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Title: A Novel Breath-Actuated Integrated Vortex Spacer Device increases relative lung bioavailability of Fluticasone/Salmeterol in Combination

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Abstract:**Background**

Spacer devices facilitate respirable drug delivery. A novel breath actuated antistatic spacer with integrated vortex chamber (Synchro-Breathe™) device has been developed, which is compact, portable and user friendly as compared to conventional spacers which are bulky and cumbersome. The relative bioavailability to the lung of inhaled fluticasone and salmeterol combination is primarily dependant on respirable dose delivery and can be reliably quantified using adrenal suppression and early fall in serum potassium (marker of systemic beta-2 adrenoreceptor response) as surrogate markers for delivered lung dose.

Aims & Objectives

To compare the in vivo relative bioavailability to the lung of Hydrofluoroalkane (HFA) Seretide™ delivered via Synchro-Breathe(SB); an optimally prepared 750 ml large volume plastic spacer, Volumatic™ (VM); and conventional Evohaler pMDI (EH).

Methods

Nineteen Healthy Volunteers completed the study using a randomised double blind, double dummy crossover design. Single doses of placebo or Seretide HFA 250 (total dose ex-valve: Fluticasone 2000 mcg/ Salmeterol 200mcg) were administered via SB, VM and EH. Overnight urinary cortisol/ creatinine (OUCC) and serum potassium (K) were measured at baseline and after each dose as systemic surrogates of relative respirable dose delivery for the fluticasone and salmeterol moieties respectively.

Results

Significant suppression of OUCC and K occurred from baseline with SB and VM but not EH devices (geom. mean fold suppression, 95%CI, p and arithmetic mean fall mmol/l, 95%CI respectively); EH: 1.51(0.43 to 1.01), p=0.06 ; VM: 2.52(1.57 to 4.04) p<0.001; SB: 2.66(1.57 to 4.49), p<0.001 (equating to 33.8%, 60.2% and 62.3% falls respectively). For K, the falls for EH were -0.09 (-.25 to .07), p=0.69; VM: -0.27(-.46 to -.08) p=0.003; SB: -0.32(-.53 to -.11) p=0.002 (equating to 2.2%, 6.8%, and 8.06% fall respectively). There were no significant differences between SB and VM.

Conclusion:

The breath actuated Synchro-Breathe device was comparable to an optimally prepared Volumatic spacer, and resulted in commensurate improvement in relative lung bioavailability for both Fluticasone and Salmeterol moieties compared to pMDI.

Key Words: Spacer; Lung bioavailability; Inhalers; Asthma; Seretide

ACCEPTED MANUSCRIPT

Introduction:

The primary aim of inhaled therapy is to attain objective improvements in airway caliber and function by targeted drug delivery. The delivery of respirable particles may be achieved by the use of pressurized metered dose inhalers (pMDI), which are notoriously difficult to coordinate.[1, 2] Spacer devices or valved holding chambers are recommended to further enhance respirable dose delivery, avoid problems with coordination [3, 4] and are dependent on specific drug device interactions.[5, 6]

Combination inhalers which consist of long acting beta agonists (LABA) and inhaled corticosteroids (ICS) are now recommended at step 3 of asthma management guidelines[3, 4] They provide the dual benefit of targeting airway inflammation and bronchodilation with a single device, thus potentially encouraging compliance. The use of combination inhalers results in clinically relevant improvements in symptoms, lung function, and exacerbations and is superior when compared to doubling the dose of ICS in asthmatics.[7-10]

The relative degree of lung dose delivery of inhaled drugs can be quantified by measuring the systemic bioavailability, which in turn is dependant on pulmonary and gut absorption. Systemic bioavailability also determines the propensity for systemic side effects. Salmeterol(SM) is a highly lipophilic compound and is approximately 8 times as potent as salbutamol (weight per weight).[11-13] The swallowed fraction of this drug contributes to 28-36% of its systemic bioavailability[14] Fluticasone (FP) on the other hand has negligible oral bioavailability on account of its near complete hepatic first pass mechanism of the swallowed fraction[15, 16] Therefore with regard to systemic bioavailability, with fluticasone it depends entirely on lung absorption[16], while for salmeterol it depends predominantly on lung absorption.

