6).

	Ethyl alcohol–saline (%)					
	0	10	20	30	40	50
Nebulization rate/min						
1st min	4.8 ± 0.8%	5.0 ± 0.6%	6.2 ± 0.8%	8.8 ± 1.0%	8.0 ± 0.8%	6.4 ± 0.5%
10th min	4.3 ± 0.5%	4.2 ± 0.4%	5.4 ± 0.6%	8.2 ± 0.6%	7.5 ± 0.6%	6.1 ± 0.5%
15th min	4.1 ± 0.5%	4.1 ± 0.2%	Dry	Dry	Dry	Dry
Nebulized fraction (%)	29.6 ± 3.5	37.2 ± 4.4	55.3 ± 6.5	68.6 ± 5.8	68.3 ± 6.5	69.3 ± 7.5

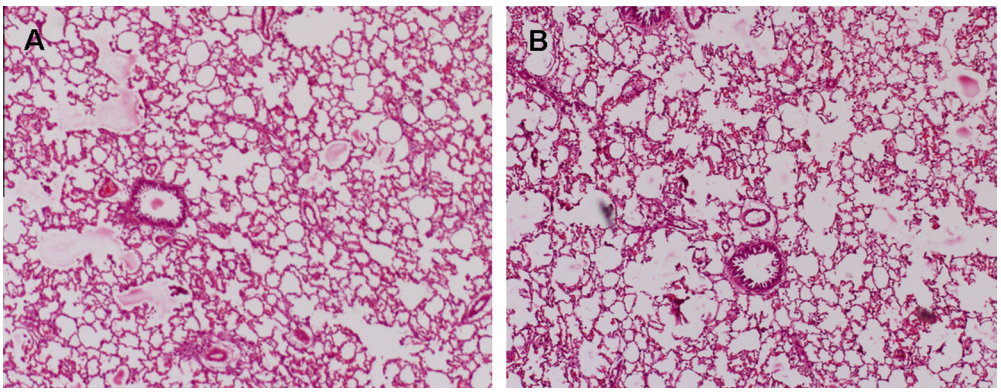

Figure 1 Comparison of lung micrograph of (A) control and (B) treatment group rabbit nebulized with submicronic 5% arginine in 30% ethanol–saline (four times the intended human pharmacological dose) twice a day for 7 weeks showing no apparent hemorrhage or edema in the tissue in both cases. (H.E. × 20 magnification).

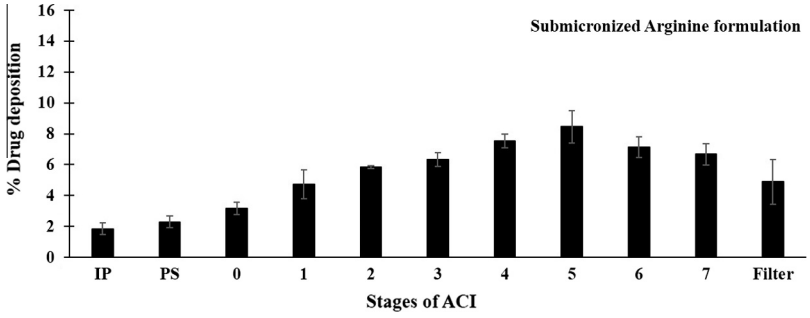

Figure 2 ACI study comparison for percentage deposition in each stage for submicronic arginine respiratory fluid (nebulization time = 10 min; $n = 6$).

measure of the amount of drug likely to be delivered to the lungs (Mittal et al., 2014).

3.4. Clinical study

3.4.1. Gamma scintigraphic study

Table 3 shows quantitative data using gamma scintigraphy with respect to relative percentage of the inhaled drug (a) deposited in different lung compartments of a human volunteer, (b) amount retained in oropharynx, and (c) amount remaining in nebulizer assembly. Although nearly 78% of the drug was retained in nebulizer assembly at the end of nebulization, two-third of the inhaled portion reached the lungs, resulting in a high respirable fraction for the nebulized drug. Whole lung deposition was in the range of 15 ± 2%, while only 7 ± 2% of the nebulized drug was deposited in oropharynx compartment, representing oropharynx, esophagus and stomach. It is much less in comparison with that reported for micronized drugs, where nearly two-third of the drug is deposited in the oral region and is not available to the lungs (Bhavna et al., 2009). The ratio of peripheral to central lung zone deposition for submicronic arginine respiratory fluid was 0.8.

Visual examination of the lung scintigraphy scans showed an appreciably deep lung penetration of the submicronic drug, with majority of the drug being deposited in central and peripheral regions and less accumulation in oropharynx, trachea, esophagus and stomach at 5, 30 and 60 min intervals (Fig. 3A). In case of COPD patients, similar observations were made although their lung uptake was obviously much less

Table 3 In vivo gamma scintigraphic evaluation of total and regional pulmonary deposition of submicronic L-arginine aerosols after inhalation of ^{99m}Tc-arginine respiratory fluid.

