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Research Paper

Determination of the Relative Bioavailability of Salbutamol to the Lungs Following Inhalation from Dry Powder Inhaler Formulations Containing Drug Substance Manufactured by Supercritical Fluids and Micronization

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Purpose. The relative lung bioavailability of salbutamol sulfate particles produced using supercritical fluids (SEDS™) and delivered by dry powder inhaler (DPI) was compared with the performance of a conventional micronized drug DPI using the same device design (Clickhaler™, Innovata Biomed).
Materials and Methods. Twelve healthy volunteers and 11 mild asthmatic patients completed separate four-way randomised cross-over studies, assessing the relative bioavailability of salbutamol sulfate (urinary excretion method), formulated as SEDS™ particles (three batches) and micronized particles (Asmasal™ inhaler, UCB Pharma Ltd). Post-treatment improvements in patient lung function were assessed by measuring FEV_1. Physicochemical evaluation of the three SEDS™ batches revealed inter-batch differences in particle size and shape.
Results. There was no significant difference in the relative lung bioavailability of salbutamol and its bronchodilator response between the best performing SEDS™ formulation and the Asmasal™ inhaler in volunteers and patients, respectively. SEDS™ salbutamol sulfate showing wafer like morphology gave greater fine particle dose, relative lung bioavailability and enhanced bronchodilation compared to other SEDS™ batches containing elongated particles.
Conclusions. Active Pharmaceutical Ingredient (API) manufactured using supercritical fluids and delivered by DPI can provide similar lung bioavailability and clinical effect to the conventional micronized commercial product. Product performance is however notably influenced by inter-batch differences in particle characteristics.

KEY WORDS: dry powder inhalers; fine particle dose; relative lung bioavailability; supercritical fluids; urinary salbutamol.

INTRODUCTION

Dry powder inhalers (DPIs) are breath-actuated devices, which are dependent on the inspiratory effort of individuals for effective dose emission and subsequent deposition of drugs in the human lung. It is the energy derived from the patient's inspiration which is used as the sole means of aerosol generation and subsequently the entrainment and dispersion of drug particles in the airways (1). Not only does the inspiratory flow of individuals influence the magnitude of the emitted dose, but it has an effect on the deaggregation of drug from the surface of carrier particles and the subsequent respirable dose (2). The resistance of the inhalation device controls the flow inside the device and determines the amount of effort required to aerosolize the particles. However, for binary systems containing drug and larger carrier particles, the magnitude of cohesive and adhesive forces acting on the powder will determine the degree of separation during inhalation and the ultimate deposition efficiency. The particle size distribution of the active component and the interaction between drug and carrier will notably influence the respirable dose delivered and will be dependent on the particulate properties and processing history of both active pharmaceutical ingredient (API) and excipients (3,4).

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ABBREVIATIONS: ACI, Andersen cascade impactor; ANOVA, Analysis of variance; API, Active pharmaceutical ingredient; DPI (s), Dry powder inhaler(s); FEV_1, Forced expiratory volume in 1 s; FPD, Fine particle dose; GSD, Geometric standard deviation; HPLC, High performance liquid chromatography; MMAD, Mass median aerodynamic diameter; RSD, Relative standard deviation; SD, Standard deviation; SEDS™, Solution enhanced dispersion by supercritical fluids; SEM, Scanning electron microscopy; TOF, Time of flight.

For orally inhaled dosage forms, size distribution has in the past generally been controlled using conventional methods of micronization such as fluidized bed opposed jet milling or spiral jet milling. Although important for the production of powders for inhalation, micronization can produce particles which are highly heterogeneous, charged and cohesive, with the potential to cause problems in downstream processing and product performance (5,6). More recently, manufacturers of inhaled dosage forms have considered the use of alternative techniques such as spray drying (7) and supercritical fluid technology (8) to combat some of problems associated with the more traditional methods of micronization. In the field of supercritical fluid processing, the single step solution enhanced dispersion by supercritical fluids (SEDS™) process has been shown to produce micron sized budesonide particles with increased surface smoothness and reduced surface free energy resulting in improved content uniformity and fine particle dose when compared to conventionally micronized material (9). Potential differences in drug substance characteristics between micronized and SEDS™ materials such as the degree of crystallinity, hygroscopicity, density and morphology, for example, have the potential to influence lung deposition following administration by DPI. Factors such as these are known to affect the forces of interaction and aerodynamic properties of aerosolized particles, which in turn can influence fluidization, dispersion, delivery to the lungs, and deposition in the peripheral airways (10–13). It is therefore necessary to evaluate the clinical impact of changes in processing technology, where these changes have led to modifications in particulate properties. In particular, this article focuses on the effect of particle size and morphology on the delivery of drug from a dry powder inhaler.

The major drawback of dry powder inhalers is that effective drug delivery is dependent on the patient's ability to generate sufficient inspiratory flow to enable therapeutic amounts of drug to deposit in the human lung. The inhalation rate must be sufficient to allow de-aggregation of the drug-carrier complex and re-disperse agglomerated drug particles following inspiration (14,15). Past developments in device technology have, therefore, led to the advent of inhalers which can deliver precise and uniform doses at inhalation rates sustainable by young children.

