Pulmonary Pharmacology & Therapeutics 52 (2018) 7–17

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

Effect of a spacer on total systemic and lung bioavailability in healthy volunteers and *in vitro* performance of the Symbicort® (budesonide/formoterol) pressurized metered dose inhaler

Michael Gillen[a,*], Pablo Forte[b], Jan Olof Svensson[c,d], Rosa Lamarca[e], Joanna Burke[f,g], Karolina Rask[c], Ulrika Larsdotter Nilsson[c], Göran Eckerwall[c]

[a] AstraZeneca Quantitative Clinical Pharmacology, Gaithersburg, USA
[b] PAREXEL International Limited, PAREXEL Early Phase Clinical Unit, Level 7, Northwick Park Hospital, Watford Road, Harrow, Middlesex, HA1 3UJ, UK
[c] AstraZeneca, Pepparedsleden 1, 431 50, Mölndal, Gothenburg, Sweden
[d] Chalmers University of Technology, SE-412 96, Gothenburg, Sweden
[e] AstraZeneca, Avenida Diagonal, 615, 08028, Barcelona, Catalonia, Spain
[f] JB Stats Ltd, Reading, UK
[g] AstraZeneca, Academy House, 136 Hills Road, Cambridge, CB2 8PA, UK

ARTICLE INFO

Keywords:
Spacer
Bioavailability
Budesonide
Formoterol
pMDI
Charcoal

ABSTRACT

Introduction: Many patients with chronic obstructive pulmonary disease or asthma experience difficulties in coordinating inhalation with pressurized metered-dose inhaler (pMDI) actuation. The use of a spacer device can improve drug delivery in these patients. The aim of this study was to establish the relative bioavailability of single doses of Symbicort® (budesonide/formoterol) pMDI 160/4.5 μg/actuation (2 actuations) used with and without a spacer device. In addition, an *in vitro* study was conducted to characterize performance of the inhaler when used in conjunction with a spacer device.

Methods: A Phase I, randomized, open-label, single-dose, single-center, crossover study in 50 healthy volunteers (NCT02934607) assessed the relative bioavailability of single-dose Symbicort® pMDI 160/4.5 μg/actuation (2 actuations) with and without a spacer (AeroChamber Plus® Flow-Vu®). Inhaled doses were administered without or with activated charcoal (taken orally) to estimate total systemic exposure and exposure through the lung, respectively. The *in vitro* study characterized the effect of the spacer with respect to delivered dose, fine particle dose, and dose during simulated breathing of budesonide and formoterol.

Results: In terms of total systemic exposure, use of the spacer increased the relative bioavailability determined by $AUC_{(0\text{-last})}$ and C_{max} by 68% (spacer:no spacer treatment ratio, 167.9%; 90% CI, 144.1 to 195.6) and 99% (ratio, 198.7%; 90% CI, 164.4 to 240.2) for budesonide, and 77% (ratio, 176.6%; 90% CI, 145.1 to 215.0) and 124% (ratio, 223.6%; 90% CI, 189.9 to 263.3) for formoterol, respectively, compared with pMDI alone. Similarly, the lung exposure of budesonide and formoterol increased ($AUC_{(0\text{-last})}$ and C_{max} by 146% [ratio, 246.0%; 90% CI, 200.7 to 301.6] and 127% [ratio, 226.5%; 90% CI, 186.4 to 275.4] for budesonide, and 173% [ratio, 272.8%; 90% CI, 202.5 to 367.4] and 136% [ratio, 236.2%; 90% CI, 192.6 to 289.6] for formoterol, respectively) when the pMDI was administered through the spacer.

When assessed by $AUC_{(0\text{-last})}$ quartile without spacer, subjects in the lowest exposure quartile (indicating poor inhalation technique) with Symbicort® pMDI 160/4.5 μg/actuation (2 actuations) had markedly increased total systemic and lung exposure when the same dose was administered with the spacer. In contrast, for subjects in the highest exposure quartile with pMDI alone, total systemic and lung exposure of formoterol and budesonide was similar with and without the spacer.

In the *in vitro* study, the fine particle dose (< 5 μm) of both budesonide and formoterol from the spacer at delay time (i.e. pause period after actuation) = 0 s (instantaneous) after actuation was similar to the fine particle dose when not using the spacer. The delivered doses of budesonide and formoterol from the spacer were both

* Corresponding author. Quantitative Clinical Pharmacology, Early Clinical Development, IMED Biotech Unit, AstraZeneca, One MedImmune Way, Gaithersburg, MD 20878, USA.

E-mail addresses: michael.gillen@astrazeneca.com (M. Gillen), pablo.fortesoto@parexel.com (P. Forte), janolof.svensson@astrazeneca.com (J.O. Svensson), rosa.lamarca@astrazeneca.com (R. Lamarca), joanna.burke1@astrazeneca.com (J. Burke), karolina.rask@astrazeneca.com (K. Rask), ulrika.larsdotter@astrazeneca.com (U. Larsdotter Nilsson), goran.eckerwall@astrazeneca.com (G. Eckerwall).

https://doi.org/10.1016/j.pupt.2018.08.001
Received 4 December 2017; Received in revised form 16 July 2018; Accepted 1 August 2018
Available online 02 August 2018
1094-5539/

lower compared with the doses administered without the spacer. There was also a decrease in delivered dose with increasing delay time.

Conclusions: The clinical study demonstrated that in subjects with poor inhalation technique the use of the AeroChamber Plus® Flow-Vu® spacer increased the bioavailability of Symbicort® pMDI to a level observed in subjects with good inhalation technique without a spacer. The findings from the *in vitro* study support the fine particle dose characteristics of Symbicort® pMDI with the AeroChamber Plus® Flow-Vu® spacer.

1. Introduction

Observational studies have shown there is a significant relationship between the quality of inhaler use and control of symptoms in patients with chronic obstructive pulmonary disease (COPD) and asthma [1]. Indeed, previous studies have demonstrated that around 75% of patients with COPD had poor inhalation technique when using a pressurized metered-dose inhaler (pMDI) [2]. Similar results have been observed in patients with asthma [3,4]. Specifically, studies evaluating inhalation technique have identified that the most common critical handling errors with pMDIs are related to coordination of inhalation with inhaler actuation [5]. Poor inhalation technique leads to decreased medication delivery to the lungs and, in turn, poor disease control [6]. In patients who experience difficulties in coordinating inhalation with pMDI actuation, the use of a spacer device (also known as a valved holding chamber device) can improve the efficiency of drug delivery [2,7,8].