Lung deposition of inhaled drugs occurs by inertial impaction (in the large conducting airways and mouth) and by gravitational sedimentation in the smaller conducting airways and alveoli. Spacer devices work on the principle of reducing aerosol velocity and droplet size, thus causing a reduction in large particle deposition in the mouth. Hence, they improve the fine particle respirable dose (FPD) and reduce local side effects like oral candidiasis and dysphonia associated with inhaled steroids. Conventional spacer devices are however bulky, cumbersome and plastic spacers in particular have inherent electrostatic charge which impairs spacer performance and consequent respirable drug delivery.[17-19]

A novel palm sized breath-actuated spacer device with an integrated vortex chamber (Synchro-Breathe[SB], Vortran Medical Technology Inc, Sacramento, CA, USA) (Figure 1) has been developed and is more compact than a conventional valved holding chamber with pMDI, and has none of the problems with electrostatic charge. The SB has a reduced actuator orifice diameter with a unique vortex extension that creates turbulent ex-valve flow producing a slower plume velocity when compared to a pMDI alone. This also alleviates gagging due to throat impaction.

The systemic bioavailability of inhaled FP depends on absorption via the lungs and this is in turn reliant on delivered lung dose. Therefore sensitive surrogates of relative lung bioavailability help assess relative lung dose delivery of inhaled FP from different spacer devices. Suppression of overnight urinary cortisol creatinine ratio(OUCC), is a sensitive surrogate for systemic bioavailability and relative lung dose delivery.[5, 20-22] For example in a previous study we found that the SB device increased the respirable dose delivery (as adrenal suppression) of inhaled FP by 1.6 fold in mild to moderate asthmatics, when compared to an optimally used pMDI. The relative respirable delivery of beta-2 agonists like SM can be quantified by measuring the early fall in serum potassium(K) as a systemic beta-2 adrenoreceptor response, and therefore can be used as a marker of relative lung dose.[23] In this regard, the early fall in serum K reflects predominantly lung rather than gut absorption, especially when using a spacer device which reduces its systemic bioavailability from the swallowed fraction.

We are not aware of any published studies looking at the relative bioavailability of both active components of Seretide to the lungs from spacer devices compared to a pMDI, in terms of measuring the simultaneous effects on OUCC and K. The aim of the present study was therefore to compare the relative bioavailability of FP and SM moieties of Hydrofluoroalkane (HFA) Seretide (FP/SM) 250 delivered to the lungs via the Synchro-Breathe device and Evohaler pMDI. In addition we elected to use a 750 ml large volume plastic Volumatic™ (VM) spacer as the standard comparator.

Methods:

Patients: Twenty non-smoking healthy subjects between the ages of 18 to 65 years were recruited from our existing database of research volunteers. The Tayside Committee on Medical Research Ethics scrutinized and approved the protocol

Study Design: This was a single centre, randomized double-blind, double-dummy study using a three way crossover design. The details of the randomization and blinding process are given below. Each subject had 2 placebo pMDI canisters and 1 active Seretide pMDI canister which were independently labelled, blinded and packed under supervision of the clinical trial pharmacist into separate treatment packs based on the computerised randomization sequence available online at www.randomization.com. Evohaler actuators were used throughout except when using the Synchro-Breathe. The Placebo/active canisters were independently inserted into the Evohaler actuator and the Synchro-Breathe device prior to use. As the Volumatic, Evohaler and Synchro-Breathe are visually different, further blinding of the trial via the double dummy technique was carried by using the pMDI via each of the three devices at each study visit. Thus the participants and the researchers had no way of identifying which device was delivering the active drug even though they were all being used at each study visit.

At their initial screening suitability was checked and informed consent was obtained. All volunteers had a full physical examination, spirometry, evaluation of baseline biochemistry at screening and if deemed suitable were randomized into the study. A pre-labeled container with written instructions on overnight urine collection was handed to the volunteers. This was brought in on the morning of the first study visit from the volunteer's residence by a taxi service, so as to minimize patient visits and encourage compliance.