The Clickhaler™ is a widely used multiple dose reservoir powder inhaler developed by Innovata Biomed (Tewkesbury, UK), which is used to deliver dry powders comprising drug and inert carrier particles. It is available with standard or large reservoir and requires no coordination between actuation and inhalation. It offers the advantage of a metering system that prevents accidental multiple dosing, a dose counter and end of life lock out system. It can be provided with a variety of different metering cones to provide a range of doses, from 60 to 200 μg per dose (16). Clickhalers containing salbutamol (albuterol, Asmasal™) are licensed in the UK and Europe for use in adults and children as young as six and have been shown to demonstrate a high level of reproducibility of fine particle and emitted dose across a range of flow rates in *in vitro* studies (17). It has also been demonstrated that asthmatic patients aged 6 or more can produce sufficient air flow to operate the Clickhaler™ and receive sufficient fine particle dose to produce the required therapeutic outcome (18,19).

For precision devices such as the Clickhaler™, although the componentry is designed to provide minimal variability in dose emission, the characteristics of the formulation, particularly particle properties, still have major potential to impact inhaler performance and subsequent lung deposition. When considering the use of alternative processing technology for the manufacture of APIs, such as the SEDS™ process, it is necessary to evaluate the impact of changes to particle characteristics on subsequent in vivo performance. This study has therefore aimed to investigate the influence of API manufacturing process and inter-batch differences in particulate characteristics on the lung bioavailability of salbutamol delivered by Clickhaler™. The major goal was to compare the relative lung bioavailability of salbutamol (20) manufactured by supercritical fluids to the commercially available Asmasal™ formulation.

MATERIALS AND METHODS

Materials

α-Lactose monohydrate (Pharmatose™ 325 M) was a gift from DMV International, Veghel, The Netherlands. Inhalation grade micronized salbutamol sulfate (MS) was supplied by Glaxo Manufacturing Services, Ware, UK. Three batches of salbutamol sulfate (SEDS™1, SEDS™2 and SEDS™3) prepared using supercritical fluid technology, were supplied by Nektar Therapeutics, Bradford, UK.

Empty Clickhaler™ components were supplied by ML Laboratories PLC, Farnham UK. Commercial salbutamol sulfate dry powder inhalers (Asmasal™ Clickhalers™) were supplied by Medeva Phamaceutical Ltd, Leatherhead, UK. All other chemicals used were of analytical grade. This included salbutamol, bamethane sulfate and terbutaline used as standards for high performance liquid chromatography (HPLC) analysis.

Particle Characterisation of Salbutamol Sulfate Samples

Particle Size Analysis

Particle size distribution was determined for SEDS™ batches and compared to a typical batch of micronized salbutamol sulfate using a time of flight (TOF) analyser (Aerosizer™ with Aerodisperser, Amherst Process Instruments Inc, USA) in accordance with the manufacturers instructions. Samples were analysed for 300 seconds using normal deagglomeration conditions, feed rate of 5,000 particles per second and shear force of 0.5 psi.

Scanning Electron Microscopy (SEM)

Scanning electron micrographs showing particles from the micronized and SEDS™ salbutamol sulfate batches were collected using a Hitachi S520 SEM (Hitachi, Tokyo, Japan). Samples were mounted on a graphite layer on an aluminium stub. A Polarcon SEM cool sputter coater (Polaton, Watford, UK) was then used to apply the gold sputter coat. Micrographs

of samples from each batch were then collected using a range of magnification settings.

Dry Powder Inhaler (DPI) Preparation

DPIs of nominally identical quantitative and qualitative composition to the commercial Asmasal™ Clickhaler™ were prepared using the 3 batches of salbutamol sulfate prepared using supercritical fluid methodology. Each SEDS™ batch was blended with inhalation grade lactose (Pharmatose 325 M) to a target salbutamol sulfate concentration of 3.8% w/w. Batches were manufactured at a scale of approximately 2 g with mixing for 30 s using the Spiramix S, roller mixer (Sussex, UK). The active agent content (approximately >99% of nominal) and the uniformity of content of the mixes (<2% RSD) were confirmed for five samples from each batch by HPLC. The resultant mixes were tested for fungal and microbial contamination then were filled into empty Clickhalers™ by hand. The Clickhaler™ devices used, contained the standard reservoir and standard metering cone to give formulations with nominally identical doses to the commercial Asmasal™ formulation. Each metered actuation of 3 mg of powder for inhalation contained 114 μg of salbutamol sulphate, which is equivalent to 95 μg of salbutamol base.

In vitro Evaluation

Dose emission measurements were determined using the dose sampling unit based on the design of Byron and Hindle (21). The emitted dose from each formulation was determined by drawing air through the inhaler onto a filter fitted into the dose sampling unit using an air flow rate of 28.3 l/min. A GAST 1023 pump (Brook Crompton, Doncaster, UK) was used to pull 4 L of air through the cascade for each determination. Drug collected in the dose-sampling unit and on the filter, was recovered by rinsing with distilled water containing bamethane (500 μg/l) as the internal standard. Salbutamol sulfate levels were then quantified by HPLC. The emitted dose and associated relative standard deviation (%RSD) were determined for 18 replicates (six actuations from three different devices) for each formulation.