Symbicort®, a combination of the inhaled corticosteroid (ICS) budesonide and the rapid and long-acting β_2-adrenoceptor agonist formoterol, is commercially available as a dry powder inhaler (DPI) (Symbicort® Turbuhaler®) and pMDI (Symbicort® pMDI). It has been shown to be effective in reducing exacerbations and improving symptoms in patients with moderate-to-severe COPD or asthma, either *via* a DPI or a pMDI [9–14]. Budesonide provides a high local anti-inflammatory effect within the lungs and airways, while formoterol results in a rapid and long-acting relaxation of bronchial smooth muscle in patients with reversible airway construction [15]. Budesonide undergoes rapid, extensive and reversible esterification in the lungs, readily dissolves in bronchial secretions, and is rapidly and extensively absorbed with maximum plasma concentration reached within 30 min [16]. Formoterol is rapidly absorbed and a maximum plasma concentration is reached within 10 min [16].

The present study was designed to establish the relative bioavailability of Symbicort® (budesonide/formoterol) pMDI with and without a spacer device (AeroChamber Plus® Flow-Vu®; Trudell Medical International, London, Ontario, Canada) in healthy volunteers. In addition to the clinical study, an *in vitro* study was conducted to characterize the AeroChamber Plus® Flow-Vu® spacer when applied together with Symbicort® pMDI, with regard to how the use of the spacer affects the fine particle dose (< 5 µm) delivered by Symbicort® pMDI according to standard methods [17].

2. Materials and methods

2.1. Clinical study

This was a randomized, open-label, single-center, crossover study in healthy volunteers (ClinicalTrials.gov identifier: NCT02934607). The primary objective was to establish the relative bioavailability of Symbicort® (budesonide/formoterol) pMDI 160/4.5 µg/actuation (2 actuations) with and without a spacer (AeroChamber Plus® Flow-Vu®), when administered without or with activated oral charcoal to estimate total systemic exposure and exposure through the lung, respectively. The secondary objectives were to characterize the pharmacokinetic (PK) profiles of budesonide and formoterol delivered *via* a pMDI with and without a AeroChamber Plus® Flow-Vu® spacer without or with activated charcoal, and to assess the safety of single doses of Symbicort® 160/4.5 µg/actuation (2 actuations) pMDI in healthy subjects.

Subjects were screened for a maximum of 28 days before randomization, which was followed by four treatment periods during which the subjects were resident in the Phase I Unit from the afternoon before dosing with Symbicort® pMDI until at least 24 h after dosing. At each treatment visit, subjects received (under fasting conditions) two actuations of Symbicort® pMDI (160/4.5 µg/actuation) delivered with or without the AeroChamber Plus® Flow-Vu® spacer without or with activated charcoal, resulting in four treatment groups: A: pMDI alone, no charcoal; B: pMDI + AeroChamber Plus® Flow-Vu® spacer, no charcoal; C: pMDI with charcoal; D: pMDI + AeroChamber Plus® Flow-Vu® spacer with charcoal (Table 1). Device and inhalation training using a placebo pMDI (identical to Symbicort® pMDI) with or without the AeroChamber Plus® Flow-Vu® spacer was conducted on admission to each treatment period and prior to dosing on Day 1 of each treatment period.

Blood samples were taken pre-dose and at 5, 20, and 40 min and at 1, 2, 4, 8, 10, 12, 18, and 24 h after Symbicort® pMDI dosing for determination of plasma concentrations of budesonide and formoterol. A final visit occurred 5 to 7 days following the last drug administration. Between each treatment period, there was a minimum washout period of 3 days.

The charcoal block technique, as described by Thorsson et al. [18], was used to assess lung exposure. Activated charcoal was prepared as a charcoal-water suspension (approximately 10 g charcoal in 80 mL of water) and given orally at repeated intervals: immediately before inhalation, immediately after dosing (following the mouth rinse), 1 h after administration, and 2 h after inhalation. Subjects rinsed their mouths with water after each charcoal administration, but this water was not swallowed. Subjects were trained by study personnel to perform inhalation techniques correctly on admission to each treatment

Table 1
Treatment groups.

Treatment group	Symbicort® (budesonide/formoterol) pMDI 160/4.5 µg/actuation (2 actuations)	AeroChamber Plus® Flow-Vu® spacer	Charcoal block	
A				Total systemic exposure
B				
C				Lung exposure
D				

Comparison B versus A (reference) evaluated the effect of the spacer on total systemic exposure; comparison D versus C (reference) evaluated the effect of the spacer on lung exposure.

Table 2
Participant baseline demographics.

Parameter	Subjects (N = 50)
Mean age, years (SD)	42.2 (16.3)
Males, n (%)	28 (56.0)
Race, n (%)	
White	36 (72.0)
Black or African American	5 (10.0)
Asian	4 (8.0)
Other	5 (10.0)
Mean highest FEV_1% predicted (SD)	104.8 (13.8)
Mean FEV_1, L (SD)	3.7 (1.0)
Mean BMI, kg/m^2 (SD)	24.4 (2.9)

BMI, body mass index; SD, standard deviation.

period and prior to dosing, in order to achieve reproducibility of inhalation. Subjects rinsed their mouths twice with 25 mL of water after administration of Symbicort® pMDI.

The safety endpoints for this study were the occurrence of adverse events (AEs) and laboratory/vital signs abnormalities, summarized by treatment and overall. AEs were coded using the MedDRA dictionary version 19.1. A treatment-emergent AE was defined as an AE with onset after first dose, including AEs occurring during the washout between successive treatment periods.

The study was performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and was consistent with the International Council for Harmonisation Good Clinical Practice guidelines. The clinical study protocol was approved by an Independent Ethics Committee (South Central – Berkshire B Research Ethics Committee, Bristol HRA Centre, Level 3, Block B, Whitefriars, Lewins Mead, Bristol BS1 2NT, UK; Reference 16/SC/0461) and the Medicines and Healthcare Products Regulatory Agency.

2.1.1. Subjects

Healthy male and female non-smokers aged ≥18 years with a body mass index of 18–30 kg/m^2, and weight ≥50–≤100 kg were eligible for participation. Subjects were required to have a forced expiratory volume in 1 s (FEV_1) of ≥80% of the predicted value, and FEV_1/forced vital capacity ratio ≥70%. No concomitant medication or therapy was permitted (with the exception of oral paracetamol and hormone replacement therapy/systemic contraceptives in females). Females were required to have a negative pregnancy test at screening and on first admission, and to not be lactating.

