Submicronic Salbutamol Respiratory Fluid: A Novel Formulation for Treating Broncho Contrictive Diseases

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ABSTRACT

Context: Inhalation of drugs like salbutamol sulphate (SBS) suffers from low pulmonary deposition due to its micronized size. Objective: (a) to develop submicronic-SBS respiratory fluid, (b) pre-clinical toxicity study, (c) in-vitro and in-vivo evaluation in terms of respiratory fraction, and (d) clinical study to assess safety and efficacy. Methods: Formulation was optimized on basis of particle size and in-vitro nebulization rate. Anderson cascade impaction (ACI) was done to validate its advantage in terms of respirable fraction. Effect on cardiopulmonary parameters (spirometry, pulse-oxymetry, echocardiography and 6-minute walk test) was evaluated and compared with control (n=12). In-vivo pulmonary deposition pattern was compared using gamma scintigraphy. Results: Formulation was optimized with average particle size of 410nm. SBS was radiolabeled with Tc-99m and ^{99m}Tc-SBS was found suitable for estimating in-vivo SBS deposition. Preclinical toxicity studies in two-animal species showed no biochemical or behavioural toxicity. In-vitro ACI data showed high respirable fraction (81.7±7.1%) in comparison to control (42.5±3.8%). In-vivo scintigraphy suggested significantly higher pulmonary deposition for test formulation in comparison to control. Better improvement in cardiopulmonary parameters was seen in test treated group. Conclusions: As demonstrated for SBS, nano-sizing may enhance regional pulmonary deposition and provide an attractive therapeutic option for bronchopulmonary disorders like COPD.

Keywords: Chronic obstructive pulmonary disease; submicronic salbutamol sulphate; nebulization; gamma scintigraphy

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are common respiratory disorders. It is reported that approximately 300 million people worldwide are suffering from asthma and 10% of the population above 40 years may have COPD.[1] Airway obstruction is a characteristic feature of both diseases and inflammatory process affects the respiratory tract up to peripheral airways which are less than 2mm diameter.[2] These constricted airways play an important role in pathogenesis of various respiratory diseases and also in efficacy of applied inhaled therapies.[3] Inhaled short-acting ß2-agonists are suggested for the acute relief of airway obstruction and exercise associated bronchospasm. Asthma and COPD have a key feature of reversible airway obstruction and the short-acting ß2-agonists are usually used for the management of respiratory symptoms associated with these disorders. Salbutamol sulphate (SBS) is the most widely used short-acting ß2 -agonist that causes smooth muscle relaxation.[4, 5] It is prescribed for bronchial asthma, bronchitis, bronchiolitis, COPD and other respiratory diseases. SBS is administered orally or through inhalation, though the inhalation route is preferred because of its local action, early therapeutic effect and for reducing systemic side effects. Inhalation of SBS aerosols is done by any of the 3 methods available: (a) inhalation of wet aerosols produced by a combination of electric air-jet generator and nebulization chamber, (b) metered dose inhaler (MDI), and (c) dry powder inhalation (DPI).[6] For wet aerosol method, SBS respiratory fluid is available as 0.25 % (2.5 mg/mL) solution in normal saline. It is used as such, or after dilution in saline. However, a lot of drug is wasted during the nebulization process and never reaches the lungs.[7] It is reported that on an average only 10% of the dose placed in nebulizer is delivered to lungs.[8] We have found that if the above formulation is made in 30% ethyl alcohol in place of water, and when a large spacer is attached in series to the nebulizer assembly, several advantages over the conventional method are imparted, particularly in treatment of hypoxemic hypoxia, pulmonary hypertension, asthma, and COPD. 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[9]. 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 and correct inhalational technique.[1] Small sized particles tend to travel farther and settle in the deeper compartments of lungs.[10] Thus in the management of asthma and COPD, one of the recent strategies has been the reduction of drug particles size to enhance penetrability, particularly in the presence of inflammatory, obstructive or constrictive factors so that the drug could be

targeted to the desired area in pharmacological dose.[11,12] This approach has been successfully used in our laboratory for the development of various novel inhalational formulations for different clinical indications.[13-17] In the present work we report the development and characterization of a submicronic respiratory formulation of SBS. Aim was to prepare submicronized SBS particles in the range of 400-500 nm followed by clinical evaluation of the developed formulation with respect to its safety and pulmonary deposition pattern in patients of obstructive airways disease using gamma scintigraphy and its comparison with commercially available micronized SBS formulation. Significance of gamma scintigraphy technique in evaluation of drug deposition pattern of various drug formulations has already been established by researchers from our laboratory and elsewhere.[4,13,18,19]

MATERIALS AND METHODS

Salbutamol sulphate (SBS) was obtained from Cipla Ltd. (Mumbai, India). Stannous chloride dihydrate ($SnCl_2.H_2O$) was procured from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. Technetium-99m (Tc-99m) was supplied by BRIT, BARC (India). All other chemicals were purchased from Merck (India) and were of analytical grade.