Variables	Drug deposition (% radioactivity)
No. of subjects	6
Nebulizer cup	48 ± 4
Spacer, mouthpiece and T-piece	11 ± 1
Exhalation filter	19 ± 1
Emitted dose	22 ± 2
Oropharynx, esophagus and stomach	7 ± 2
Whole lung	15 ± 2
a) Central	a) 5 ± 0.6
b) Intermediate	b) 6 ± 0.8
c) Peripheral	c) 4 ± 0.5
P/*C* ratio	0.8

Data are presented as mean ± S.D.
Whole-lung deposition is the sum of central, intermediate, and peripheral values.

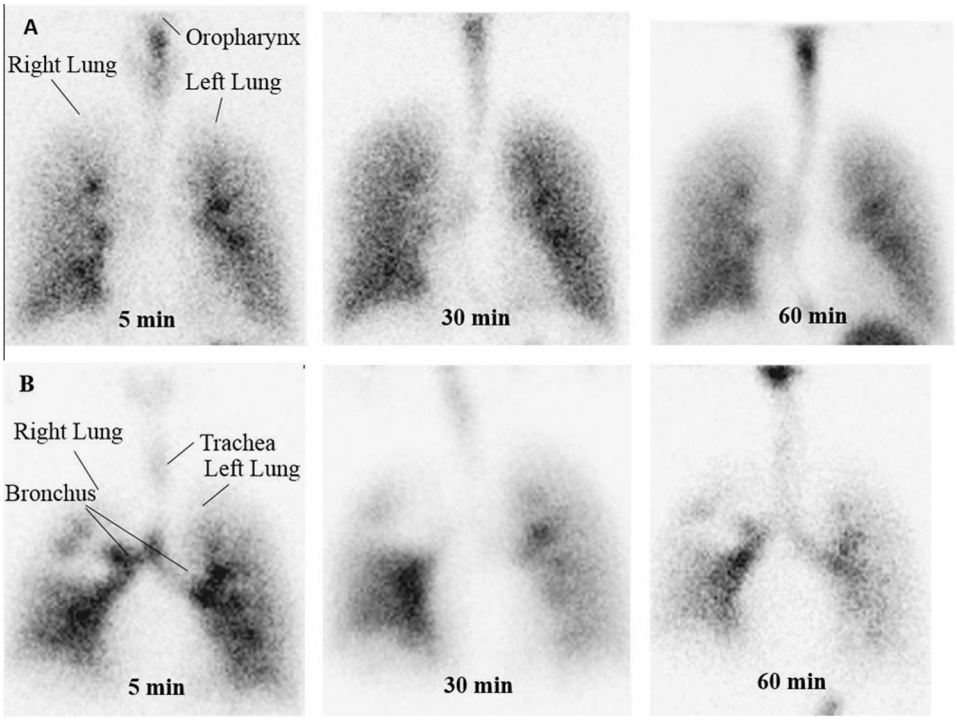

Figure 3 Gamma scintigraphy showing in vivo pulmonary deposition pattern of submicronic 1.25% arginine respiratory formulation in (A) healthy volunteer, (B) COPD patient, showing distribution of the drug at 5, 30 and 60 min after inhalation.

than healthy volunteers due to their diseased condition (Fig. 3B).

3.4.2. Limited efficacy study in COPD patients

It may be reasoned that elevated lung deposition, associated with more absorption through alveoli may cause higher systemic effects and therefore an increased risk/benefit ratio. Therefore, serial measurements of respiratory functions, heart rate, SPO2, mPAP and 6-min walk test were used to assess the immediate effects of submicronic arginine inhalation in healthy/diseased subjects. No significant change in physiological parameters was seen in healthy volunteers after inhalation of the test formulation, confirming its safety.

Any beneficial effect after inhalation of submicronic arginine respiratory formulation with respect to their cardiopulmonary status was studied in 15 COPD patients. The results are depicted in Fig. 4. There was a significant change in SPO_2 levels and heart rate ($p < 0.05$) before and at the end of 4-day treatment period. There was also a significant improvement in subjects' mean pulmonary artery pressure (mPAP) before (27.91 ± 2.75 mmHg) and after (25.7 ± 4.78 mmHg) the treatment. Spirometry parameters such as FEV_1 (Forced expiratory volume in 1 s) and FVC (Forced vital capacity) also improved in all the subjects at the end of 4-day study period (48.3 ± 10.88% and 63.53 ± 9.13% of predicted respectively) as compared to at the beginning (45.2 ± 10.98% and 60.13 ± 6.22% of predicted respectively). In 6-min walk test the average distance covered by patients at the start of study was 395 ± 53.88 m. Post-treatment, the patients were able to walk an average distance of 437.7 ± 66.28 m. Thus, all the subjects were able to show a marked improvement in their cardiopulmonary status at the end of 4-day study period. Furthermore, no side effects or any kind of discomfort was reported by the volunteers and in general the patient acceptability was quite good. Subjective relief was reported by nearly 90% of the 15 COPD patients. Arginine is a precursor of NO and inhalation of NO has been shown to reduce PAP, besides improving exercise tolerance and oxygenation (Perez-Penate et al., 2001; Vonbank et al., 2003; Yung et al., 2001). These reports validate the findings of our study, which strongly suggest advantage of submicronic arginine nebulization in improving cardiopulmonary complications in COPD and/or other respiratory condition.