2.1.2. Pharmacokinetic measurements

The primary PK parameters for relative bioavailability criteria were maximum observed plasma concentration (C_{max}) and area under the plasma concentration-time curve from time zero to time of last quantifiable concentration ($AUC_{0\text{-last}}$). The secondary PK parameters included area under the plasma concentration-time curve from zero to infinity (AUC), time to reach maximum observed plasma concentration (t_{max}), half-life associated with terminal slope (λz) of semi-logarithmic concentration-time curve ($t_{½\lambda}$), apparent volume of distribution during the terminal phase (extravascular administration) (V_z/F), and apparent total body clearance of drug from plasma after extravascular administration (CL/F).

2.1.3. Statistical analysis

All subjects who received at least one dose of Symbicort® pMDI, and for whom any safety post-dose data were available were included in the safety analysis.

The PK analysis set consisted of all subjects in the safety analysis set for whom at least one of the primary PK parameters for a given analyte could be calculated, and for whom no important protocol deviations thought to impact on the analysis of PK data occurred.

The purpose of this study was to estimate any difference in the PK of

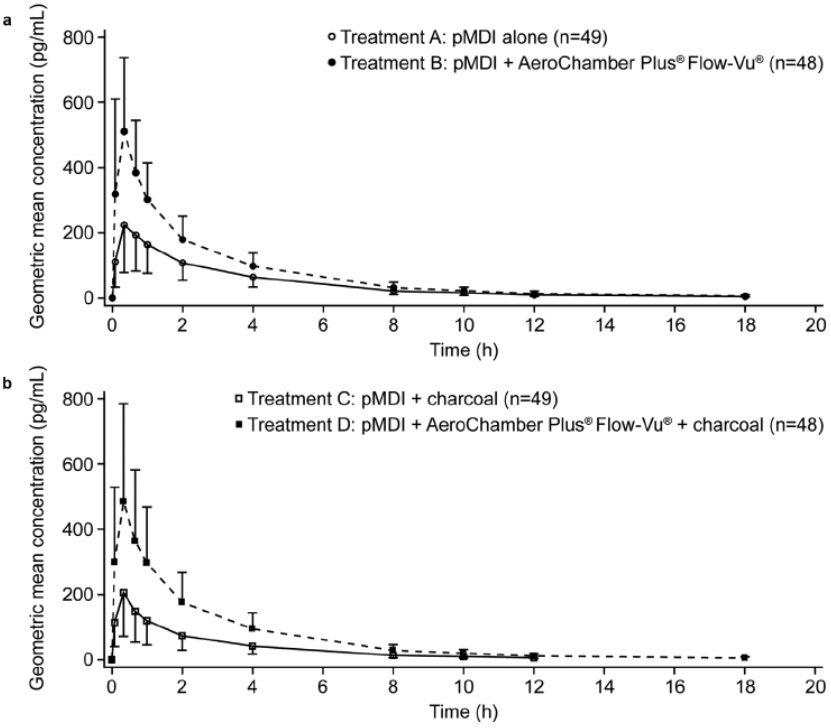

Fig. 1. Plasma budesonide concentration-time profiles following administration of Symbicort® (budesonide/formoterol) pMDI 160/4.5 μg/actuation (2 actuations) with and without a spacer, a) total systemic exposure (no charcoal block) and b) lung exposure (with charcoal block) (pharmacokinetic analysis set, linear scale). Bars represent geometric standard deviation.

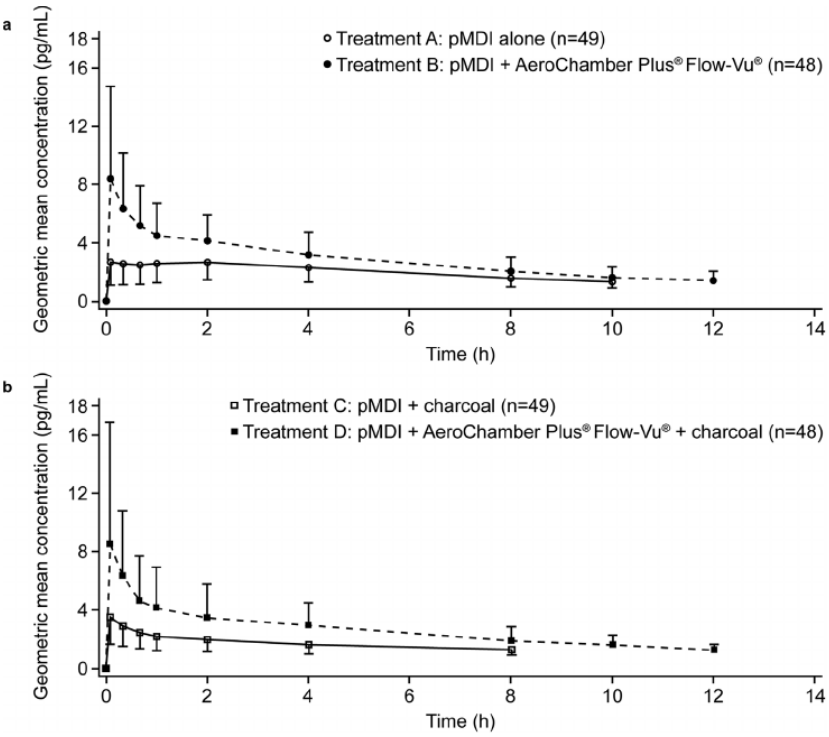

Fig. 2. Plasma formoterol concentration-time profiles following administration of Symbicort® (budesonide/formoterol) pMDI 160/4.5 μg/actuation (2 actuations) with and without a spacer, a) total systemic exposure (no charcoal block) and b) lung exposure (with charcoal block) (pharmacokinetic analysis set, linear scale). Bars represent geometric standard deviation.

Table 3
Secondary pharmacokinetic parameters.