Making test formulation

Three millilitre solution of 0.25% SBS in normal saline or in different concentrations of ethanol-saline (10-50%) or tween-80 (0.5-20%) were taken as the potential test formulation. In vitro nebulization rate and nebulization fraction of potential test formulations was determined by a method previously reported from our laboratory.[20] 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) 0.25% SBS 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 micron filter.

Preclinical toxicity study

Though SBS is an approved drug via inhalation route, no inhalation formulation of SBS is available in combination with ethanol. Therefore, an acute animal toxicity study was performed to evaluate the local effect of the 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 duly constituted for the purpose (INM/IAEC/2009/IX). Male albino rats (2–3 months; 200–250 g) and New Zealand

white rabbits (1.5–2 years; 2–2.25 kg) were selected for the study. As per the guidelines, three incremental doses (0.25, 0.5 and 1%) of the test formulation, including the intended therapeutic dose were inhaled to different group of animals (n=6) using an animal inhalation chamber. Nebulization was carried out twice a day for 7 weeks. The animals were observed daily with respect to general health, food and 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.

In vitro estimation of respiratory fraction

Respiratory fraction of the developed formulation was measured using Anderson Cascade Impactor (ACI) (Copley Scientific, Notttingham, UK). Three millilitres of test SBS formulation (0.25% SBS in 30% ethanol-saline) was nebulized by jet nebulizer (American Bantex Corp., Alphaneb Plus nebulizer, NJ, USA) through a spacer that was in turn connected to mouth piece (initiation port) of the ACI. Nebulization was done for 10 min and the wash solutions from initiation port, pre-separator deposit, and various impactor stages of ACI were collected and quantified for drug content by high-performance liquid chromatography (Quarternary HPLC system, Shimadzu, Japan) by the method reported by Rotta et al.[21] The amount deposited at the various stages was expressed as percentage SBS per nebulization. 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 in to the lungs.

Clinical study

The study protocol was approved by the Institutional Human Ethics Committee, duly constituted for the purpose (INM/TS/IEC/015/07). Written informed consent was taken from all participants. The clinical study was done in (a) healthy volunteers (n=6; mean age 27.7±2.9 years); as part of the safety study and to evaluate and compare the pulmonary drug deposition pattern of the developed formulation vis-à-vis commercially available SBS respiratory fluid using gamma scintigraphy, (b) patients with obstructive airway disease (COPD; n=6; mean age 41.8±8.2 years); as part of limited efficacy study. The patients belonged to the outpatient department of Rajanbabu Institute of Pulmonary Medicine and Tuberculosis, Delhi with documented diagnosis of asthma or COPD. Critically sick patients or those having mixed disease pattern, or those likely to develop severe grade of dysnea, children and pregnant females were excluded from the study.

Gamma scintigraphy based study

Radiolabeling of SBS with Tc-99m

For in vivo evaluation of total and regional lung deposition of nebulized SBS by gamma scintigraphic method, SBS was radio labeled with Tc-99m using stannous based reduction protocol reported previously.[22] Radiolabeling efficiency and stability of Tc-99m radiolabeled SBS (^{99m}Tc-SBS) was evaluated by means of standard procedures involving instant thin layer chromatography (ITLC) and a gamma counter (Capintec, USA).[20,23] Nebulization rate of SBS and ^{99m}Tc-SBS (analyzed by HPLC and radiometry) was correlated to establish bio-equivalence between the two methods. Gamma scintigraphy study was carried out in twelve subjects (six healthy volunteers and COPD patients each), using a dual head gamma camera system (Symbia T2, Siemens, Erlangen, Germany). Three millilitres of test SBS formulation (0.25% SBS in 30% ethanol-saline) containing tracer quantity of ^{99m}Tc-SBS was nebulized through a spacer to the subjects. 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. For comparison three millilitres of commercially available 0.25% SBS respiratory fluid along with tracer quantity of ^{99m}Tc-SBS was inhaled to other group of subjects and the scintigraphy procedure was repeated as described above. Regions of interest were drawn around the oropharynx, esophagus, stomach and whole lung. The counts within these regions were corrected for background radioactivity, radioactive decay and tissue attenuation of gamma rays.[24] The lung region was further sub-divided into central, intermediate and peripheral sections, representing mainly the respiratory tree, mixed and alveolar region respectively.[25] Lung images were also visually compared to observe movement of the deposited radiolabeled drug particles with time from one compartment to another.

Effect on cardiopulmonary status in patients

The study was carried out in 12 patients with chronic obstructive pulmonary disorder. Three millilitres of test SBS formulation/ commercially available micronized SBS respiratory formulation was nebulized to six patients each twice a day for 3 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-minute walk test were performed on patients before and after inhalation of test/ control SBS formulation to assess and compare the effect on their cardiopulmonary status. Spirometry and pulse-oxymetry data was collected for different time intervals (30, 60 and 120 min). Echocardiography and 6-minute walk

test were performed at 60 min post-inhalation.