The aerodynamic particle size distribution of salbutamol sulfate delivered from the four different DPI variants was determined using the Andersen Mark II Non-Viable1 ACFM Ambient cascade Impactor (ACI, Copley Scientific, Nottingham, UK) operated at 28.3 l/min and fitted with the United States Pharmacopeia (USP) induction port. For each formulation the deposition profile was measured using four actuations. Doses were delivered from three different devices, which were tested in triplicate.

Impactor plates were coated with silicone spray to prevent particle bounce and particle re-entrainment. The impactor was assembled with the throat, preseparator, stages 0–7 and terminal filter. The preseparator also contained 10 ml of deionised water for each determination to remove re-entrainment errors. A rubber mouthpiece was used to connect each DPI device to the throat of the ACI. The relevant sections of the inhalers and impactor were washed with distilled water containing bamethane (500 μg/l). The resultant solutions were then analysed for salbutamol content by HPLC.

The particle size distributions were fractionated into the amount of drug deposited in the mouthpiece of the DPI, rubber mouthpiece, ACI induction port, eight stages of the ACI and the terminal filter. The proportion of the total dose deposited from stage 2 to the terminal filter (corresponding to particles less than 5.8 μm) was considered to be the fine particle dose (FPD).

In vivo Evaluation

The relative lung bioavailability of salbutamol delivered by the different DPIs was evaluated using the previously validated 30 min urinary excretion method (20). The reverse phase HPLC method used for the analysis of the urine samples was modified to allow elution of terbutaline sulfate and bamethane sulfate as internal standards. The mobile phase for this analysis was also changed to include a reduced level of potassium dihydrogen phosphate (5 mmol/l) adjusted to pH 2.5 using phosphoric acid (0.5 M). The organic content of mobile phase was additionally modified to contain acetonitrile (10% v/v), tetrahydrofuran (8% v/v) and methanol (14% v/v). The modified analytical method was subsequently validated and shown to demonstrate suitable accuracy, precision, linearity and sensitivity.

Healthy Volunteers

Local research ethics committee approval was obtained for this four-way randomised cross-over study in 12 healthy non-smoking volunteers (six women). The volunteers were assessed prior to the study and shown to suffer from no reported medical complaints. The study followed the tenets of the Declaration of Helsinki promulgated in 1964. Prior to recruitment, the nature of the study was explained both verbally and in writing to potential volunteers. Each volunteer gave written informed consent prior to inclusion in this programme and was trained in the correct inhalation technique. Gender, age, height and weight were recorded prior to commencing the study. There was a three day wash-out period before the start of the study during which all volunteers abstained from drinking tea, coffee or alcohol. On each study day (at least 7 days apart) the volunteer's heart rate and blood pressure were recorded. They then received 10 g of activated charcoal in 80 ml of water to exclude any salbutamol that may be absorbed via the gastrointestinal tract. This method has previously been shown to be effective as a means of preventing the gastrointestinal absorption of salbutamol (22). Dosing of the charcoal was followed by 2×95 μg inhaled doses of salbutamol from one of the 4 different DPI formulations (Asmasal™, SEDS™1, SEDS™2 and SEDS™3) with subsequent administration of a further 15 g of activated charcoal delivered in 80 ml of water. Urine samples were collected half an hour prior to administration then at 0.5, 1, 2, 4, 6, 9, 12 and 24 h post inhalation, which were analysed for levels of salbutamol and its sulfate ester metabolite by HPLC. Heart rate and blood pressure were recorded before dosing and 15, 30 and 60 min after inhalation.

Mild Asthmatic Patients

Hospital ethics committee approval was obtained for this four-way randomised cross-over study in 11 moderate

asthmatic patients showing 60–80% of predicted FEV_1. The study followed the tenets of the Declaration of Helsinki promulgated in 1964. Prior to recruitment, the nature of the study was explained both verbally and in writing to potential subjects. Each patient then gave written informed consent prior to inclusion in this programme and was trained in the correct inhalation technique. Gender, age, height, weight and FEV_1 were recorded prior to commencing the study. Their normal salbutamol inhaler was replaced with a Terbutaline inhaler at least 14 days before commencing the study. Each patient was also asked to avoid the usage of any inhaler therapy for at least 8 h before receiving the experimental DPIs on the various study days. There was a three day wash-out period before the start of the study during which all patients abstained from drinking tea, coffee or alcohol. On each of the study days, which were at least 7 days apart, the patients received 2×95 μg inhaled doses of salbutamol from one of the four different DPI formulations (Asmasal™, SEDS™1, SEDS™2 and SEDS™3). Ethics committee approval was not given for the oral delivery of activated charcoal to patients as this approach is known to affect the absorption of orally administered medicines. Under these circumstances, the amount of salbutamol excreted in urine over the first 30 min after inhalation is known to be the most reliable indicator of lung bioavailability (20), whereas urinary excretion of drug and metabolite over the 24 h period post-inhalation would have notable contribution from oral absorption. Urine samples were therefore collected half an hour prior to administration then at 30 min, 1 and 2 h post inhalation, which were analysed for levels of salbutamol and its sulfate ester metabolite by HPLC. Samples were collected at the 1 and 2 h time points to enable the assessment of Tmax (time to maximum urinary excretion of salbutamol and metabolite) following inhalation. Heart rate, blood pressure, actual FEV_1 and % predicted FEV_1 were recorded before treatment and 15, 35, 65, 90 and 125 min after inhalation.

