Original article

Pharmacodynamics of dry powder formulations of salbutamol for delivery by inhalation

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Background: Salbutamol is a β_2-selective adrenoceptor agonist used as a bronchodilator. Delivery by inhalation has many advantages over oral dosage for the treatment of asthma. It offers rapid onset of action with low systemic side effects.
Objective: Evaluate the relationship of *in vitro* particle size characteristics and pharmacodynamics of formulations of inhaled salbutamol dry powder.
Methods: Three formulations contained micronized salbutamol and a lactose carrier with different size ranges (40-80, 20-40, and 10-20 μm for formulations 1, 2, and 3, respectively). Following formulation of the drug, resultant powders were characterized using scanning electron microscopy and the aerosolization performance determined using an Andersen Cascade Impactor analysis. A high-performance liquid chromatography method was used for measuring the salbutamol drug content. The *in vivo* pharmacodynamics of the formulations was monitored in 12 healthy and 12 asthmatic volunteers.
Results: The percentage of the fine particle fractions (FPF) for formulations 1, 2, and 3 were 24.87±0.52%, 33.82±3.80%, and 41.50±2.86%, respectively. The mass median aerodynamic diameters (MMAD) were around 3 μm for all formulations. The pharmacodynamic parameters, forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and mid expiratory flow (FEF_{25-75}), were indices for evaluation of the bioavailability of the bronchodilatory drug. All formulations improved the FEF_{25-75} value in asthmatics, while FVC and FEV_1 were not altered.
Conclusion: The formulations of salbutamol dry powder aerosols with a fine lactose carrier produced a high deposition in the lower regions of the respiratory tract. Although the FEF_{25-75} value in asthmatics was improved, the value did not correlate well with the FPF of the salbutamol dry powder.

Keywords: Dry powder inhaler, lactose carrier, pharmacodynamics, salbutamol, size characterization

Salbutamol is a β_2-agonist used as a bronchodilator in chronic obstructive pulmonary diseases like asthma. Delivery by inhalation has many advantages over oral dosage such as its direct action on the airways, low dose, low systemic side effects, and avoidance of first pass metabolism.

A dry powder inhaler is one of three types of pulmonary drug delivery that generally consists of a drug agglomerated on a carrier. The mechanism of dry powder release from the inhaler is breath actuated, inspiration is needed to aerosolize, disperse, and deliver the powder to the site of action (lung). The efficacy of the drug relates to fine particle dose (FPD). It is the amount of drug delivered to the lower respiratory airways. The Andersen cascade impactor (ACI) can identify particles delivered at each stage to provide comparisons for each site of the respiratory areas. A suitable particle size for treatment of the lower respiratory tract in terms of mass median aerodynamic diameter (MMAD) must be in the range 0.5 to 5 μm [1-4]. If only 10 to 20% of the drug can be delivered to the respiratory airways in combination with a low dose, the plasma level of the drug would be very low and difficult to detect [5, 6].

The pharmacodynamic data of inhaled bronchodilators are generally obtained by spirometry using the measure of improvements to lung function after inhalation. They are reported in terms of forced vital capacity (FVC), forced expiratory volume

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in one second (FEV_1) and mid expiratory flow (FEF_{25-75}) [7-9]. Bronchoprovocation is an alternative method. Methacholine or histamine is first inhaled to induce bronchoconstriction, and the protective effect of the inhaled bronchodilator against the allergen is assessed [7, 8]. Although the clinical response data are considered for assessments of efficacy and safety of the inhaled products, they have shown less sensitivity [7].

It is important to develop a dry powder formulation of salbutamol for delivery by inhaler for the treatment of asthma. Weda et al. [10] measured three salbutamol dry powder formulations for their aerodynamic particle size distribution and fine particle doses (FPDs) using four impactors (Glass impinger, Metal impinger, Multistage liquid impinger, and Andersen cascade impactor). The same formulations were administered to 12 asthmatics. The FEV_1 was measured at the baseline and 15 minutes after each dose (50-400 µg). The *in vitro* results of all impactors showed large differences between the FPDs of the three preparations, but the *in vivo* measurements did not show significant differences in efficacy of those preparations [10].