Parameter, unit	Symbicort® pMDI alone (A) (N = 49)	Symbicort® pMDI + AeroChamber Plus® Flow-Vu® (B) (N = 48)	Symbicort® pMDI alone (C) (N = 49)	Symbicort® pMDI + AeroChamber Plus® Flow-Vu® (D) (N = 48)
	—— Total systemic exposure ——		—— Lung exposure ——	
Budesonide				
AUC, h*pg/mL[a]	788.2 (70.4)	1308.0 (33.3)	608.4 (77.1)	1253.0 (47.0)
t_{max}, h[b]	0.3 (0.1, 1.0)	0.3 (0.1, 1.0)	0.3 (0.1, 0.7)	0.3 (0.1, 0.7)
$t_{½\lambda_z}$, h[c]	3.4 (1.0)	3.7 (1.2)	2.9 (0.9)	3.3 (1.1)
V_z/F, L[c]	2253.0 (1469.0)	1308.0 (390.9)	2546.0 (1676.0)	1284.0 (725.8)
CL/F, L/h[c]	503.6 (392.4)	258.2 (91.9)	660.3 (462.5)	290.7 (222.2)
Formoterol				
AUC, h*pg/mL[a]	66.5 (29.7)	62.2 (29.9)	66.8 (NC)	52.4 (24.7)
t_{max}, h[b]	0.3 (0.1, 8.0)	0.1 (0.1, 0.7)	0.1 (0.1, 0.7)	0.1 (0.1, 0.7)
$t_{½\lambda_z}$, h[c]	5.8 (1.5)	6.3 (1.5)	5.8 (1.7)	5.9 (1.7)
V_z/F, L[c]	1096.0 (181.2)	1278.0 (276.3)	1601.0 (NA)	1357.0 (150.8)
CL/F, L/h[c]	140.1 (39.7)	150.3 (42.2)	134.7 (NC)	176.1 (39.5)

Data shown as [a]geometric mean (CV%); [b]median (min, max); [c]arithmetic mean (SD). Comparison B versus A (reference) evaluated the effect of the spacer on total systemic exposure; comparison D versus C (reference) evaluated the effect of the spacer on lung exposure. AUC, area under the plasma concentration-time curve from time zero extrapolated to infinity; CL/F, apparent total body clearance of drug from plasma after extravascular administration; CV, coefficient of variation; pMDI, pressurized metered-dose inhaler; NC, not calculable; $t_{½\lambda_z}$, half-life associated with terminal slope (λz) of a semi logarithmic concentration time curve; $t_{max,}$ time to reach maximum observed plasma concentration; V_z/F, apparent volume of distribution during the terminal phase (extravascular administration).

budesonide and formoterol with adequate precision; all analyses were considered to be descriptive in nature. Assuming an intra-subject coefficient of variation of 33% (based on the variability of AUC_{0-12h} for budesonide and AUC_{0-12h} and C_{max} for formoterol observed in a similarly designed crossover study in healthy adults) [19] and an expected difference of 0, forty four (44) evaluable subjects provided at least 80% power to show that the 90% confidence interval (CI) for the treatment effects fell entirely within the range 0.8–1.25, i.e., ruled out a 20% change (on a log scale) in exposure to budesonide and formoterol. It was considered appropriate to base the sample size on AUC_{0-12h} for budesonide rather than C_{max} (which has higher variability) because the effects of ICS are more likely related to total systemic exposure (i.e., AUC) rather than to acute exposure (i.e., C_{max}). Additional subjects were randomized to ensure that at least 44 evaluable subjects completed the study.

A two-sided 90% CI approach based on a repeated measures analysis of variance (ANOVA) model was used to assess the primary PK parameters, including period and treatment as fixed effects, and participant as a random effect. All PK parameters were log-transformed prior to analysis. The estimated treatment differences and the 90% CIs on the

Table 4
Budesonide and formoterol plasma pharmacokinetic parameters in healthy volunteers following single-dose Symbicort® (budesonide/formoterol) pMDI 160/4.5 μg/actuation (2 actuations) with or without spacer (AeroChamber Plus® Flow-Vu®) for total systemic and lung exposure.

Parameter, unit Geometric mean (CV%)	Symbicort® pMDI alone (A) (N = 49)	Symbicort® pMDI + AeroChamber Plus® Flow-Vu® (B) (N = 48)	Pairwise comparison B vs A Ratio, % [90% CI]	Symbicort® pMDI alone (C) (N = 49)	Symbicort® pMDI + AeroChamber Plus® Flow-Vu® (D) (N = 48)	Pairwise comparison D vs C Ratio, % [90% CI]
	Total systemic exposure			Lung exposure		
Budesonide						
C_{max}, pg/mL	254.8 (105.5)	524.9 (40.1)	**198.7** [164.4, 240.2]	231.7 (95.9)	495.4 (51.3)	**226.5** [186.4, 275.4]
$AUC_{(0\text{-last})}$, h*pg/mL	740.9 (84.8)	1284.0 (35.1)	**167.9** [144.1, 195.6]	527.6 (103.7)	1212 (45.3)	**246.0** [200.7, 301.6]
Formoterol						
C_{max}, pg/mL	4.2 (73.2)	9.3 (52.1)	**223.6** [189.9, 263.3]	4.6 (56.7)	9.2 (58.7)	**236.2** [192.6, 289.6]
$AUC_{(0\text{-last})}$, h*pg/mL	19.9 (115.6)	34.6 (60.6)	**176.6** [145.1, 215.0]	13.6 (119.1)	32.4 (57.7)	**272.8** [202.5, 367.4]

Comparison B versus A (reference) evaluated the effect of the spacer on total systemic exposure; comparison D versus C (reference) evaluated the effect of the spacer on lung exposure.
$AUC_{(0\text{-last})}$, area under the plasma concentration-time curve from time zero to the last quantifiable concentration after dosing; CI, confidence interval; C_{max}, observed maximum plasma concentration; CV, coefficient of variation; pMDI, pressurized metered-dose inhaler.

log scale were back-transformed to obtain the G_{mean} ratios for each pair of treatments. The least square means (and 95% CIs), G_{mean} ratios, and 90% CIs were tabulated for each comparison and analyte (budesonide and formoterol).

An additional ANOVA model including sequence, subject within sequence, period, and treatment as fixed effects (with no random effects) was incorporated in the analysis.

The treatment ratio of each of the test formulations (i.e., with the spacer) were compared with the reference formulations (i.e., without the spacer) for both budesonide and formoterol. In order to determine total systemic exposure, statistical analyses were conducted comparing treatments without charcoal, whereas to determine lung exposure, analyses were conducted comparing treatments with charcoal (see Table 1).

An additional analysis was included post database lock, assessing subjects split by $AUC_{(0\text{-last})}$ quartile during no spacer treatment. Spacer:No spacer ratios were calculated for $AUC_{(0\text{-last})}$ and C_{max} for each quartile. Quartile 1 had the lowest $AUC_{(0\text{-last})}$ values, indicative of poor inhalation technique, and Quartile 4 had the highest $AUC_{(0\text{-last})}$ values, indicative of good inhalation technique.