Statistical Analysis

Statistical analysis of both in vitro and in vivo data was carried out using the SPSS (version 11) computer program. One-way analysis of variance (ANOVA) with application of Bonferroni correction of multiple comparisons was used to determine any significant differences in *In vitro* and *in vivo* performance between the different formulations tested. The mean differences (95% confidence interval) in data were assessed and a probability value of $p<0.05$ was considered to be significant.

RESULTS

Particle Characteristics of Salbutamol Sulfate Powders

Table I details the results of TOF particle size analysis for the micronized salbutamol sulfate and SEDS™ produced materials. The mean aerodynamic particle size of the typical batch of micronized drug substance was markedly heterogeneous and typically smaller than the SEDS™ produced salbutamol sulfate. The particle size determined by number distribution was similar for each of the SEDS™ batches. The particle size for the SEDS™3 samples determined by volume distribution was however slightly greater than measured for SEDS™1 and SEDS™2, suggesting that there was a proportion of particles from SEDS™3 with larger diameter than the other SEDS™ batches. The particle images shown in Fig. 1 indicate that these larger particulates are probably agglomerates.

The SEM micrographs of the processed salbutamol sulfate samples are presented in Fig. 1 showing the SEDS™ material to contain particulates, which are generally larger than those from a typical batch of micronized drug substance. The micronized samples exhibit irregular shape, whist the SEDS™ samples demonstrate a more defined and regular morphology. SEDS™1 and 2 were predominantly composed of elongated shards, having approximate aspect ratio of 10–20. Material from SEDS™3 was wafer-like in appearance and predominantly comprised of particles within the size range 1–5 μm, which appeared to form agglomerates of less than 10 μm.

In vitro Evaluation

Table II shows the mean, standard deviation (SD) and relative standard deviation (%RSD) of the emitted dose recovered for the formulations tested using the dose sampling unit. All four formulations provided emitted doses ranging from 75 to 86% of nominal dose delivered (100 μg). There was significant difference ($p<0.001$ ANOVA) in emitted dose between the SEDS™3 and the other three formulations, with this formulation giving greatest dose emission.

Table I. Particle Size Data for Processed Salbutamol Sulfate Samples (n=3)

Sample	Mean (SD) Aerodynamic Diameter (μm)					
	By Number			By Volume		
	d_{10}	d_{50}	d_{90}	d_{10}	d_{50}	d_{90}
MS	0.37 (0.005)	0.60 (0.01)	0.94 (0.02)	0.56 (0.003)	0.89 (0.008)	1.38 (0.06)
SEDS™1	0.55 (0.01)	0.99 (0.05)	1.93 (0.12)	1.17 (0.07)	2.41 (0.21)	4.99 (0.79)
SEDS™2	0.56 (0.04)	0.98 (0.06)	1.73 (0.11)	1.10 (0.12)	2.57 (0.91)	6.91 (1.86)
SEDS™3	0.54 (0.02)	0.92 (0.06)	2.52 (0.64)	3.57 (0.34)	6.91 (0.53)	12.25 (0.81)