In this study, we evaluated the correlation of *in vitro* particle size characteristics and pharmacodynamics of inhaled salbutamol dry powder formulations. A randomized double-blind crossover design was employed. Three formulations with different FPF were administered via an in-house made inhaler device to 12 healthy volunteers and 12 asthmatics whose gastrointestinal (GI) absorption had been blocked by co-administration of charcoal. After inhalation, lung function tests (FEV_1, FVC, and $FEF25_{-75}$) were carried out 30 min before and after drug administration. The *in vitro* - *in vivo* correlation was investigated.

Materials and methods
Materials

The (±)-salbutamol (free base) and (-)-Phenylephrine HCl were obtained from Sigma Chemicals (Sigma Chemicals, St. Louis, USA). Salbutamol sulfate was purchased from Allchem International Ltd (Berkshire, UK). Lactochem® lactose was obtained from Borculo Whey Ltd. (Zwolle, Netherlands). Triethylamine, sodium carbonate and silica gel 60 were obtained from Fluka Chemie (Buchs, Switzerland). All high-performance liquid chromatography (HPLC) reagents were purchased from Rathburn (Walkerburn, Scotland).

Preparation of dry powder formulations

The formulations were prepared by mixing micronized salbutamol sulfate (0.2 g) with different size ranges (40-80, 20-40, and 10-20 µm) of lactose carrier (13.5 g) in a Turbula mixer (Basel, Switzerland) for one hour. They were designated formulation 1, formulation 2, and formulation 3, respectively. Blended materials were tested for their uniformity of salbutamol sulfate content [11]. For each formulation, ten replicated samplings were taken at the top, middle, and bottom of the powder blends and the salbutamol content was analyzed by HPLC. Then, 27.4 mg of each blend (equivalent to 400 g drug) was weighed into capsules. Each formulation was delivered by an in-house made device [12].

HPLC analysis of salbutamol sulfate

Salbutamol sulfate was analyzed by HPLC (Waters, Waters Associates Inc, Boston, USA) using a diode array detector at a wavelength of 276 nm. The mobile phase consisted of water, acetonitrile, and formic acid in a ratio of 600: 400: 2 by volume, respectively. The stationary phase was a silica column (Spherisorb® S5W). A flow rate of 1 mL/min was set at ambient conditions. The injection volume was 100 µL. Phenylephrine hydrochloride was used as an internal standard at a concentration of 1.5 µg/mL. This method was validated for suitability with respect to linearity, accuracy, and precision.

Particle characterization by scanning electron microscopy

Salbutamol sulfate, lactose monohydrate and all formulations were analyzed for their qualitative characteristics by scanning electron microscopy (SEM) (JSM-5800LV, Joel, Tokyo, Japan). The sample was scattered gently and uniformly on two-sided adhesive tape attached to an aluminum stub, and coated with a 15-20 nm gold layer before characterization. Electron micrographs were taken randomly across the sample using a scanning electron microscope (Joel, Tokyo, Japan).

In vitro measurement of aerosol characteristics

The dry powder formulation (equivalent to salbutamol sulfate 400 µg) was delivered to an Andersen Cascade Impactor (ACI) by an in house made glass inhaler device [12]. The device had a No.29

Quickfit® socket to fit into the glass throat of an ACI. The dry powders were liberated from the capsule container into the sample port. A round disc plastic grid, mesh size 74 μm (Biodis®) was fitted to the device to separate the aggregated powder. The bleed holes served to reduce the resistance of the device and to generate a turbulent air flow for aerosolization and de-aggregation of the formulation. The apparatus was operated at a flow rate of 60 L/min for five seconds. The drug deposited on each stage of the apparatus; inhaler device (id), inlet (it), pre-separator (ps), stage 0-7 (S_0-S_7), were collected and analyzed by HPLC as described above.

The fine particle fraction (FPF) was calculated from the amount of the drug deposited on stage 2 to stage 7 of the ACI. The size was in range of 0.3 to 4.7 μm. The aerodynamic particle size distribution was further investigated as the mass median aerodynamic diameter (MMAD). The percentage of drug deposited at each stage was transformed to the Z-distribution, and plotted against particle size on a logarithmic scale. The MMAD was achieved at Z=0, and the geometric standard deviation (GSD) was obtained using the division of size at Z=1 by the size at Z=0. The measurements were repeated five times for all formulations.