There were three study visits in total after screening and were held 5-7 days apart to enable a steroid free washout period with FP.[24] Optimum inhaler technique was demonstrated by the researcher at screening and rechecked at each study visit. The study visits all took place at 4 pm. An intravenous cannula was inserted in to the forearm ante-cubital vein and was flushed with 10 ml of normal saline to maintain patency. After thirty minutes of rest, at 4.30 pm, a baseline sample (5ml) of blood was collected and the following treatments were administered under supervision in a randomized double blind, double-dummy fashion. At any given study visit only one of the devices delivered the active treatments: (1) Eight puffs of HFA placebo or active FP/SM combination (Seretide™ 250: FP 250 mcg ex-valve and SM 25 mcg ex-valve per actuation (total dose ex-valve FP 2000 mcg & SM 200 mcg) via the Evohaler [EH] pMDI, Volumatic[VM], and Synchro-Breathe[SB] spacer devices

respectively. Although the dose used does not reflect standard clinical practice in asthma, it was chosen to ensure an adequate systemic signal, in terms of suppression of OUCC and K was detectable to enable comparisons between spacer devices.

The pMDIs used in the study were all primed by shaking them and discharging 5 puffs prior to use. The volunteers were then asked to exhale to residual volume, and the inhaler was actuated at the beginning of inhalation and steady slow inhalation was maintained till total lung capacity and was followed by breath holding for ten seconds or for as long as possible. After 30 seconds, the procedure was repeated for the remaining doses. The pMDI canister was inserted in to the SB device and used exactly like a conventional pMDI. The principal difference being that the SB device was breath actuated. The VM spacer was pre-washed in lukewarm mild soapy detergent and allowed to drip dry prior to use so as to minimize electrostatic charge and improve respirable drug delivery.[17, 25] Single puffs were inhaled without delay between actuation and inhalation to optimize lung dose deposition.[17-19] There was a 30 second delay between inhalations and the procedure was repeated for each of the eight inhalations.

Measurements:

Assessment of systemic FP lung bioavailability: On the night prior to the first study visit, the volunteers were instructed to collect 10 hour overnight urine in pre-labeled sealed container. The volunteers were given written instructions and asked to empty their bladder at 10:00 PM and to collect all of the voided urine till 8:00 AM. The total volume of urine sample was recorded, and aliquots were taken for urinary cortisol and creatinine for overnight (10 hour) urinary cortisol creatinine measurements. The procedure was repeated on the night following completion of drug inhalation at each of the study visits.

Assessment of serum K for Salmeterol: An intravenous cannula was inserted in to the ante-cubital vein at the start of the study visit and was flushed with 10 ml bolus of normal saline to maintain patency. Baseline Serum K (5ml) was collected and was repeated 1 hour post study drug inhalation to measure the early fall in K which predominantly reflects lung rather gut absorption .

Assessment for adverse events: Automated Heart rate, blood pressure, and visual tremor score scales were monitored at 15 minute intervals for 1 hour period to help monitor any immediate adverse effects to Salmeterol

Assays:

All assays were performed in duplicate in a blinded fashion. The urinary cortisol was measured using a commercial radioimmunoassay kit which has no cross reactivity for FP (Diasorin Ltd, Wokingham, Berkshire, UK). The intra assay coefficient of variation was 4% and the inter assay coefficient of variation was 8%. Urinary creatinine was measured on a Cobas-Bio auto analyzer (Roche Products, Welwyn Garden City, UK). The inter and intra assay coefficient of variation was 3% and 4.6 % respectively. Serum Potassium was analyzed using the Roche Modular Multi ISE 1800 auto analyzer and the co-efficient of variation of the test was 1.2%

Statistical Analysis:

The primary endpoint was overnight urinary cortisol-creatinine ratio. A sample size of 16 completed patients per protocol was chosen to power the study at 80% to detect a 20% difference in overnight urinary cortisol-creatinine ratio, using data from a previous study which detected a 50% difference between spacer and pMDI with a sample size of 14 completed patients.[22]

Data sets were analyzed for patients who completed the crossover study per protocol. All data was tested for normality prior to analysis. The OUCC data was log transformed, and K data was analyzed without transformation in view of its normal distribution. Comparisons were made using repeated measures General Linear Model [GLM] Analysis of Variance with Bonferroni correction for multiple comparisons, set with 95% confidence intervals for differences. All effects are reported as being significant $<.05$ (2 tailed) and violation of sphericity of within subject effects was tested with the Mauchly's test. The analysis was carried out using SPSS Version 13.