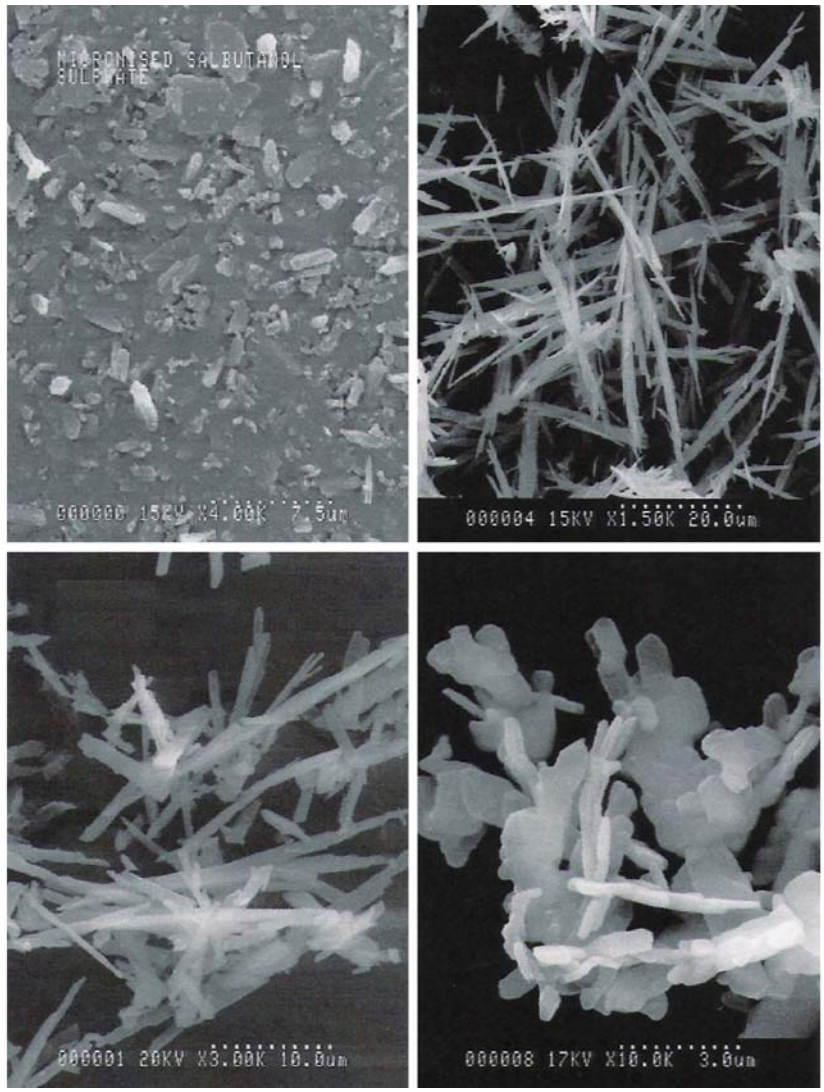

Fig. 1. SEM photomicrographs of **a** micronized, **b** SEDS™1, **c** SEDS™2 and SEDS™3 salbutamol sulfate powders

The aerodynamic particle size distributions measured for the Asmasal™ and SEDS™ DPIs were assessed by ACI and the results given in Table III. The results show the emitted dose (amount deposited on the impactor), FPD (cumulative amount <5.8 μm) and the total amount of salbutamol recovered which equates to the emitted dose plus the amount recovered from the inhaler mouthpiece. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) have also been included for each DPI formulation. Figure 2 shows the deposition of drug on each stage of the ACI as percentage of the emitted dose and indicates that for each of the formulations tested, the largest proportion of the FPD was deposited on stages 3 and 4 of the ACI in the size range 2.1–4.7 μm.

The mean (SD) total recovery for Asmasal™, SEDS™1, SEDS™2 and SEDS™3 formulations was 81.35 (10.15) μg, 87.98 (4.32) μg, 92.78 (1.93) μg and 105.25 (5.98) μg, respectively. Comparison of emitted dose (from ACI) supports the findings of the experiments undertaken using the dose sampling unit with the SEDS™3 formulation demonstrating highest emitted dose of 88.08 (4.86) μg and significantly greater dose emission that the other three formulations ($p<0.05$). The SEDS™3 formulation was shown to give greater FPD than both the SEDS™1 ($p<0.001$) and SEDS™2 ($p<0.01$) formula-

Table II. Emitted Dose of Salbutamol Sulfate (μg) for Each of the Formulation Variants Tested (n=18)

Attribute	Asmasal™	SEDS™1	SEDS™2	SEDS™3
Emitted Dose (μg)	75.33	75.91	78.47	85.63
SD (μg)	4.76	7.37	4.85	4.74
RSD (%)	6.32	9.70	6.18	5.53

Table III. Andersen Cascade Impactor (ACI) Results for the 4 DPI Formulations Measured Using an Air Flow Rate of 28.3 L min^{-1} (n=9)

Particle Size Attribute	Asmasal™	SEDS™1	SEDS™2	SEDS™3 DPI
Emitted Dose (μg)	70.87 (8.66)	73.93 (3.60)	76.65 (1.30)	88.08 (4.86)
Fine Particle Dose (μg)	25.58 (3.03)	17.21 (1.57)	20.50 (1.17)	26.04 (1.81)
Total Recovered (μg)	81.35 (10.15)	87.98 (4.32)	92.78 (1.93)	105.25 (5.98)
MMAD (μm)	3.08 (0.09)	3.38 (0.13)	3.74 (0.04)	4.19 (0.04)
GSD (μm)	1.68 (0.01)	1.63 (0.07)	1.48 (0.02)	1.79 (0.02)

tions but not the Asmasal™ formulation, which demonstrated similar FPD. Although the SEDS™3 formulation gave high FPD, it also produced the largest MMAD of 4.19 μm (0.04) with GSD of 1.79 μm (0.02). Of the four formulations tested, Asmasal™ gave lowest MMAD of 3.08 μm (0.09) with a GSD of 1.65 μm. This finding is supported by the TOF particle size analysis and SEM, which showed smaller particle size for the typical batch of micronized salbutamol sulfate compared to the SEDS™ based materials.

In vivo Evaluation

Healthy Volunteers

Mean data for urinary excretion of salbutamol over the first 30 min post inhalation and the cumulative amount of salbutamol and its metabolite excreted in urine 0–24 h post dose, for each DPI formulation are given in Table IV. The mean (SD) urinary excretion rate time profiles (0–24 h), for salbutamol and metabolite following inhalation from each formulation are given in Fig. 3. The mean amount of salbutamol excreted in the urine of volunteers over the first 30 min after inhalation was 5.21 (1.69), 3.16(1.06), 3.43(1.26) and 5.07 (2.23) μm for the Asmasal™, SEDS™1, SEDS™2 and SEDS™3 formulations, respectively. There was no significant difference in the amount of salbutamol recovered in urine, 30 min post-inhalation, between the Asmasal™ and SEDS™3 formulations or between the SEDS™1 and SEDS™2 formulations. Significantly more salbutamol was however excreted in the first 30 min following inhalation by the Asmasal™ and the SEDS™3 formulations compared to the SEDS™1 formulation (p<0.05).