In vivo evaluation of salbutamol aerosols

The experiment was a randomized double-blind crossover study design. Two groups of subjects were employed in this study, 12 healthy volunteers, and 12 patients with asthma (12 males and 12 females). Healthy volunteers were recruited from non-smokers, aged between 20 and 60 years, without evidence of heart disease. Pregnant and breast feeding females were excluded from the study. Participants were also free of kidney disease (creatinine clearance 125±13 mL/min), and showed no evidence of any hepatic disease from blood chemistry tests. Lung function tests (FVC, FEV_1, FEF_{25-75}) were carried out with a Compact II Spirometer (Vitalograph, Buckingham, UK) before administration of the drug. Healthy volunteers were excluded if their FEV_1 was less than 75% of the predicted value. During the study period, volunteers were not allowed to take any other medication (including contraceptive pills). For recruitment of asthmatics, patients with a predicted value of FEV_1 between 50-75%, as specified in the Asthma Management Handbook of National Asthma Council Australia, were selected [13].

All volunteers gave written consent prior to participating in this study. The protocol was approved by the Faculty of Pharmaceutical Sciences Ethics Committee, Prince of Songkla University. All procedures were carried out according to the principles of good clinical practice [14]. The volunteers were all in a normal fed state throughout the study.

All of the volunteers received one dose of the salbutamol dry powder formulation (400 μg of salbutamol sulfate) on each visit, with a one-week washout period. One hour before aerosol administration, all volunteers received 520 mg activated charcoal to prevent the absorption of swallowed drug [15]. Lung function tests (FEV_1, FVC, FEF_{25-75}) were carried out with a Compact II Spirometer (Vitalograph, Buckingham, UK), 30 minutes before and after drug administration. Volunteers were all fed normally during this study. Blood pressure and pulse rate were also monitored 30 minutes after drug administration.

Statistical analysis

The paired *t*-test was employed. Statistical significance was set at p = 0.05 unless otherwise stated. Data were expressed as means±SD.

Results

Figure 1 shows SEM images of salbutamol sulfate, lactose carriers and salbutamol formulations.

As shown in Figure 1, Salbutamol sulfate particles were irregular in shape, both in aggregates and individual forms (A). Particles of lactose obtained from formulation were in cylindrical, cuboid or tomahawk shape. The lactose carriers from formulation 2 (C) and formulation 3 (D) were in aggregate forms. Medium-sized or fine particles adhered to the surface of larger sized lactose particles. Some medium-sized particles aggregated in fine particles, or some fine particles aggregated with themselves. The aggregation may be caused by a reduction of surface free energy of small particles. Electron micrographs of salbutamol formulations indicated that most of the drug particles uniformly adhered to the surface of a large carrier. For example, in formulation 1 (E and F), there were four types of aggregation. These are separate fine drug particles, aggregation of fine drug particles, fine drug aggregated on a separate carrier and aggregation of drug particles and carriers as previously described [16]. The most desirable form was drug particles adhering to the carrier surface and other formulations showed similar characteristics.
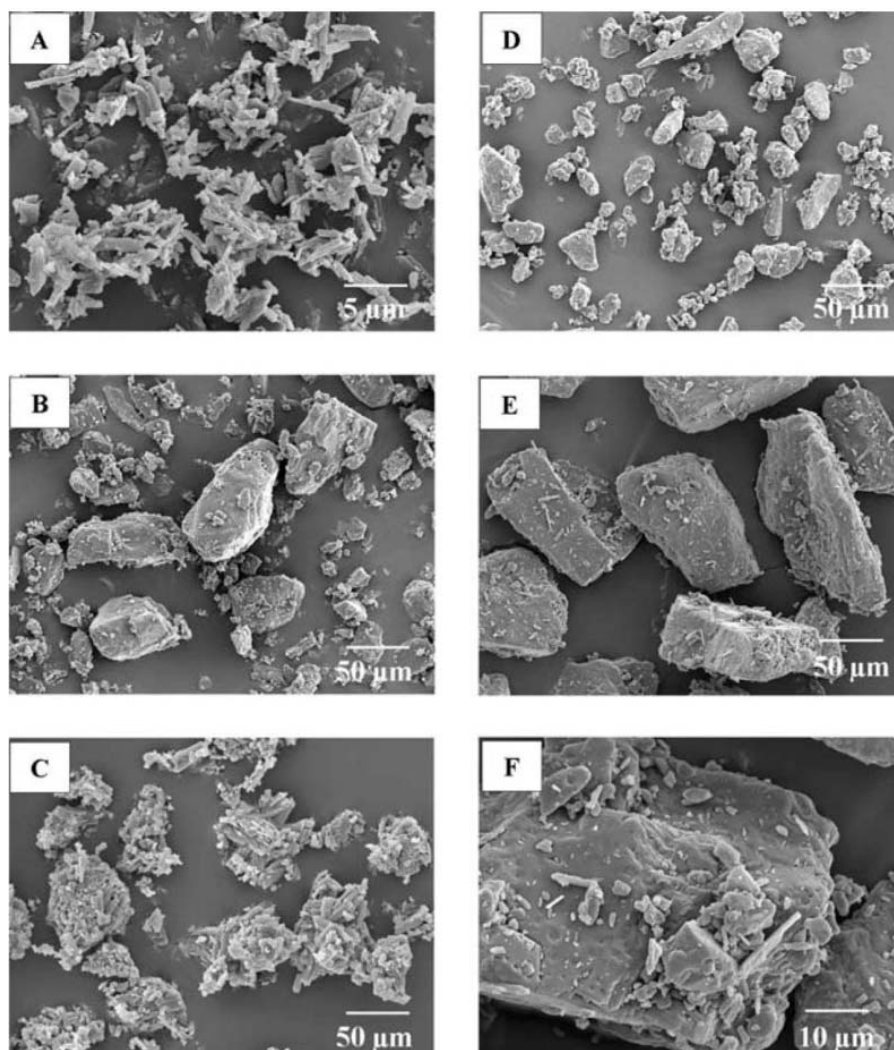
Figure 1. Scanning electron micrographs of salbutamol sulfate, lactose carriers and salbutamol formulations. **A:** irregular morphology of salbutamol sulfate, **B:** particles of lactose from Formulation 1, **C:** Formulation 2, **D:** Formulation 3, **E** and **F:** salbutamol sulfate aggregated on lactose carrier, Formulation 1