Statistical analysis

Data are expressed as mean ± S.D. Unpaired 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.

RESULTS AND DISCUSSION

The present study describes optimization of 0.25% SBS in 30% ethanol-saline respiratory formulation; in vitro and in vivo validation and comparison of its respiratory fraction vis-à-vis commercially available SBS formulation; and clinical study to evaluate pulmonary deposition pattern and efficacy in healthy volunteers/ COPD patients.

Optimization of test formulation

A good correlation was obtained between nebulization patterns of ^{99m}Tc-SBS and SBS (Table 1), corroborating the results previously reported from our laboratory[20] and validating radiometry as a suitable technique for estimating amount of SBS nebulized or deposited. For this, SBS was firstly radio labeled with Tc-99m using already established method of using stannous based reduction protocol.[22] The drug was successfully radiolabeled (^{99m}Tc-SBS) with a high radiolabeling efficiency of >95%. Serum stability studies showed 92% and 90% radiolabeling efficiency of SBS after 6 and 24 h respectively, indicating the stability of the labeled product. Table 2 shows the nebulization rates and nebulized fraction of the prospective formulations studied using radiometry. Laser optometry revealed the following MMAD: a) control formulation (0.25% SBS in normal saline): 8.6±0.8μm, b) different SBS-ethanol preparations containing 10-50% ethanol: 5.5±0.7μm, 2.2±0.4μm, 1.2±0.3μm, 1.2±0.2μm and 0.9±0.2μm respectively, c) control formulation through spacer: 0.84±0.2μm, and d) final test formulation (0.25% SBS in 30% ethanol-saline) passing through spacer: 0.41±0.1μm. On the basis of the results obtained and considering the potential throat irritability by ethanol at higher concentrations, 0.25% SBS 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 the conventional method like easy availability of smaller sized particles for inhalation and less wastage of drug aerosols to surrounding environment.[20] It is well known that reduction in drug aerosol size leads to deeper penetration in the lungs upon inhalation.[26-28] However, submicronic aerosols tend to be exhaled back and the nano-inhalation therapy has not been clinically used to the desired extent. We have proposed the concept of 'Inhalation Therapy for Alveolar Deposition'

(ITAD), the conceptual premise behind which is that inhaled sub-micron or nano-sized drug aerosols need to be deposited and retained in lung spaces in pharmacological quantities to be of therapeutic value. For this, submicron sized drug particles are produced by adding ethanol as an excipient and attaching a large volume spacer in series with the nebulization chamber. Ethanol was added to increase the nebulized fraction by lowering surface tension; reduce the aerosol size due to increased drying; and enhance retention of drug at the site of deposition.[14,20]

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

Time	^{99m}Tc-SBS in nebulization chamber	SBS in nebulization chamber
0 min	100%	100%
5 min	80.8 ± 4%	81.9 ± 3.5%
10 min	75.2 ± 3.9%	74.3 ± 3.2%
20 min	66.3 ± 3.2%	65.8 ± 3.5%

Correlation value (r) = 0.903

Table 2: Effect of excipients on nebulization rate/min and nebulized fraction using ^{99m}Tc-SBS (n=6)

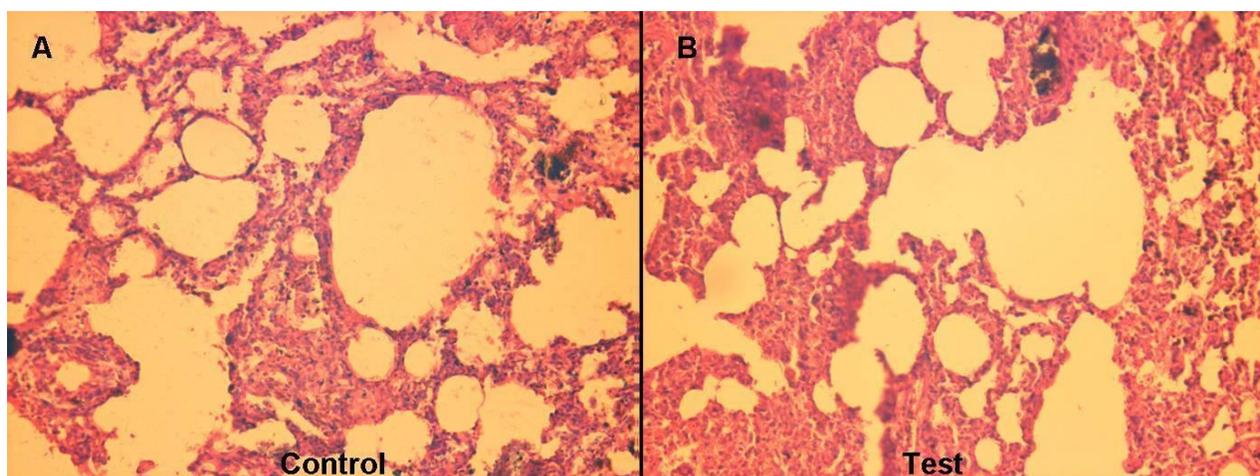| Nebulization rate/min	Ethyl alcohol-saline (%)						Tween-80 (%)		
	0	10	20	30	40	50	0.5	5	20
1st	5.2±0.6%	5.3±0.9%	5.5±0.7%	8.3±0.7%	7.8±0.8%	7.3±0.6%	6.8±0.6%	5.0±0.4%	Nil
10th	4.8±0.6%	4.8±0.5%	5.3±0.5%	8.3±0.6%	7.7±0.9%	7.0±0.6%	6.5±0.5%	4.9±0.4%	Nil
15th	4.7±0.7%	4.6±0.6%	Dry	Dry	Dry	Dry	6.6±0.5%	5.1±0.5%	2.1±0.3%
Nebulized fraction (%)	31.5±3.8	40.2±3.4	56.4±6.1	64.8±6.5	65.3±6.2	67.3±7.3	31.0±3.6	26.2±2.8	10.4±1.1