The mean amount of salbutamol and metabolite excreted in urine over the 24 h period post-inhalation was 54.08 (14.80), 35.07 (11.49), 32.54 (12.12) and 51.74 (15.13) for the respective Asmasal™, SEDS™1, SEDS™2 and SEDS™3 formulations. When expressed as a percentage of the nominal dose, the respective amounts of salbutamol excreted over 24 h were 28.46% (7.79), 18.46% (6.05), 17.13% (6.38) and 27.23% (7.96). For the 24 h time point, significantly more salbutamol and metabolite was excreted for the Asmasal™ (p<0.01) and SEDS™3 (P<0.05) formulations compared to the SEDS™1 and SEDS™2 formulations. There was no significant difference in the amount of salbutamol and metabolite recovered in urine over the 24 period post-inhalation, between the Asmasal™ and SEDS™3 formulation and no difference between the SEDS™1 and SEDS™2 formulations. Due to co-administration of oral charcoal, these values are representative of the total lung deposition of salbutamol from each formulation.

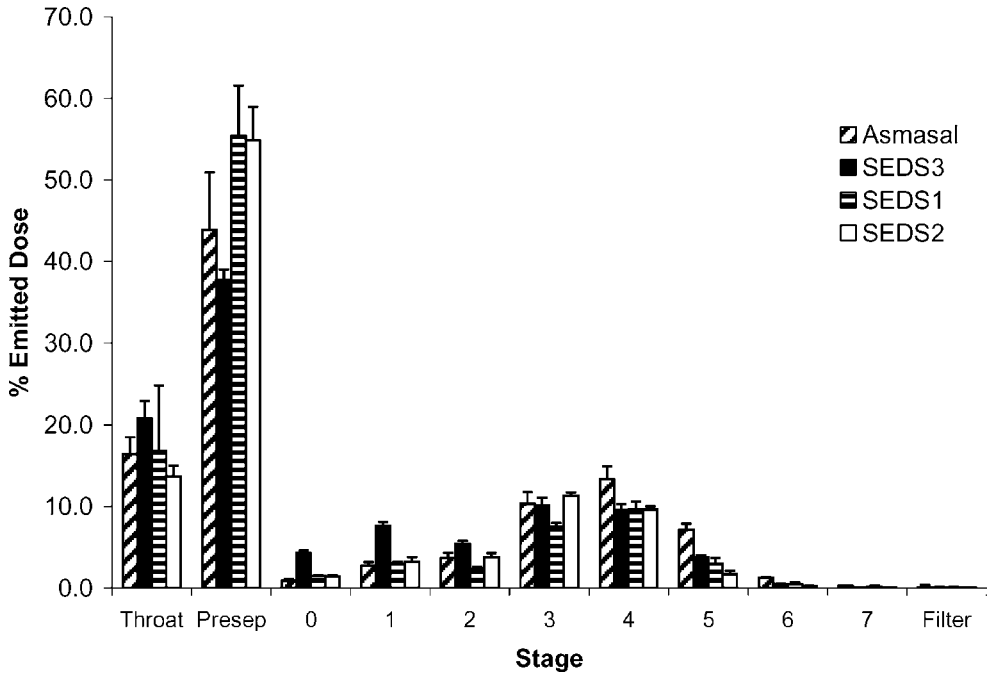

Fig. 2. ACI Deposition profiles for the Asmasal™, SEDS™1, SEDS™2 and SEDS™3 formulations showing mean (SD) percent emitted dose deposited on each stage of the ACI using airflow of 28.3 l/min (n=9)

Table IV. Mean Urinary Salbutamol Excretion Data for Each DPI Formulation in Healthy Volunteers and Patients

Mean Urinary Salbutamol Excretion (SD)	Asmasal™	SEDS™1	SEDS™2	SEDS™3
First 30 min Post-inhalation (Volunteers)	5.21 (1.69)	3.16 (1.06)	3.43 (1.26)	5.07 (2.23)
24 h Period Post-inhalation (Volunteers)	54.08 (14.80)	35.07 (11.49)	32.54 (12.12)	51.74 (15.13)
First 30 min Post-inhalation (Patients)	1.71 (1.06)	0.90 (0.63)	0.81 (0.61)	1.77 (1.12)

Patients

The mean amount (μg) of urinary salbutamol excreted over the first 30 min for each DPI formulation in patients is given in Table IV. The levels of drug recovered in urine at 30 min after inhalation in patients were low and typically less than those observed in healthy volunteers. There was no statistically significant difference ($P>0.05$ ANOVA) in urinary salbutamol excretion at 30 min post-inhalation between any of the four DPI formulations, although the trends in data were similar to those observed for urinary salbutamol excretion in healthy volunteers.

The mean percentage increase in FEV_1 from the pre-treatment baseline at 15, 35, 65, 90 and 125 post-inhalation following delivery of salbutamol sulfate from the different DPI preparations is given in Table V. The clearest differences in FEV_1 increase were typically observed at 125 min after inhalation. The mean increases in FEV_1 from the baseline at 125 min following administration of the Asmasal™, SEDS™1, SEDS™2 and SEDS™3 formulations were 15.55% (7.01), 8.45% (2.88), 8.64% (3.75) and 14.64% (7.59), respectively. There was no significant difference in FEV_1 increase between the Asmasal™ and the SEDS™3 formulations. There was also no significant difference between the SEDS™1 and SEDS™2 formulations ($P>0.5$). There were however differences between the Asmasal™ ($p<0.001$) and the SEDS™1 and 2 formulations, and also the SEDS™3 formulation when compared to the SEDS™1 ($p<0.01$) and SEDS™2 ($p<0.001$) formulations.