The three different formulations contained the same dose of salbutamol, but the lactose carrier was different in size range (40-80, 20-40, and 10-20 μm for formulation 1, 2, and 3, respectively). All formulations were uniform in their content of salbutamol (99.17±1.64%, 101.93±1.32%, and 100.54±1.67%, respectively) with % coefficient of variation (CV) of 1.7, 1.3 and 1.7 (n=10). These were in the acceptable range of 85-115% stipulated in the British Pharmacopoeia [11].

The Andersen cascade impactor (ACI) can separate the particles by their aerodynamic size; each stage collects a particle size larger than the effective cut-off diameter while smaller sizes pass through to the lower stage. The size distribution of the drug from three formulations at a flow rate 60 L/minute is shown in **Figure 2**.

Drug particles were deposited as far as stage 5 in formulation 1, while in formulation 2 they were deposited on S_0-S_4. It indicates that these two formulations were poor in delivery, because no drug particles were found at stages 6 and 7 that represent the bronchioles and alveoli, respectively. Drug particles from formulations 2 deposited mainly on stages 0-3. Therefore, they can be expected to deliver the drug primarily to the upper airways. Only a small amount of drug was deposited on stages 4 and 5 (<2%). Drug particles from formulation 3 were deposited on stages
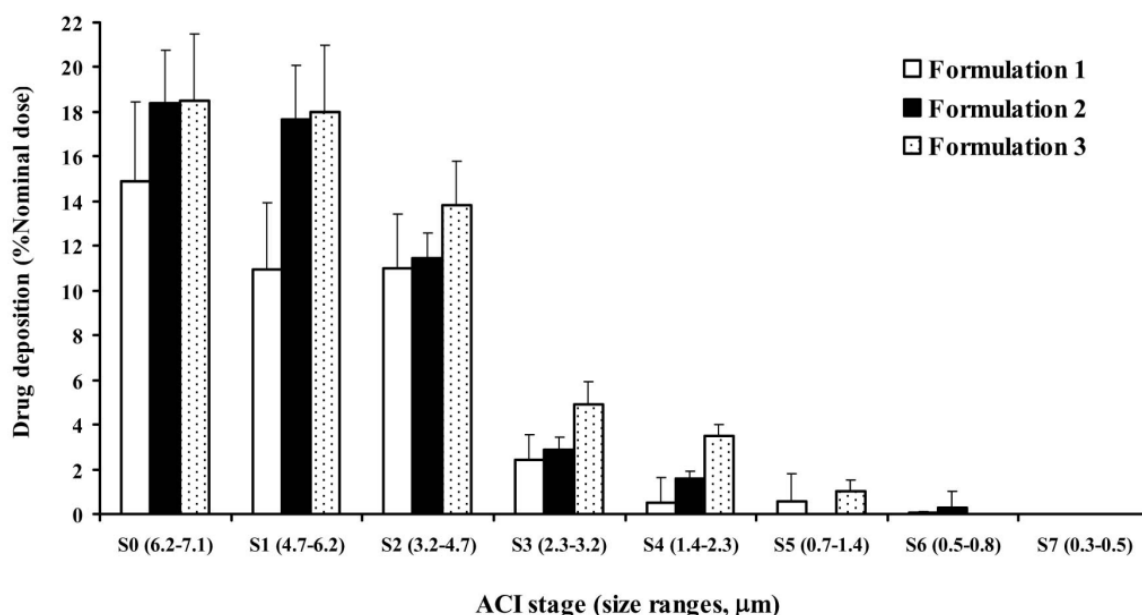
Figure 2. Deposition pattern following the aerosolization of salbutamol dry powder formulations at a flow rate of 60 L/min. HPLC was used to quantify the fraction of salbutamol sulfate reaching the various stages of the Andersen cascade impactor (ACI). (mean SD, n =5)