Results:

Twenty subjects were randomized in to the study and 19 completed the study per protocol (Table1). The mean age was 25.6 years (SE 2.2). Of the 19 subjects, ten were women and 9 were men. One subject (No.16) was completely excluded from overall analysis due to non compliance with protocol. The serum potassium samples of three subjects were haemolysed and hence 16 paired samples for K were analyzed (n=16).

Significant suppression of the OUCC (geom. mean fold suppression, 95%CI, p) and Serum K (arithmetic mean fall mmol/L, 95%CI, p) from baseline (Tables 2&3) occurred with both SB and VM, but not with EH pMDI: For OUCC, these were: 1.51(0.43 to 1.01), $p=0.06$ for EH; 2.52 (1.57 to 4.04) $p<0.001$ for VM; 2.66 (1.57 to 4.49), $p<0.001$ for SB (equating to 33.8%, 60.2% and 62.3% suppression respectively). For Serum K, the mean falls were: -0.09 (-0.27 to 0.08), $p=0.69$ for EH;

-0.28(-0.46 to -0.09) $p=0.003$ for VM; -0.33(-0.54 to -0.11) $p=0.002$ for SB (equating to 2.2%, 6.8%, and 8.06% fall respectively).

Significant differences in OUCC and K were demonstrated between EH pMDI and SB and VM (Table 4). When compared to the EH pMDI, the SB device resulted in 1.75 geometric mean fold greater suppression of OUCC 95 % CI (1.04 to 2.95), $p=0.03$ and resulted in a 0.23 mmol/L greater fall in K 95%CI (-0.40 to -0.06), $p=0.007$ (equating to a 43% and 6% greater fall in OUCC & K respectively). Similarly when compared to EH pMDI, the geometric mean fold suppression in OUCC with the VM spacer was 1.66 (0.97 to 2.25), $p=0.07$ and the arithmetic mean fall in K was 0.18mmol/L (-0.36 to -0.002), $p=0.04$ (equating to a 39.9% and 4.7% greater fall in OUCC and K respectively).

There was no significant difference between the SB and VM for the two moieties of FP and SM. For OUCC, the geometric mean fold differences was 1.05(0.62 to 1.76), $p=1$ and for K the arithmetic mean fall (95%CI) was -.05 mmol/l (-0.25 to 0.15), $p=1$.

The following adverse events were reported by the study participants. Three patients reported palpitations, although there was no objective evidence of tachycardia or change in blood pressure recorded at the time. Eight individuals experienced mild tremors as observed on Visual Analogue Scale: one with Synchrobreathe only; three with Volumatic only; three with both Volumatic and Synchrobreathe; one with all three devices.

Discussion:

This is to the best of our knowledge the first in vivo study to demonstrate commensurate improvements in the relative bioavailability to the lung of both active moieties of FP/SM in combination via a spacer device compared to pMDI alone. Pointedly, the compact breath actuated Synchro-Breathe device produced commensurate improvements in relative bioavailability to the lung and therefore improved respirable dose delivery which were comparable to the reference device, namely an optimally used 750 ml large volume Volumatic spacer (Figure 2&3). In this regard the numerical difference between Synchro-Breathe and Volumatic amounted to a 1.05 fold difference (equating to 5.2% difference) in OUCC and a 0.05 mmol/L difference in K (equating to 1.3% difference). Dempsey et al([22] in healthy volunteers had previously demonstrated that the use of VM spacer with CFC-FP via pMDI increased the suppression of OUCC by nearly 1.9 fold ,as compared to a value

of 1.66 fold greater suppression with HFA-FP in the present study. In this regard a greater degree OUCS suppression with CFC vs. HFA-FP has been previously documented[26, 27] when using pMDI alone in either healthy volunteers or asthmatics.