DISCUSSION

The total amount of unmetabolized and metabolized salbutamol recovered in urine up to 24 h post treatment in healthy volunteers, when charcoal is co-administered, is considered to be an indicator of the total amount of salbutamol delivered to the lung. Evaluation of the 24 h urinary excretion data in volunteers has indicated that the lung bioavailability of salbutamol following administration by Clickhaler™ was similar for the Asmasal™ and the SEDS™3 DPIs, which performed better than the SEDS™1 and SEDS™2 formulations. Evaluation of data for urinary salbutamol excretion over the first 30 min after inhalation also indicated that the lung bioavailability for the Asmasal™ and SEDS™3 formulations did not differ significantly. Although these formulations were shown to perform better when compared to the SEDS™1 DPI, the data did not indicate a statistically significant difference in urinary salbutamol excretion over the first 30 min, when compared to the SEDS™2 formulation.

For the early time point of 30 min, factors such as drug dissolution, absorption and renal elimination will limit the appearance of salbutamol in the urine of volunteers and so influence the magnitude of differences between the urinary salbutamol levels observed for the different formulations. The majority of salbutamol, which has deposited in the lungs, is likely to have been absorbed within the 24 h period after inhalation as demonstrated by the low salbutamol excretion rates at the later time points shown in Fig. 3. In this example, where volunteers have received activated charcoal to exclude gastrointestinal absorption of salbutamol, the measurement of urinary levels of salbutamol and its sulfate metabolite at 24 h after inhalation should provide the more reliable indicator of relative lung bioavailability for each of the formulations studied.

For the four formulations studied, post-treatment improvements in patient lung function followed a similar trend to the indices of relative lung bioavailability determined in healthy volunteers. The delivery of salbutamol from each DPI formulation was shown to provide pre-treatment improvements in FEV_1. There was however, no significant difference in FEV_1 increase between the Asmasal™ and

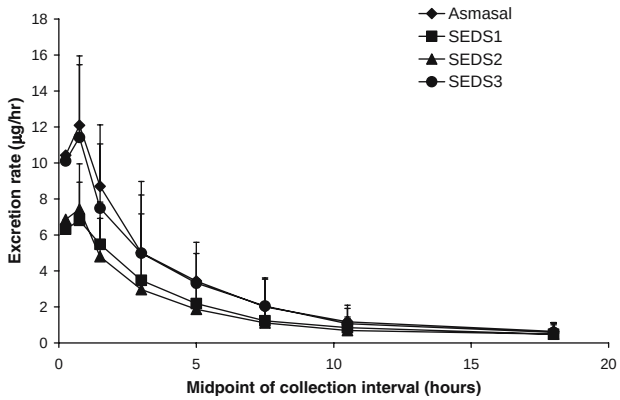

Fig. 3. Mean (SD) urinary excretion rate time profile for salbutamol and metabolite following inhalation of Asmasal™, SEDS™1, SEDS™2 and SEDS™3 DPI plus charcoal

Table V. Percentage Increase in FEV_1 in Patients Compared to the Pre-treatment Baseline Over the 125 min Period Post-inhalation

Pharmacokinetic Measurement	Asmasal™	SEDS™1	SEDS™2	SEDS™3 DPI
Mean FEV_1 Increase (%) at 15 min	13.55	10.73	7.73	14.91
SD (%)	4.74	5.69	3.69	6.61
Mean FEV_1 Increase (%) at 35 min	15.91	9.91	8.45	13.82
SD (%)	5.43	4.13	3.30	6.94
Mean FEV_1 Increase (%) at 65 min	16.36	11.82	8.73	12.82
SD (%)	5.39	6.00	4.34	7.05
Mean FEV_1 Increase (%) at 90 min	15.36	10.09	8.73	13.36
SD (%)	5.33	3.94	3.13	7.39
Mean FEV_1 Increase (%) at 125 min	15.55	8.45	8.64	14.64
SD (%)	7.01	2.88	3.75	7.59

SEDS™3 formulations. There was also no difference between the improvements in FEV_1 for the SEDS™1 and SEDS™2 formulations. There were however significant differences between the Asmasal™ and the SEDS™1 and 2 formulations, and also the SEDS™3 formulation when compared to the SEDS™1 and 2 formulations.

The amount of salbutamol recovered from the urine of patients 30 min after inhalation was in general markedly lower and more variable than that measured for the healthy volunteers after administration of equivalent formulations. Lipworth and Clark have previously demonstrated that the lung bioavailability of inhaled salbutamol is dependent on the severity of disease and the associated airway calibre of asthmatic patients (23). It is probable therefore, that patients have received lower lung doses of salbutamol in this study than the healthy volunteers. However, the increases in FEV_1 from the pre-treatment baseline suggest that sufficient salbutamol is reaching the lungs to demonstrate a bronchodilator effect. The differences in FEV_1 increase observed for the different formulations can therefore be ascribed to related differences in lung deposition for the formulations studied.