0-5 without being found on stages 6-7, but their deposition on stages 3-5 was the highest compared to the other two formulations. This formulation should be an ideal bronchodilator because the drug may deposit mainly on the stages, which represent the bronchi.

The amount of salbutamol deposited at each stage of the ACI was used to calculate the fine particle fraction (FPF) and the mass median aerodynamic diameter (MMAD). The FPF, which were used to predict the lower airway deposition, were $24.87 \pm 0.52\%$, $33.82 \pm 3.80\%$, and $41.50 \pm 2.86\%$ for formulation 1, 2, and 3, respectively. The MMADs were 3.0 ± 0.08, 3.06 ± 0.03, and 2.98 ± 0.15 μm with GSD at 1.59, 1.56, and 1.87 for formulation 1, 2, and 3, respectively. Although the MMADs for all formulations were around 3 μm, the FPFs were different (24.9, 33.8, and 41.5% for formulations 1, 2, and 3, respectively). The higher FPF was obtained from the formulation with the fine lactose carrier. In addition, this result shows that the three formulations had a geometric standard deviation (GSD) of more than 1.2 indicating that the formulations have a polydisperse distribution.

Table 1 shows the mean baseline characteristics of healthy control and asthmatics. The pulse rate and blood pressure of this group were slightly different from those of the healthy volunteers, but these were not statistically significant (p >0.05). FEV_1 and FEF_{25-75} baseline values showed significant differences between the healthy and asthmatics groups (p <0.05). The mean FEV_1 of this group was 64% (50-74) that showed mild asthma according to the criteria of The American Thoracic Society.

Pharmacodynamic parameters, (FVC, FEV_1, FEF_{25-75}) were the indices used to evaluate the bioavailability of the bronchodilator drug. There were no differences in pulse rate, blood pressure and FVC of volunteers before and after administration of salbutamol aerosols. This result was similar in both groups and for all formulations. The percent improvement and mean value of FEV_1 and FEF_{25-75} at pre- and 30 minutes post-inhalation of formulation 1, 2, and 3 in healthy volunteers and asthmatics are shown in **Table 2**.

Figure 3 shows FEF_{25-75} values before and after inhalation of dry powder aerosol formulation in healthy volunteers and asthmatics. It is important to note that only FEF_{25-75} shows a significant improvement in healthy volunteers and asthmatics for all formulations. Changes of more than 15% in FEV_1 were found in only two cases of asthmatics. Six, seven, and eight of 12 asthmatics showed an improvement in FEF_{25-75} greater than 15% after inhalation of dry powder

formulation 1, 2, and 3, respectively. Only four to six healthy volunteers improved in FEF_{25-75} more than 15% with any of the three formulations. Interestingly, FEF_{25-75} is a sensitive index of small airways and can be used to confirm the efficacy of a bronchodilator .

Figure 4 shows correlation of fine particle fraction and FEF_{25-75} in healthy volunteers and asthmatics. We note that the percentage improvement of FEF_{25-75} in asthmatics was similar in all three formulations given to volunteers. Whereas, there is a steep rising of FEF_{25-75} in healthy volunteers, although it was less than 15% improvement.