2.2. In vitro study

The AeroChamber Plus® Flow-Vu® spacer was used after removing from the package, without any pre-treatment, according to the manufacturer's instructions. Symbicort® (budesonide/formoterol) pMDI 160/4.5 μg/actuation (2 actuations) was primed prior to use (according to the patient instruction leaflet) and 10 waste actuations were pressed, similar to the handling of the pMDIs used in the clinical study. The Symbicort® pMDI was shaken before each of the two test actuations. Delay times (i.e. pause periods after actuation) of 0 s (instantaneous), 2 s, and 5 s were applied after each actuation, before extraction of the dose from the AeroChamber Plus® Flow-Vu® spacer. The delivered dose from the spacer was collected on Respirgard® disposable filters (Vital Signs, Totowa, New Jersey, USA) at a flow rate of 30 L/min. For each delay time, three doses were collected from each of three fixed Symbicort® pMDI/AeroChamber Plus® Flow-Vu® spacer combinations (one single pMDI was always combined with the same spacer), resulting in a total of nine doses collected for each delay time (one dose per filter was collected). The dose was then recovered from the filter using an internal standard technique. The simultaneous quantification of budesonide and formoterol was performed using high-performance liquid chromatography (HPLC) with a C18-column (XTerra RP-18 3.5 μm, 50 × 4.6 mm) at 25 °C with acetonitrile/25 mM sodium phosphate buffer pH 3.0 + 15 mM sodium octanesulfonic acid 30/70 (vol/vol) as mobile phase and a flow rate of 2 mL/min using a detection wavelength of 214 nm and a runtime of 6 min. The aerosol particle size distribution was assessed using a Next Generation Impactor. Three sample doses from each of three fixed Symbicort® pMDI/AeroChamber Plus® Flow-Vu® spacer combinations were collected at a flow rate of 30 L/min and analyzed by HPLC assay using same conditions as above. Linear interpolation of the discrete values bordering the mass median aerosol diameter in a log-probability plot was used for determining the median mass aerodynamic diameter from impactor stage data. The breathing profile generator F-SIG 6300 (AB FIA, Södra Sandby, Sweden) was used to generate the two different adult breathing patterns used, according to Canadian Standards Association Guidance Z264.1–02 [20]. One breathing pattern (Adult 1) used the following parameters: tidal volume 770 mL, frequency 12/min, inspiratory/expiratory (I/E) ratio 1:2 and minute volume 9240 mL. The second pattern (Adult 2) used tidal volume 500 mL, frequency 13/min, I/E ratio 1:2 and minute volume 6200 mL. The pMDI was actuated into the AeroChamber Plus® Flow-Vu® spacer immediately before the start of the inhalation part of the breathing cycle and the dose was collected ex-spacer using the breathing profile.

3. Results

3.1. Clinical study

3.1.1. Subjects

A total of 50 healthy subjects were randomized and received the study medication; all of whom were included in the safety and PK analysis sets. Subjects' demographics are presented in Table 2. Two individuals discontinued after their first treatment (one subject failed the drugs of abuse test on admission to treatment period 2 and the other subject withdrew consent after treatment period 1. The latter was not due to safety reasons); the remaining 48 subjects completed the study.

3.1.2. Pharmacokinetics

Plasma concentration-time profiles for budesonide and formoterol are depicted in Figs. 1 and 2, and the secondary plasma PK parameters are summarized in Table 3. Absorption of both budesonide and formoterol was rapid following administration of Symbicort® pMDI with and without the spacer and without or with charcoal (reflecting total systemic and lung exposure, respectively) (Figs. 1 and 2).