Figure 1: Comparison of lung micrograph of (A) control, and (B) treatment group rabbit nebulized with 1% submicronic SBS in 30% ethanol-saline (four times the human pharmacological dose) twice a day for 7 weeks showing no apparent hemorrhage or edema in the tissue in both cases. (H.E. x 150 magnification)

Preclinical toxicity study

No biochemical or behavioral toxicity was found in animals exposed to test SBS formulation. Figure 1 shows histopathology of the lungs in control and test animals exposed to highest SBS concentration. No local pulmonary or systemic toxicity was noted except congested microcirculation (and consequently 5-7% heavier organ weight) in lungs, spleen, liver and kidneys in animals receiving the highest dose.

In vitro **estimation of respiratory fraction**

ACI details of submicronic SBS respiratory formulation are given in Figure 2. The submicronic formulation had a high respirable fraction **(**81.7±7.1%**)** in comparison to control formulation (42.5 ± 3.8%). The respirable fraction was calculated as ratio of total drug deposited in the lower stages of the ACI (stage 1 to filter) to total theoretical dose, and it is a measure of the amount of drug likely to be delivered to the lungs.[14]
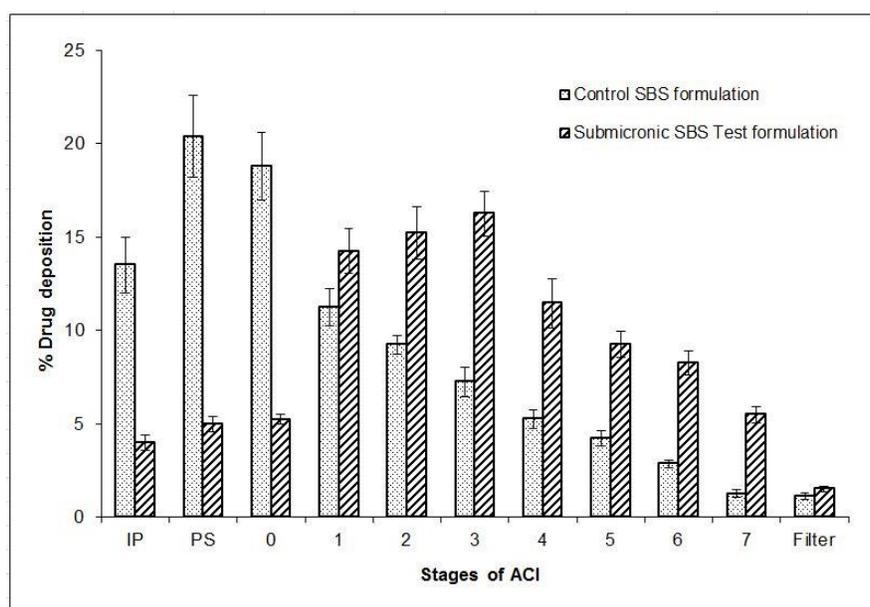

Figure 2: Andersen cascade impaction (ACI) results for control and submicronic SBS nebulization at inspiratory flow rate of 28.3L min^{-1} (n = 6)