Table 1. Baseline characteristics of healthy volunteers (n=12) and asthmatics (n=12)

	Mean data of healthy volunteers (maximum/minimum)	Mean data of asthmatics (maximum/minimum)
Age (year)	33.1 (41/20)	33.4 (58/20)
Weight (kg)	56.75 (78/36)	57.38 (94/48)
Sex (male/female)	6/6	6/6
FEV_1 (L)	2.67 (3.70/2.11)	2.01 (2.80/1.06)*
% Predicted	83.21 (100.40/74.50)	64.37 (74.1/50.50)
Pulse rate (beat/minute)	73.67 (100/51)	70.58 (120/44)
Blood pressure systole/diastole (mmHg)	115/69	116/75
FVC (L)	2.96 (4.21/2.11)	2.65 (3.80/1.71)
PEF (L/sec)	6.61 (8.85/4.43)	5.59 (7.36/3.10)
FEF_{25-75} (L/sec)	3.56 (4.70/2.25)	1.72 (2.35/0.53)*

FEV_1 =force expiratory volume in one second, FVC = force vital capacity, PEF=peak expiratory flow, FEF_{25-75} = mid expiratory flow. *$p < 0.05$ values compared with healthy volunteers

Table 2. Lung function (Mean FEF_{25-75} and FEV_1) for pre- and post inhalation of salbutamol dry powder formulations (F) in 12 healthy volunteers and 12 asthmatics

	Healthy control						Patient with asthma					
F	Mean of FEF_{25-75} (L/sec)		% IP	Mean of FEV_1 (L)		% IP	Mean of FEF_{25-75} (L/sec)		%IP	Mean of FEV_1 (L)		% IP
	Pre	Post		Pre	Post		Pre	Post		Pre	Post	
1	3.56	3.82	7.3*	2.67	2.70	1.1	1.72	2.05	19.2**	2.01	2.15	7.0
2	3.39	3.82	12.7*	2.70	2.80	3.7	1.70	2.02	18.8**	2.01	2.13	6.0
3	3.17	3.66	15.5*	2.59	2.69	3.9	1.65	1.98	20.0**	1.93	2.09	8.3