Evaluation of pharmacokinetic and pharmacodynamic data from the respective studies suggest that there is a link between FPD for each formulation measured using the ACI, the urinary excretion of salbutamol and metabolite 24 h after inhalation in volunteers and post-treatment improvements in FEV_1 in mild asthmatic patients across all time points, but particularly at 125 min after inhalation. Both the Asmasal™ and SEDS™3 formulations demonstrated greatest FPD and subsequently gave highest relative lung bioavailability in healthy volunteers and greatest bronchodilator response in patients. For these formulations, the largest proportion of the FPD was deposited on stages 3 and 4 of the ACI in the size range 2.1–4.7 μm as shown in Fig. 2. In accordance with the findings of Usmani et al. (24), it is probable that the particles in this size range have deposited primarily in the central and intermediate airways, giving rise to improved bronchodilator responses in patients. Usmani et al. (24) using monodisperse aerosols of salbutamol showed that particles with MMAD of 6 and 3 μm gave notably greater bronchodilation than smaller 1.5 μm particles. Using gamma scintigraphy, this was ascribed to greater proximal deposition of the 3 and 6 μm particles, where the conducting airways, comprising bronchial smooth muscle are considered to be the appropriate site of action for short acting β2-agonists. Although the 1.5 μm particles showed greater total lung deposition, these smaller particles were primarily delivered distally within the lungs to the alveolar region.

In previous studies of aerosol deposition, researchers have investigated the relationships between mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD) and aerosol lung deposition (25,26). When comparing the median particle diameter (d_{50}) from TOF analysis (number distribution) shown in Table I and the MMAD from ACI measurements given in Table III, it is clear that notable agglomeration of API with itself or fine particle lactose has occurred during preparation of the formulations, which has influenced dose emission and aerodynamic performance of the different DPIs. For each material studied, the d_{50} for particles dispersed in the air stream during TOF analysis was typically less than 1 μm, which would suggest the potential for notable exhalation with some alveolar deposition (27). The MMAD of each formulation measured using the ACI was however greater than 3 μm leading to marked lung deposition and subsequent bronchodilator responses. However, although there were differences in MMAD for each formulation, there was no discernible relationship between these factors and the *in vivo* indices of lung deposition. In particular, it is clear that the SEDS™3 formulation, which demonstrated highest MMAD and GSD, performed similarly in the in vivo experiments to the commercial Asmasal™ product. This suggests that in this instance, FPD is the more appropriate indicator of *in vivo* performance.

The FPD measured by ACI was shown to be similar for both Asmasal™ and SEDS™3 DPIs. Although fine particle fraction was greater for Asmasal™ than for SEDS™3 (36.1 versus 29.6%), the emitted dose for the former was markedly lower. The low total recovery for Asmasal™ (81.35 μg) shown in Table III suggests that a notable proportion of drug remains in the dosing chamber following actuation of this formulation. Data for the SEDS™1 and SEDS™2 formulations also demonstrated lower dose emission and total recovery than the SEDS™3 DPI.

It is probable that the larger primary particle size of SEDS™3 salbutamol sulfate, was responsible for enhanced flow characteristics and subsequent improved release from the dosing chamber of the Clickhaler™. For the SEDS™1 and SEDS™2 formulations, the elongated particles, such as those shown in Fig. 1 are likely to suffer from poorer flow (28) than the wafer like SEDS™ particles and as a consequence provide less desirable dose emission. Accounting for the low

dose emission of the Asmasal™ DPI, Feeley et al. have suggested that micronization of salbutamol sulfate increases the particle surface energy leading to more cohesive powders with less favourable flow characteristics (29).

Although the emitted doses for SEDS™1 and SEDS™2 DPIs were similar to the commercial Asmasal™ inhaler, the fine particle fraction measured for these systems was markedly lower than determined for the commercial product at 23.3% and 26.7% respectively. In studies of the SEDS process, Schiavone et al. suggested that increased surface smoothness and reduced surface free energy of SEDS™ materials are presumed to minimize irreversible drug-carrier particle interactions resulting in more efficient drug detachment during aerosolization (9). Furthermore, Podczeck has suggested that fine carrier particles in interactive mixtures may form agglomerates with micronized drug and deposit in the airways according to the aerodynamic properties of the agglomerates rather than that of primary drug particles (4). In this study, it possible that micronized salbutamol sulfate with its greater surface free energy has interacted more effectively with fine lactose particles than the SEDS™1 and 2 based materials. This will have resulted in greater deposition of drug on the lower stages of the ACI, improved FPD and therefore enhanced relative lung bioavailability for the Asmasal™ product versus the two SEDS™ based systems.

CONCLUSIONS

This study has shown that salbutamol sulfate produced by the SEDS™ process can be delivered to the lungs of human volunteers and asthmatic patients by DPI with similar effectiveness to the commercial Asmasal™ product. FPD measured using the ACI operated at a flow rate of 28.3 l/min appears to be a good predictor of *in vivo* lung bioavailability and clinical effect for the formulations studied.

Whilst, the SEDS™ process could be considered as a suitable alternative to conventional micronization, the dose emission, lung bioavailability and clinical effect appear to be dependent on inter-batch differences in particle characteristics such as size, shape and probably surface free energy. Of the three SEDS™ batches studied, salbutamol sulfate with largest particle size and wafer-like morphology gave best FPD, greatest relative lung bioavailability and most improved lung function in patients.

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