Gamma scintigraphy has been widely used to assess pulmonary deposition of inhaled drugs (Ali et al., 2013, 2009; Bhavna et al., 2009; Kumar et al., 2011; Sultana et al., 2014). Our results suggest a high deposition of submicronized arginine particles in various regions of lung, a pattern which was observed till the period for which the scintigraphy study was conducted. Moreover, a lower oropharyngeal deposition seen with this formulation may also negate the disadvantage of systemic side-effects like tachycardia and tendency of esophagitis associated with presently available options like micronized salbutamol inhalation. The results suggest submicron sized arginine formulation can ensure a high local availability of the drug in the lungs. The probable reason for this could be that submicron sized particles are largely spared from undergoing phagocytosis by macrophages stationed in the respiratory tract, while the larger micronized particles readily undergo phagocytosis (Edwards et al., 1998; Fels and Cohn, 1986; Oberdorster et al., 2005). Since number of macrophages is too high in case of airway diseases such as COPD and asthma, this may cause suboptimal pharmacological action of micronized drug particles in comparison with submicron

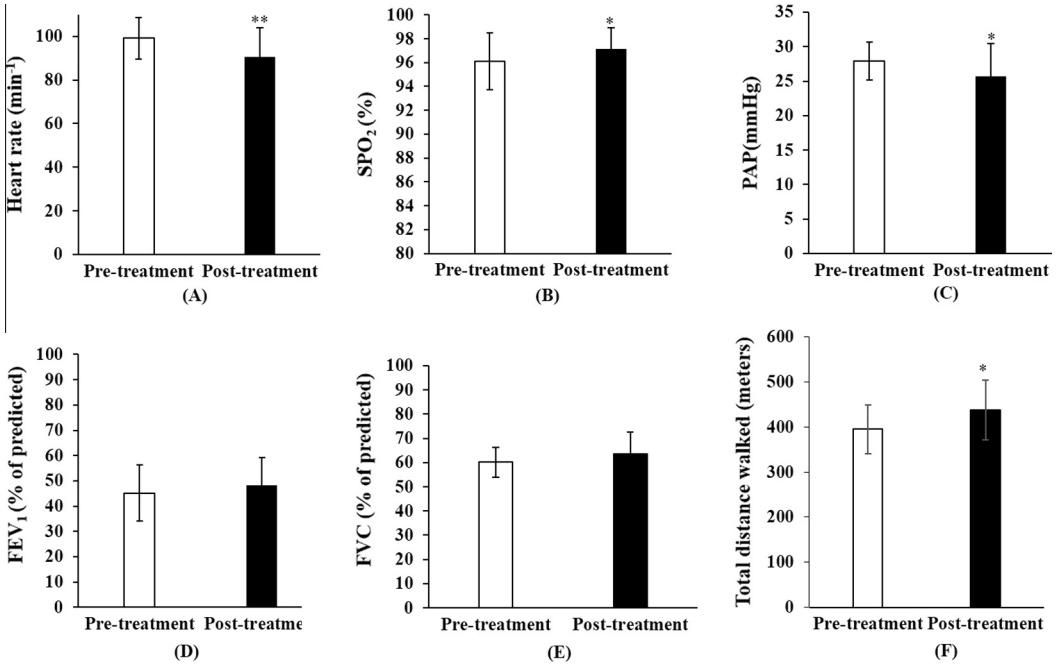

Figure 4 Effect of submicronic L-arginine inhalation on cardiopulmonary status of COPD patients [FVC-Forced vital capacity, FEV_1-Forced expiratory volume in 1st second, mPAP-mean pulmonary artery pressure; data presented as mean ± S.D.; * indicates significant difference ($p < 0.05$) between values measured before and after treatment in patients with cardiopulmonary complications].

sized particles. Although micronized drug particles may get released from the macrophages eventually, but their concentration at that time would be suboptimal for any clinical benefits. Another advantage of submicronic formulation is that smaller particles deposit more in peripheral lungs and from there move toward the center by mucociliary action resulting in sustained action of the drug. Respiratory diseases such as COPD and asthma are characterized by airway inflammation and increased mucus production, which leads to further narrowing of the airways with low drug delivery and less bioavailability in lungs. Smaller particles are more likely to pass through these constricted airways in comparison with larger particles resulting in their greater bioavailability.

4. Conclusion

Results of the present study suggest that submicronic 1.25% arginine in 30% ethanol–saline respiratory formulation may be developed into a potential therapeutic option in treating bronchopulmonary diseases such as COPD. Further clinical studies with larger sample size are however required to confirm the efficacy of the test formulation.

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