F=formulation, %Improvement = %IP, *$p < 0.05$ and **$p < 0.01$

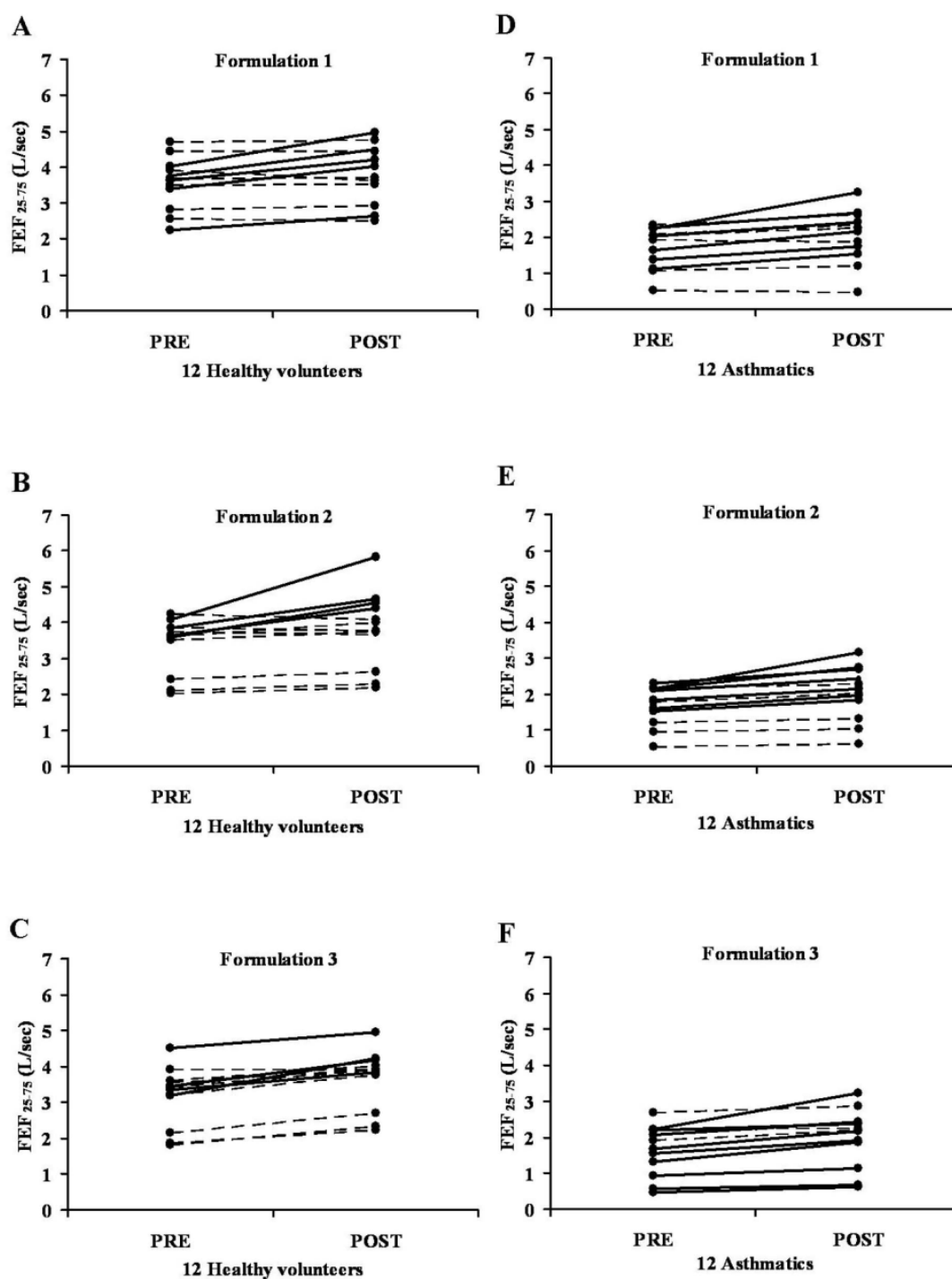**Figure 3.** FEF$_{25-75}$ values at pre- and 30 minutes post inhalation of dry powder aerosol formulation 1 (**A, D**), formulation 2 (**B, E**) and formulation 3 (**C, F**) in 12 healthy volunteers and asthmatics, respectively. (The bold line represents 15% improvement in FEF$_{25-75}$)
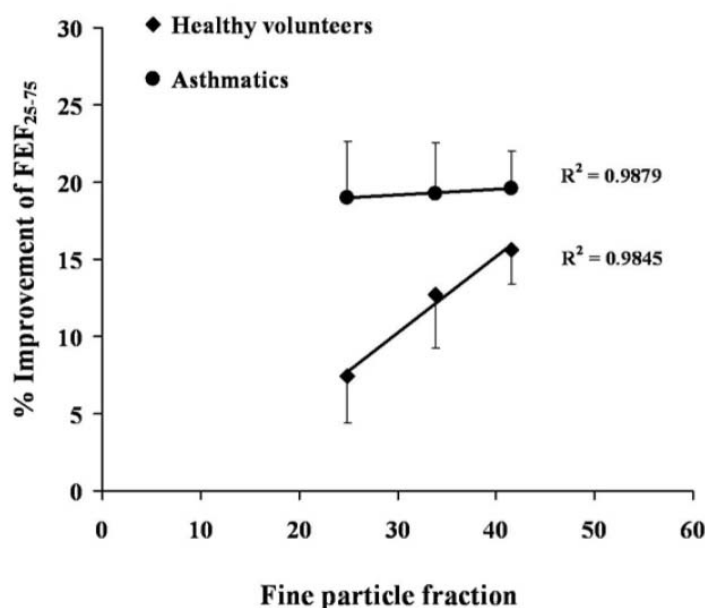
Figure 4. Correlation of fine particle fraction and FEF_{25-75} in healthy volunteers (♦) and asthmatics (•)

Discussion

All three formulations had good uniformity but differences in FPF from *in vitro* deposition in the ACI. However, some pharmacodynamic parameters (FVC, FEV_1) revealed that it was not a significant change in either healthy volunteers or asthmatics for these three formulations. This is in agreement with previous studies where these two parameters were not sensitive to small changes of lung improvement [15]. Another parameter (FEF_{25-75}) was well correlated with the dose. Although the percent improvement did not change in the case of asthmatics in healthy volunteers, it was clearly indicated that as the FPF increased, the FEF_{25-75} was significantly improved. On the other hand, the FEF_{25-75} improvement in asthmatic patients was always over 15% and did not change significantly, as the FPF increased. This can be explained from different pathophysiological viewpoints of healthy volunteers and asthmatics. The drug may reach a plateau phase of the dose response curve in the case of asthmatics from all three formulations. In order to observe changes of this parameter, it is necessary to decrease the dose delivery to the lung to a linear response level. In the case of healthy volunteers, the drug may still be in the range of the linear dose response curve so that the percent improvement did not reach the plateau phase. The physiology of healthy lung provides a larger capacity and a further increase

in FEF_{25} may not be seen even if the delivery dose is increased.