Gamma scintigraphy based study

Relative drug deposition of commercially available micronized SBS respiratory fluid and submicronic test SBS formulation in different regions of the lung was determined using gamma scintigraphy in healthy volunteers just after inhalation and at 1 h (Table 3). Lung scintigraphy showed visually appreciable difference in in-vivo distribution pattern of the test formulation compared to conventional nebulization. A major portion of the nebulized drug (71.21±8.47%) was wasted to the gastrointestinal tract with conventional procedure compared to only 49.55±11.03%

with the test protocol immediately post-inhalation. In control subjects, there was more deposition of drug in central region i.e., oropharynx, trachea, oesophagus, medial regions of lungs and stomach at 5 min. At 30 minutes, the drug was seen to move in stomach and intestines. At 60 minutes, significant drug mobilization was noted from lungs to the gastrointestinal tract. In contrast, appreciably deeper lung penetration was evident with the test formulation which showed more drug deposition in central and peripheral regions of lungs with lesser activity in oropharynx, trachea, oesophagus and stomach at 5, 30 and 60 min intervals (Figure 3). In case of COPD patients, similar observations were made although their lung uptake was obviously much less than healthy volunteers due to their diseased condition (Figure 4). The results indicated that submicronic SBS test formulation was able to retain in lungs for a significantly longer time as compared to commercially available SBS formulation. Scintigraphy imaging has been widely used to assess pulmonary deposition of inhaled drugs.[13-17] Our results suggest a significantly higher deposition of the submicronized SBS particles in various regions of lung as compared to micronized particles, a pattern which was observed till the period for which the study was conducted. Further, a significantly lower oropharyngeal deposition was seen in case of submicronic test SBS formulation, which may probably cause less systemic side-effects like tachycardia and tendency of esophagitis as compared to presently available option. The results suggest submicron sized SBS formulation can ensure a significantly higher local availability of the drug for longer period of time as compared to micronized SBS using same dose. One of the reasons for this observation could be that micronized particles readily undergo phagocytosis by macrophages stationed in the respiratory tract while submicronic particles are largely spared.[29-31] Since number of macrophages is too high in case of airway diseases like asthma /COPD, this may cause suboptimal pharmacological action of micronized drug particles in comparison to submicronized 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 is that smaller particles deposit more in peripheral lungs and from there move towards the centre by mucociliary action resulting in sustained action of the drug. Respiratory diseases like asthma and COPD 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 to larger particles resulting in their greater bioavailability.
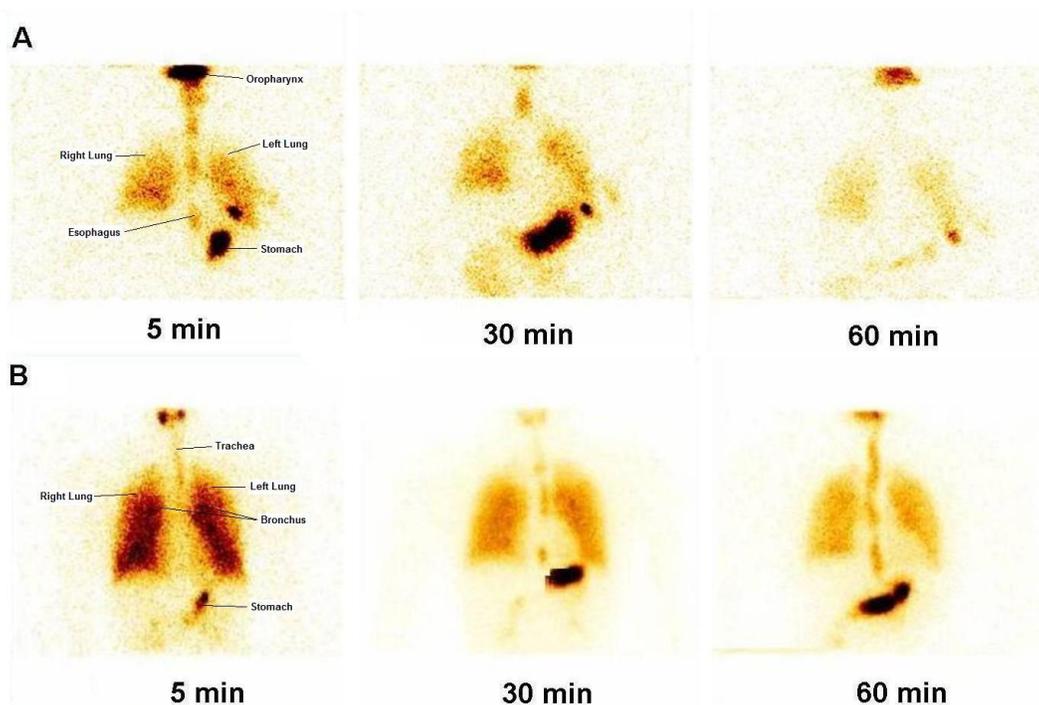
Figure 3: Gamma scintigraphy comparison of (A) control SBS respiratory formulation vs (B) submicronic 0.25% SBS respiratory formulation in two healthy volunteers showing distribution of the drug into oral cavity, tracheobronchial tree, lungs and stomach at 5, 30 and 60 min.