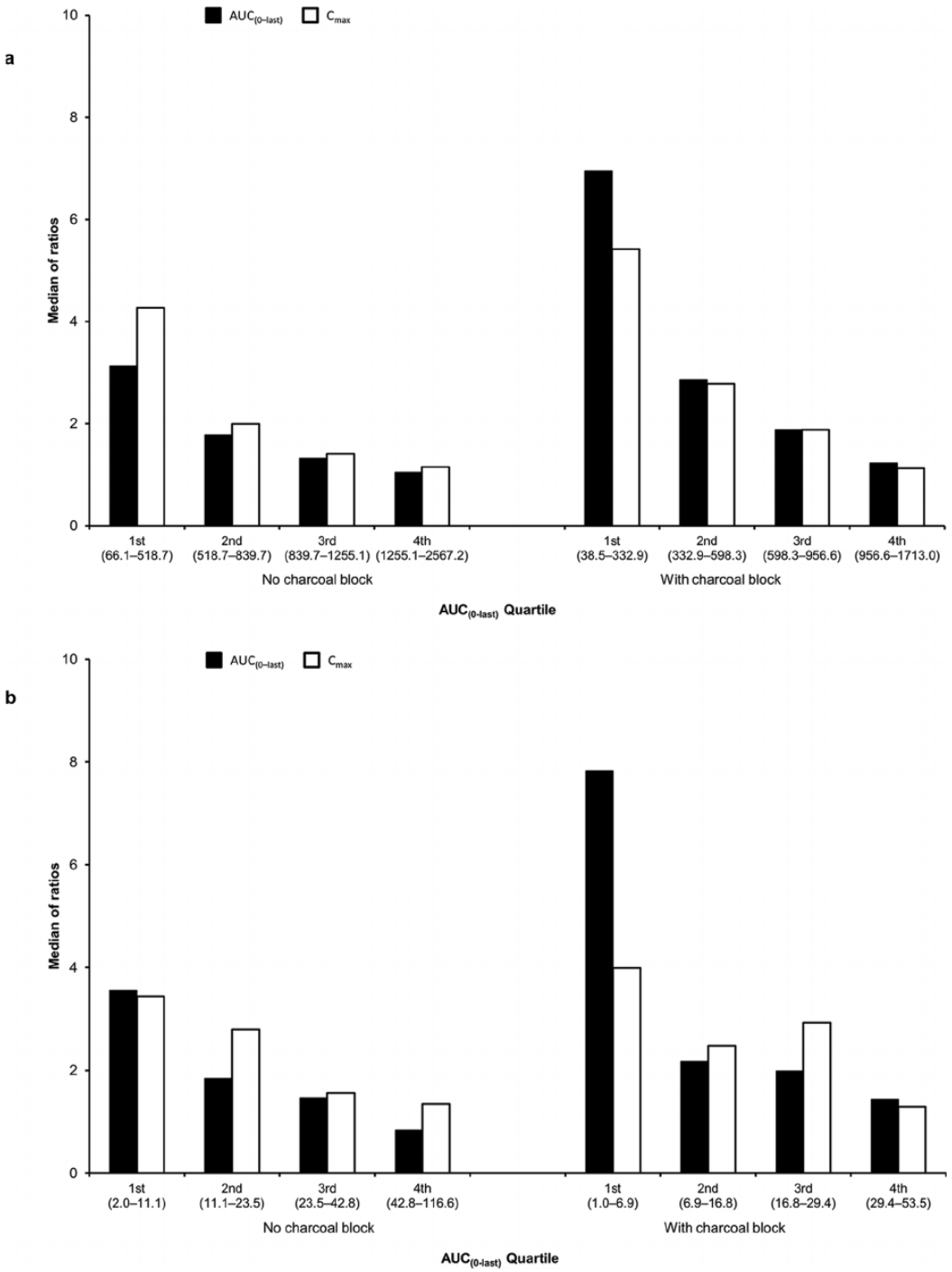

Fig. 3. Effect of the AeroChamber Plus® Flow-Vu® spacer on plasma pharmacokinetic parameters as determined by ratios of Spacer:No spacer for $AUC_{(0\text{-last})}$ and C_{max} of a) budesonide and b) formoterol according to exposure quartile (1st 4th)* (pharmacokinetic analysis set). No charcoal block corresponds to total systemic exposure; charcoal block corresponds to lung exposure.
*Data were divided into quartiles based on $AUC_{(0\text{-last})}$ during no spacer treatment.

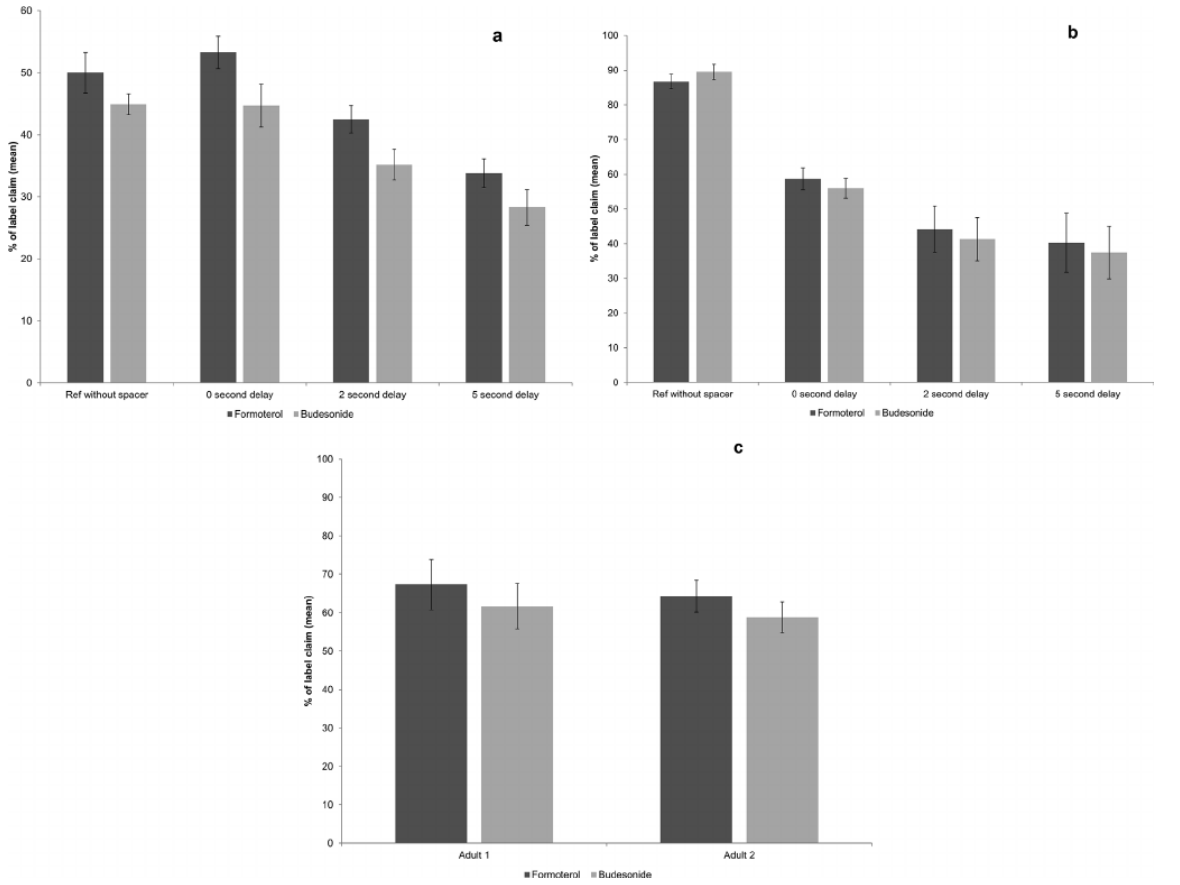

Fig. 4. Results from the *in vitro* study showing a) fine particle dose < 5 µm, with the AeroChamber Plus® Flow-Vu® spacer at different delay times, in percentage of label claim; b) dose with spacer at different delay times, in percentage of label claim; and c) dose with spacer (breathing patterns), in percentage of label claim. All data presented as mean ± SD.

For budesonide, median t_{max} occurred at 20 min post-dose both with and without the spacer and without or with charcoal block. For formoterol, the median t_{max} occurred at 20 min and 5 min post-dose with and without the spacer, respectively. When administered concomitantly with activated charcoal, the formoterol median t_{max} occurred at 5 min (range: 4 to 40 min) post-dose with or without the spacer (Table 3).

Between-subject variability (Table 4) of budesonide and formoterol was high and more pronounced in treatments without the spacer. For total systemic exposure, geometric intra-subject coefficient of variation (CV%) of AUC (Table 3), $AUC_{(0\text{-last})}$, and C_{max} ranged from 70% to 106% for Treatment A, and 33% to 40% for Treatment B for budesonide, and 116% and 73% for Treatment A and 61% and 52% for Treatment B for formoterol. Similarly for lung exposure, geometric CV % of AUC, $AUC_{(0\text{-last})}$, and C_{max} ranged between 77% and 104% for Treatment C and 45% to 51% for Treatment D for budesonide, and 57% and 119% for Treatment C and 58% and 59% for Treatment D for formoterol (Table 4).