In vitro FPF measurement cannot be the only tool that can be used to predict drug availability to the airways. It is important to consider the airway parameters as well as the physiology of the lung to adjust the effective dose regimen. This is partly due to the pathology of the disease that affects the amount of drug deposited in the lower airways.

Conclusion

The developed formulations of salbutamol were successfully prepared into dry powders by mixing the drug with different sizes of lactose carrier for asthmatic inhalers. The formulations of salbutamol dry powder aerosols prepared with the fine lactose carrier exhibited a high deposition in the lower regions of the respiratory tract. Although the FEF_{25-75} value in patients with asthma was improved, the value of FPF for the salbutamol dry powder did not correlate well with the lung responses.

References

1. Rees PJ, Clark TJ, Moren F. The importance of particle size in responses to inhaled bronchodilators. Eur. J. Respir. Dis. 1982; 63:73-8.

2. Zanen P, Go TL, Lammers JWJ. The optimal particle size for β_2-adrenergic aerosols in mild asthmatics. Int. J. Pharm. 1994; 107: 211-7.

3. Zanen P, Go TL, Lammers JWJ. Optimal particle size for β_2-agonist and anticholinergic aerosols in patients with severe airflow obstruction. Thorax. 1996; 51: 977-80.

4. Chrystyn, H., Is total particle dose more important than particle distribution? Respir. Med. 1997; 91:17-9.

5. Newman SP, Pavia D, Moren F, Sheahan NF, Clarke SW. Deposition of pressurised aerosols in the human respiratory tract. Thorax. 1981; 36:52-5.

6. Mechlor R, Biddiscomb MF, Mak VHF, Spiro SG. Lung deposition patterns of directly labelled salbutamol in normal subjects and inpatients with reversible airflow obstruction. Thorax. 1993; 48:506-11.

7. Newman SP, Wilding IR, Hirst PH. Human lung deposition data: The bridge between *in vitro* and clinical evaluations for inhaled drug products?. Int J Pharm. 2000; 208:49-60.

8. Tomlinson HS, Corlett SA, Allen MB, Chrystyn H. Assessment of different methods of inhalation from salbutamol metered dose inhalers by urinary drug excretion and methacholine challenge. Br J Clin Pharm. 2005; 60:605-10.

9. National Heart Lung and Blood Institute. National asthma education and prevention program expert panel report 3: guidelines for the diagnosis and management for asthma. 2007. [cited 2010 April 8]. Available from: http://www.nhlbi.nih.gov.

10. Weda M, Zanen P, De Boer AH, Gjaltema D, Ajaoud A, Barends DM, et al. Equivalence testing of salbutamol dry powder inhalers: In vitro impaction results versus in vivo efficacy. In J Pharm. 2002; 249:247-55.

11. British Pharmacopoeia. 2010. London: The Stationery Office (CD-ROM accessed 1 January 2010).

12. Srichana T, Suedee R, Srisudjai P. Application of spectrofluorometry for evaluation of dry powder inhalers *in vitro*. Pharmazie. 2003; 58:125-9.

13. National Asthma Council Australia. Asthma Management Handbook (Online). National Asthma Council Australia Ltd. [cited 2010 April 8].Available from: http://www.nationalasthma.org.au/cms/index.php

14. International Conference on Harmonization (ICH). Good Clinical Practice Guidelines, Minister of Public Health, Therapeutic Directorate, Health Canada, Ontario; 1996.

15. Srichana T, Suedee R, Tanmanee N, Muanpanarai D, Marriott C. The correlation of urinary levels of albuterol and its metabolites isomers following inhalation from a dry powder inhaler and in vitro particle size characterisation. Pulm.Pharmacol. 2007; 20:36-45.

16. Srichana T, Brain A, Marriot C. A study of Drug-carrier interaction in dry powder inhaler formulation using the Andersen Cascade Impactor, X-ray microanalysis and time of flight aerosol beam spectrometry (TOFABS). Chem. Pharm. Bull. 2000; 48:167-74.