Table 3: *In vivo* gamma scintigraphic comparison of total and regional pulmonary deposition pattern of commercially available micronized SBS respiratory fluid and submicronic test SBS respiratory fluid (n=6)

	Control SBS formulation (% drug deposition)	Test SBS formulation (% drug deposition)	Control SBS formulation (% drug deposition)	Test SBS formulation (% drug deposition)
	5 min	5 min	1 h	1 h
Whole lung	**28.79±7.80**	**50.44±11.15****	**20.75±7.28**	**39.22±8.62****
(a) Central	8.06±2.69	16.58±3.26**	7.30±2.46	15.55±4.14**
(b)Intermediate	15.46±3.33	22.67±5.80*	8.37±3.18	15.77±3.10**
(c) Peripheral	5.27±1.78	11.19±2.09**	5.08±1.64	7.90±1.38**
Oropharynx, oesophagus and stomach	**71.21±8.47**	**49.55±11.03****	**79.24±7.12**	**60.79±7.67****

Whole-lung deposition is the sum of central, intermediate, and peripheral values.

Data are presented as mean± S.D.

*p < 0.05 between control and test 5 min/ 1 h groups

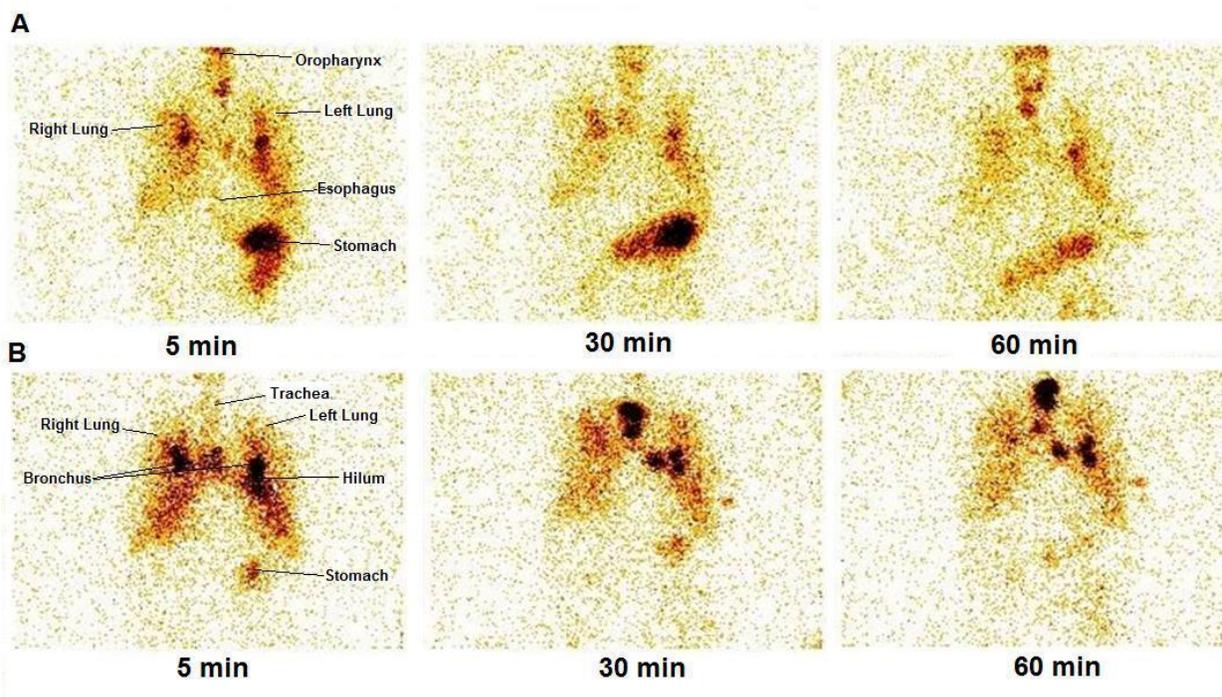**p < 0.01 between control and test 5 min/ 1 h groups

Figure 4: Gamma scintigraphy comparison of (A) control SBS respiratory formulation vs (B) submicronic 0.25% SBS respiratory formulation in two COPD patients showing distribution of the drug into oral cavity, tracheobronchial tree, lungs and stomach at 5, 30 and 60 min.

Effect on cardiopulmonary status in patients

Table 4 represents the effect on cardiopulmonary status of COPD patients after inhalation of submicronic SBS respiratory formulation in comparison to control SBS respiratory formulation. In comparison to the control formulation, inhalation of submicronic SBS resulted in much better improvement in all the subjects in terms of cardiopulmonary parameters studied at the end of study protocol. While there was a marginal increase in SPO_2 levels, a significant reduction in heart rate ($p<0.05$) was seen in patients after inhalation of the test SBS formulation. There was also an improvement in subjects' mean pulmonary artery pressure (mPAP) in the test SBS group, with mPAP of 22.08 ± 3.7mmHg. On the other hand, mPAP in subjects given conventional treatment was 24.1 ± 4.7mmHg. Spirometry parameters like FEV_1 (Forced expiratory volume in 1 sec) and FVC (Forced vital capacity) were better in subjects who were nebulized with test formulation (52.2 ± 10.5 and $70.0 \pm11.1\%$ of predicted respectively) in comparison to control group (48.1 ± 10.2 and $68.5\pm9.1\%$ of predicted respectively). In 6-minute walk test, the average distance covered by test group patients was 424.0 ± 38.2 meters, while the patients given control treatment walked 408.7 ± 37.9 meters. It is apprehended that elevated lung deposition, associated with more absorption via alveolar deposition, may cause higher systemic effects and therefore an increased