Compared with pMDI alone, use of the spacer increased the total systemic exposure of budesonide and formoterol. Thus, the relative bioavailability, as determined by $AUC_{(0\text{-last})}$ and C_{max} was 68% and 99% higher, respectively, for budesonide, and 77% and 124% higher, respectively, for formoterol (Table 4). Similarly, the lung exposure of budesonide and formoterol increased when the pMDI was administered through the spacer: the relative bioavailability, as determined by $AUC_{(0\text{-last})}$ and C_{max}, increased by 146% and 127%, respectively, for budesonide, and by 173% and 136%, respectively, for formoterol (Table 4).

An additional analysis was performed separating subjects into four groups based on exposure ($AUC_{0\text{-last}}$) quartiles with pMDI alone (Fig. 3). Subjects in the lowest exposure quartile (indicative of poor inhalation technique) had increased total systemic exposure of budesonide when Symbicort® pMDI was administered with the spacer (Fig. 3a). In contrast, for subjects in the highest exposure quartile with pMDI alone (indicative of good inhalation technique), total systemic and lung exposure of budesonide was similar with and without the spacer (Fig. 3a). Similar trends were observed for formoterol (Fig. 3b).

3.1.3. Safety

In this cohort of healthy individuals, there were no deaths, serious AEs, or AEs leading to discontinuation of treatment reported. Thirty subjects (60%) reported ≥1 AE during this study. All AEs were mild to moderate in intensity; the most common being headache and dizziness. No clinically relevant findings were observed in laboratory data or vital signs.

3.2. In vitro study

The *in vitro* study demonstrated that the AeroChamber Plus® Flow-

Table 5
Results from the *in vitro* study showing fine particle dose < 5 µm in percentage of label claim, fine particle fraction in percentage of delivered dose to impactor, median mass aerodynamic diameter and dose in percentage of label claim, all with the AeroChamber Plus® Flow-Vu® spacer at different delay times (N = 9 doses for each delay time), and dose with spacer (breathing patterns) in percentage of label claim.

Parameter, unit	Formoterol	Budesonide
Fine particle dose < 5 µm, mean % (SD)		
Ref without spacer	50.0 (3.3)	44.9 (1.7)
0 s delay	53.3 (2.6)	44.7 (3.5)
2 s delay	42.5 (2.2)	35.2 (2.5)
5 s delay	33.8 (2.3)	28.3 (2.9)
Fine particle fraction, mean % (SD)		
Ref without spacer	60.9 (0.9)	53.2 (1.7)
0 s delay	81.3 (1.2)	73.6 (1.2)
2 s delay	79.7 (2.0)	72.3 (2.0)
5 s delay	79.9 (1.1)	73.1 (0.9)
Median mass aerodynamic diameter, µm		
Ref without spacer	3.25	3.57
0 s delay	3.13	3.40
2 s delay	3.30	3.57
5 s delay	3.31	3.55
Delivered dose, mean % (SD)		
Ref without spacer	86.7 (2.1)	89.5 (2.2)
0 s delay	58.7 (3.1)	56.0 (2.9)
2 s delay	44.2 (6.7)	41.3 (6.3)
5 s delay	40.3 (8.5)	37.4 (7.6)
Delivered dose with spacer (breathing patterns), mean % (SD)		
Adult 1	67.3 (6.5)	61.7 (5.9)
Adult 2	64.3 (4.2)	58.8 (4.0)

SD, standard deviation. Nominal dose/label claim is 160/4.5 µg/actuation; a patient dose consists of two actuations.

Vu® spacer performed as expected. If the dose was withdrawn (i.e., inhaled) from the spacer according to its instruction for use, without any delay time, the fine particle dose (< 5 µm) was very similar to the fine particle dose without spacer for both active substances. There was a decrease in fine particle dose with increasing delay time (Fig. 4a; Table 5). The impactor sized distributions for Symbicort® pMDI with and without the spacer for both active substances were very similar taking into consideration the general decay over time (Fig. 5a + 5b). In addition, the cumulative distribution data for Symbicort® pMDI with and without the spacer for both active substances showed similar behavior (Fig. 6a + 6b). The delivered doses from the spacer decreased for both budesonide and formoterol compared with the doses administered with Symbicort® pMDI alone. There was also a decrease in delivered dose with increasing delay time (Fig. 4b; Table 5).

When the spacer was used with simulated breathing patterns designed to represent those of adults, the dose collected was similar for the two patterns used when the pMDI was actuated immediately before the inhalation part of the breathing cycle and similar to doses withdrawn using constant flow (Fig. 4c; Table 5). When the pMDI was actuated immediately before the exhalation part of the breathing cycle, slightly lower doses were found, similar to the doses withdrawn with constant flow after a 2- to 5-second delay.

4. Discussion

In this clinical study, the relative total systemic and lung bioavailability of Symbicort® (budesonide/formoterol) pMDI 160/4.5 µg/actuation (2 actuations) was increased when administered through the AeroChamber Plus® Flow-Vu® spacer, compared with Symbicort® pMDI alone. Indeed, administration of Symbicort® pMDI with the spacer increased the total systemic exposure ($AUC_{(0\text{-last})}$ and C_{max}) of both budesonide (68% and 99%, respectively) and formoterol (77% and 124%, respectively). For evaluation of delivery of the components to the lung, the charcoal block method was used to inhibit gastrointestinal absorption of budesonide and formoterol. In this study, the percentage increase in lung exposure with Symbicort® pMDI with spacer versus Symbicort® pMDI alone was greater than the increase in total systemic exposure; this is expected since the with-charcoal comparison reflects the change in lung delivery with use of the spacer device. In the without-charcoal comparison (i.e., total systemic exposure), the difference between exposure with Symbicort® pMDI and spacer versus Symbicort® pMDI alone is affected by the reduction in oral deposition/absorption with the spacer device, as well as the improvement in lung deposition due to improved coordination of inhalation. An increase in exposure due to improved lung delivery is therefore offset, to some degree, by a reduction in gastrointestinal absorption. In previous studies with Symbicort® pMDI, budesonide and formoterol systemic exposure ($AUC_{(0\text{-last})}$ and C_{max}) was approximately double that observed in this study, but was similar to that seen following administration of Symbicort® pMDI with the AeroChamber Plus® Flow-Vu® spacer [19,21]. This suggests that, in the present study, a proportion of subjects had poor inhalation technique, for whom the use of the spacer overcame problems with inhalation coordination, as shown by the 3 4-fold increase in exposure to budesonide and formoterol. However, for those subjects who had high exposure with Symbicort® pMDI alone (i.e., indicative of good inhalation technique), exposure was relatively unchanged by the spacer. Similar results were observed in patients treated with beclometasone dipropionate/formoterol (100/6 µg) pMDI plus AeroChamber Plus [22]. In that study, patients with poor inhalation technique experienced an increase in peak plasma concentrations with AeroChamber Plus [22]. Furthermore, in the present study, exposure with the spacer in subjects who had a high ratio with:without spacer (i.e., those in exposure quartiles 1 and 2) did not exceed that in subjects in the highest exposure quartile regardless of spacer use. Such data demonstrate that the increase in relative total systemic and lung bioavailability caused by the spacer does not result in higher exposure than in a subject who inhales properly.