There are only limited in vitro and in vivo data on the relative respirable dose delivery of inhaled SM via spacer devices.[28, 29] We believe that the early fall in serum potassium is a valid surrogate of the early lung absorption profile of inhaled beta-2-agonists support previous data showing that serum K highly co-related with plasma salbutamol levels.[23]

The results obtained from our healthy volunteer study need to be evaluated bearing in mind the effect of airway caliber on lung absorption of FP. Although higher doses of FP are often used in more severe asthmatics patients, the effects reduced airway caliber will tend to reduce lung absorption of inhaled FP, and hence are less likely to develop systemic adverse effects.[30] However the relative degree of OUCS suppression will be similar in healthy or asthmatic subjects when comparing devices, as evidenced by the 1.75 fold increase in respirable dose delivery of inhaled HFA-FP from combined FP/SM combination delivered via the SB device vs. pMDI in healthy volunteers in the present study compared to the 1.59 fold increase previously noted via the SB device vs. pMDI in asthmatics.[31] In this regard previous in vitro data have suggested that a higher respirable dose of FP and SM is delivered by the combination inhaler when delivered via a single inhaler than separate inhalers $p<0.001$.[32]

In the current study, we have used the pMDI and the Volumatic spacer under optimal conditions using the correct technique which is unlikely to be the case in real life.[33] Therefore it is likely that the performance of a new unwashed VM device with multiple puffs and a delayed actuation-inhalation sequence will be less efficient due to effects of static charge, when compared to the optimal method used in the present study.[17, 19] The observed differences between the pMDI and the Volumatic in our study are perhaps an overestimation of the true difference one would expect to see in day to day clinical practice. In this regard SB device is breath actuated and is not influenced by electrostatic charge like plastic holding chambers, so that the observed improvements in relative lung bioavailability are likely to be the same or even greater in real life, due to inherent problems with pMDI coordination.

It is also important to stress that we did not attempt to evaluate the efficacy of FP/SM combination via the different delivery devices as we only used a single dosing study in healthy volunteers. Nonetheless we believe that the observed increases in respirable dose delivery are likely to translate into commensurate improvements in clinically control or reduce the nominal dose during step down. However, one needs to be aware that increased respirable dose delivery can be a two edged sword, as it raises the possibility of increased systemic adverse effects with FP on chronic dosing. With regard to the SM moiety, any systemic adverse effects with repeated dosing are likely to be diminished due to tachyphylaxis of response due to beta2-adrenoceptor down regulation and subsensitivity of response.[34]

In conclusion, the use of a novel breath actuated integrated Synchro-Breathe device resulted in commensurate increase in the relative degree of lung dose delivery of both moieties of FP/SM in combination versus pMDI alone, and was comparable to a 750 ml large volume plastic Volumatic spacer. Further dose response studies in asthmatics are needed to assess whether the improved respirable bioavailability results in commensurate improvements in the relative therapeutic ratios of FP/SM.

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Disclosure: There are no conflicts of interest for any of the authors and the University of Dundee has had no role in the design, conduct, analysis, and preparation of this manuscript.

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Figure Legends:

1. Synchro-Breathe breath actuated integrated vortex spacer device and Volumatic device with a pMDI (Seretide™ 250 Evohaler). The Volumatic has been optimally prepared and pre-washed in mild soapy detergent and drip dried prior to use.
2. Individual values of overnight urinary cortisol creatinine ratio with geometric mean at baseline and after inhalation of a single ex-valve dose of 2000 mcg FP and 200 mcg SM via the pMDI Evohaler, Volumatic and Synchro-Breathe devices plotted on a logged scale.
3. Individual values of serum potassium with arithmetic mean at baseline and after inhalation of 200 mcg of SM and 2000 mcg of FP via pMDI Evohaler, Volumatic and Synchro-Breathe devices.

Table Legends:

Table 1: Patient Demographics at baseline.

Table 2: Relative Overnight urinary cortisol creatinine ratios (OUCC) and Potassium at baseline and after 2000 mcg of FP and 200mcg of SM combination pMDI.

Table 3: Geometric mean fold suppression of overnight urinary cortisol creatinine ratio (OUCC) and arithmetic mean fall in Serum potassium from baseline after inhalation of 2000 mcg of FP and 200mcg of SM combination pMDI via Evohaler, Volumatic and Synchro-Breathe devices.