risk/benefit ratio. However serial measurements of respiratory functions, heart rate, SPO2, mPAP, 6-minute walk test were used to assess the immediate effects of submicronic SBS inhalation in healthy/ diseased subjects. There was no significant change in physiological parameters after inhalation of the test formulation in healthy volunteers, confirming its safety. In fact, all the subjects in the test group showed much better improvement in their cardiopulmonary status. Although the results were significant only in terms of reduction in heart rate, we expect that other parameters would also had shown significant improvement had the study been carried for at least one week instead of the present three day protocol. These modifications have been kept in mind for future large cohort Phase II study. These advantages and the findings of the present study suggests that 0.25% SBS in 30% ethanol-saline respiratory formulation may be developed into a potential therapeutic option in treating various respiratory disorders, including moderate to severe bronchial asthma and COPD.

Table 4: Comparison of the effect on cardiopulmonary status of COPD patients after inhalation of submicronic test SBS respiratory formulation or control SBS respiratory formulation twice a day for 3 days

	Control SBS formulation (n=6)		Submicronic test SBS formulation (n=6)	
	Pre-inhalation	Post- inhalation	Pre-inhalation	Post- inhalation
SPO$_2$	95.2 ±1.7	96.0±1.2	95.0 ±2.9	97.2± 2.1
Heart rate (beat-min^{-1})	98.5 ±6.3	93.8±5.7	98.3 ±7.1	90.2±6.4*
FEV$_1$ (% predicted)	46.5 ±11.2	48.1 ±10.2	48.7±12.8	52.2±10.5
FVC (% predicted)	67.2±10.6	68.5±9.1	66.7±10.7	70.0 ±11.1
6-minute walk test (meters)	397.5±35.9	408.7±37.9	402.5±42.9	424.0 ±38.2
mPAP (mmHg)	24.3±3. 2	24.1±4.7	24.6±6.4	22.08 ± 3.7

where, FVC: Forced vital capacity; FEV$_1$: Forced expiratory volume in 1 second; SPO$_2$: Oxygen saturation; and mPAP: Mean pulmonary artery pressure, Data are presented as mean± S.D.*$p < 0.05$ between pre-treatment and post-treatment

CONCLUSION

Our findings suggests that submicronic-SBS particles can be generated and may represent a novel method of treating bronchopulmonary diseases such as COPD by virtue of significantly higher peripheral pulmonary deposition and mucociliary movement back to tracheo-bronchial region causing higher and more sustained drug concentration in the target area.

REFERENCES**:**

1. Capstick TGD, Clifton IJ. Inhaler technique and training in people with chronic obstructive

pulmonary disease and asthma. Expert Rev Respir Med 2012; 6:91–103.

2. Berge MVD, Ten Hacken NHT, Cohen J, Douma WR, Postma DS. Small Airway Disease in Asthma and COPD Clinical Implications. Chest 2011; 139(2): 412 – 423.

3. Burgel PR. The role of small airways in obstructive airway diseases. Eur Respir Rev 2011; 119:23–33.

4. Bhavna, Ahmad FJ, Mittal G, Jain GK, Malhotra G, Khar RK, Bhatnagar A. Nano-salbutamol Dry Powder Inhalation: a new approach for treating broncho-constructive conditions. Eur J Pharm Biopharm 2009; 71:282-291.

5. Dhand R, Goode M, Reid R, Fink JB, Fahey PJ, Tobin MJ. Preferential pulmonary retention of (S)-albuterol after inhalationof racemic albuterol. Am J Respir Crit Care Med 1999; 160(4):1136–41.

6. Geller DE. Comparing clinical features of the nebulizer, metered-dose inhaler, and dry powder inhaler. Respir Care 2005; 50(10):1313–1321.

7. O'Callaghan C, Barry PW. The science of nebulized drug delivery. Thorax 1997; 52(Suppl 2): s31–s44.

8. Labiris NR, Dolovich MB. Pulmonary drug delivery. Part II: The role of inhalant delivery devices and drug formulations in therapeutic effectiveness of aerosolized medications. Br J Clin Pharmacol 2003; 56: 600–612.

9. Tena AF, Clara PC. Deposition of Inhaled Particles in the Lungs. Arch Bronconeumol 2012; 48(7): 240–246.

10. Wedaa M, Zanenb P, Boerc AHD, Barendsa DM, Frijlinkc HW. An investigation into the predictive value of cascade impactor results for side effects of inhaled Salbutamol. Int J Pharm 2004; 287(1–2): 79–87.

11. Johnson KA. Preparation of peptide and protein powders for inhalation, Adv Drug Deliv Rev 1997; 26, 29–40.

12. Schulz H. Mechanisms and factors affecting intrapulmonary particle deposition: implications for efficient inhalation therapies. Pharm Sci Technol Today 1998; 1:336–344.

13. Ali R, Jain GK, Iqbal Z, Talegaonkar S, Pandit P, Sule S, Malhotra G, Khar RK, Bhatnagar A, Ahmad FJ. Development and clinical trial of nano-atropine sulfate dry powder inhaler as a novel organophosphorous poisoning antidote. Nanomedicine: NBM 2009; 5:55-63.

14. Sultana S, Singh T, Ahmad FJ, Bhatnagar A, Mittal G. Development of nano alpha-ketoglutarate nebulization formulation and its pharmacokinetic and safety evaluation in healthy

human volunteers for cyanide poisoning. Environmental toxicology and pharmacology 2011; 31:436–442.

15. Kumar N, Soni S, Jaimini A, Ahmad FJ, Bhatnagar A, Mittal G. Edetate calcium disodium nanoparticle dry powder inhalation: A novel approach against heavy metal decorporation. Int J Pharm 2011; 416:376-383.

16. Ali R, Mittal G, Ali R, Kumar M, Khar RK, Ahmad FJ, Bhatnagar A. Development, characterisation and pharmacoscintigraphic evaluation of nano-fluticasone propionate dry powder inhalation as potential antidote against inhaled toxic gases. J Microencapsul 2013; 30:546–558.

17. Sultana S, Bhatnagar A, Rawat H, Nishad DK, Talegaonkar S, Ahmad FJ, Mittal G. Pulmonary delivery of nanosized alendronate for decorporation of inhaled heavy metals: formulation development, characterization and gamma scintigraphic evaluation. Pharm Dev Technol 2014; 19(5): 623–633.

18. Rajpal S, Mittal G, Sachdeva R, Chhillar M, Ali R, Agrawal SS, Kashyap R, Bhatnagar A. Development of atropine sulphate nasal drops and its pharmacokinetic and safety evaluation in healthy human volunteers. Environ Toxicol Pharmacol 2009; 27: 206–211.

19. Rajpal S, Ali R, Bhatnagar A, Bhandari SS, Mittal G. Clinical and bioavailability studies of sublingually administered atropine sulphate. Am J Emerg Med 2010; 28: 143–150.

20. Mittal G, Kumar N, Rawat H, Chopra MK, Bhatnagar A. A radiometric study of factors affecting drug output of jet nebulizers. Ind J Pharm Sci 2010; 72 (1): 31-38.

21. Rotta ET, Amantéa SL, Froehlich PE, Becker A. Determination of plasma salbutamol concentrations after nebulization in a pediatric emergency department. J Pediatr (Rio J) 2007; 83(5):481-484.

22. Gulati M, Singh N, Singh AK, Chopra MK, Bhatnagar A, Agrawal SS. Development and potentials of Tc-99m salbutamol (Saltec). Ind J Nucl Med 2005; 20: 72-76.

23. Singh AK, Verma J, Bhatnagar A, Sen S, Bose M. Tc-99m Isoniazid: A specific agent for diagnosis of tuberculosis. World J Nucl Med 2003; 2:292-305.

24. Pitcairn GR, Newman SP. Tissue attenuation corrections in gamma scintigraphy. J Aerosol Med 1997; 10:187-198.

25. Newman SP, Clark AR, Talalee N, Clarke SW. Pressurized aerosol deposition in the human lung with and without an open spacer. Thorax 1989; 44: 706–710.

26. Hadinoto K, Zhu K, Reginald B. Drug release study of large hollow nano particulate aggregates carrier particles for pulmonary delivery. Int J Pharm 2007; 341: 195–206.

27. Hoet PHM, Hohlfeld IB, Salata OV. Nanoparticles—known and unknown health risks. J Nanobiotechnol 2004; 2:12.

28. Finlay WH, Stapleton KW, Zuberbuhler P. Fine particle fraction as a measure of mass depositing in the lung during inhalation of nearly isotonic nebulized aerosols. J Aerosol Sci 1997; 28: 1301–1309.

29. Oberdorster G, Oberdörster E, Oberdörster J. Nano-toxicology: an emerging discipline evolving from studies of ultrafine particles, Environ Health Perspect 2005; 113:823-839.

30. Edwards DA, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 1998; 84(2): 379–385.

31. Fels AO, Cohn ZA. The alveolar macrophage, J Appl Physiol 1986; 60:353–369.