Table 4: Between device comparisons of geometric mean fold differences in Overnight Urinary cortisol creatinine ratio (OUCC) and arithmetic mean differences in serum potassium after inhalation of 2000 mcg of FP and 200mcg of SM combination pMDI via the Evohaler, Synchro-Breathe and Volumatic devices.

Table 1: Patient Demographics at baseline

Patient no	Sex	Age (Years)	FEV ₁ (L/s)	FEV _{1%} (%predicted)	OUCC ratio (mmol/nmol)	Serum K+ mmol/L
1	F	23	2.45	93	2.35	3.93
2	M	36	5.47	118	8.98	4.03
3	M	31	3.93	89	7.46	4.03
4	F	25	2.84	89	8.05	4.1
5	M	21	4.38	97	4.22	4.2
6	F	24	3.08	108	8.42	4.07
7	M	19	4.78	109	7.61	4.2
8	F	19	2.95	95	7.86	4.2
9	F	22	3.3	86	7.9	4.13
10	F	21	3.41	104	8.7	4.03
11	M	24	4.75	109	4.61	3.97
12	F	59	2.64	110	8.92	3.93
13	M	20	4.48	110	4.46	4.13
14	F	19	3.37	103	3.69	3.87
15	M	36	3.42	81	10.08	4.1
17	F	22	2.15	75	8.07	3.9
18	M	20	4.64	98	5.79	4.33
19	M	20	4.29	99	7.38	4.5
20	F	25	2.56	87	3.82	4.13
Mean (SEM)		25.6 (2.2)	3.63 (0.22)	97.90 (2.63)	6.32* (0.58)	4.09 (.03)

Abbreviations: FEV₁: Forced expiratory volume in 1 second; OUCC: Overnight urinary cortisol creatinine ratio; K: Serum Potassium

* Geometric mean and (standard error of geometric mean)

Table 2: Relative Overnight urinary cortisol creatinine ratios (OUCC) and Potassium at baseline and after 2000 mcg of FP and 200mcg of SM combination via the pMDI Evohaler, Volumatic and Synchro-Breathe spacer devices.

	OUCC* (mmol/nmol) N=19	Potassium† (mmol/L) N=16
Baseline	6.32(0.58)	4.09(.03)
Evohaler	4.18(0.64)	4.00(.06)
Volumatic	2.51(0.33)	3.81(.07)
Synchro-Breathe	2.38(0.36)	3.76(.07)

* Geometric Mean (SEGM)

† Mean (SE)

Table 3: Geometric mean fold suppression of overnight urinary cortisol creatinine ratio (OUCC) and arithmetic mean fall in Serum potassium from baseline after inhalation of 2000 mcg of FP and 200mcg of SM combination via pMDI Evohaler, Volumatic and Synchro-Breathe devices.

Device	OUCC*	Potassium† (mmol/L)
Evohaler	1.51(0.43 to 1.01), p=0.063	-0.09(-0.25 to 0.07), p= 0.69
Volumatic	2.52(1.57 to 4.04), p<0.001	-0.28(-0.47 to -0.09), p=0.003
Synchro-Breathe	2.66(1.57 to 4.49), p<0.001	-0.33(-0.54 to -0.11), p=0.002

* Geo Mean fold suppression, 95% CI, p

† Arithmetic Mean fall mmol/L, 95% CI, p

Table 4: Between device comparisons of geometric mean fold differences in Overnight Urinary cortisol creatinine ratio (OUCC) and arithmetic mean differences in serum potassium after inhalation of 2000 mcg of FP and 200mcg of SM combination via the pMDI Evohaler, Synchro-Breathe and Volumatic devices.

Device	OUCC* N=19	Potassium (mmol/L) N=16
Evohaler vs. Volumatic	1.66(0.97 to 2.85), p=0.07	-0.18(-0.36 to -0.002), p=0.04
Evohaler vs. Synchro-Breathe	1.75(1.04 to 2.95), p =0.03	-0.23(-0.40 to -0 .06), p=0.007
Volumatic vs. Synchro-Breathe	1.05(0.62to 1.76), p=1	-0.05(-0.25 to 0.15), p=1

*Geom Mean fold differences, 95% CI, p

† Arithmetic Mean fall, 95% CI, p



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