The use of the AeroChamber Plus® Flow-Vu® spacer in the present study did not increase the total systemic exposure to budesonide and formoterol above what has been observed with Symbicort® pMDI in previous studies [19,21]. Furthermore, exposure did not exceed values observed with Symbicort® Turbuhaler®, a device which does not require inhalation coordination like a pMDI, and has a similar well-established safety profile.

The safety results from this study in healthy volunteers were consistent with the well-established safety profile of Symbicort® in subjects with COPD or asthma [10 14]. Plasma exposure to both drugs increased when using the AeroChamber Plus® Flow-Vu® spacer; however, total systemic exposure did not exceed the levels typically seen in previous Symbicort® pMDI studies [19,21]. Furthermore, exposure was relatively unchanged in subjects with good inhalation technique, so there should be no increased risk to patients with COPD or asthma when using the spacer in combination with the pMDI. Single doses of Symbicort® (budesonide/formoterol) pMDI 160/4.5 µg/actuation (2 actuations) were well tolerated when administered with or without the spacer, and without or with activated charcoal.

The *in vitro* study results confirm that the AeroChamber Plus® Flow-Vu® spacer performs as expected when used with Symbicort® pMDI. The reduction in delivered dose observed when Symbicort® pMDI was administered with the spacer can be attributed to the retention of mainly coarse particles in the device due to particle impaction in the valve and chamber, and sedimentation in the chamber. The decrease in fine particle dose with increased residence time for the aerosol in the chamber could be attributed to impaction and retention due to some remaining electrostatic charge. The increase in fine particle fraction and decrease in fine particle dose with delay has been noted in an early publication describing performance of the AeroChamber [23].

Expected dose delivery was obtained when the AeroChamber Plus® Flow-Vu® spacer was used with simulated breathing patterns designed to represent those of adults, indicating well-constructed inhalation and

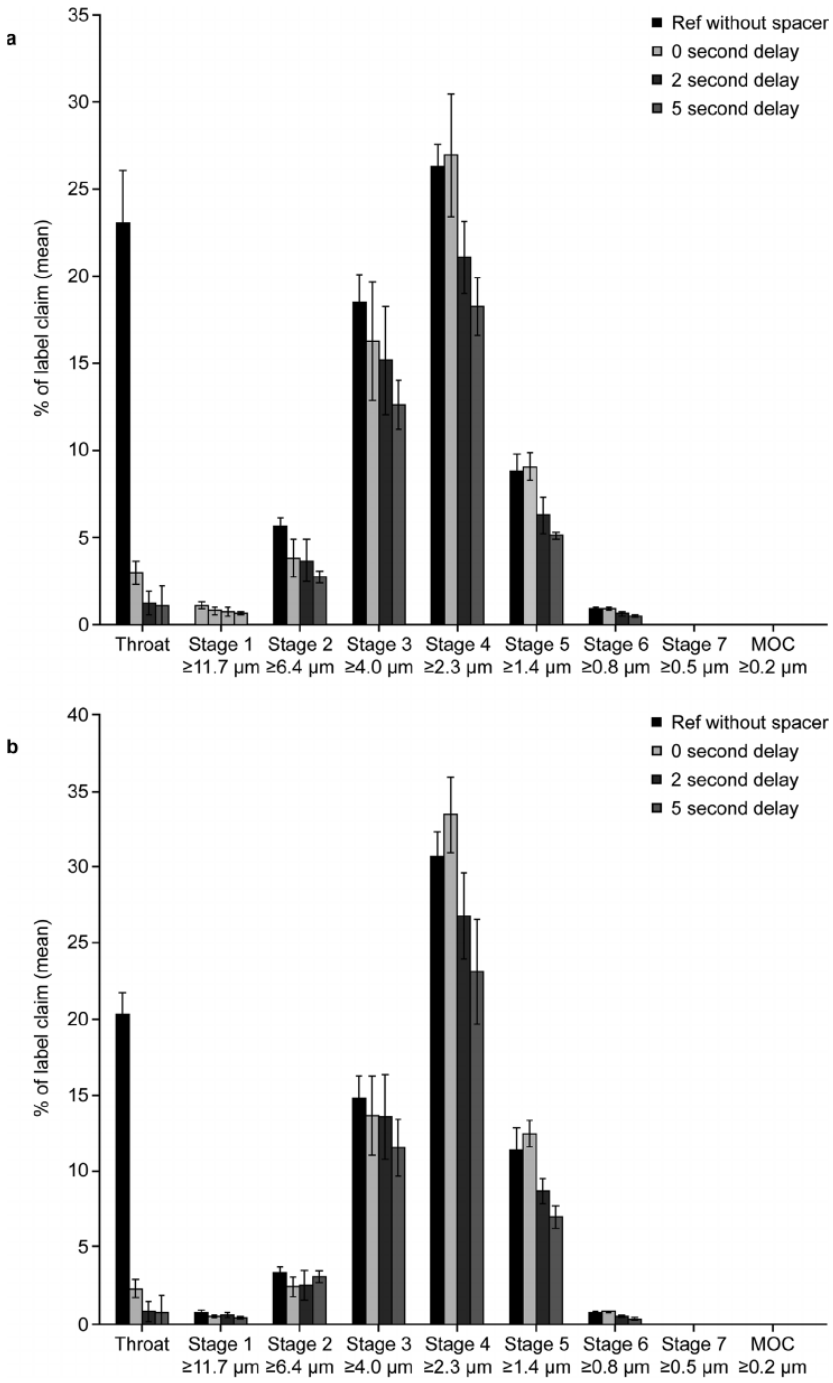

Fig. 5. Individual impactor stage data at different delay times, in percentage of label claim for a) budesonide and b) formoterol. All data are presented as mean ± 90% confidence intervals using the Student's t-distribution. MOC, micro-orifice collector.

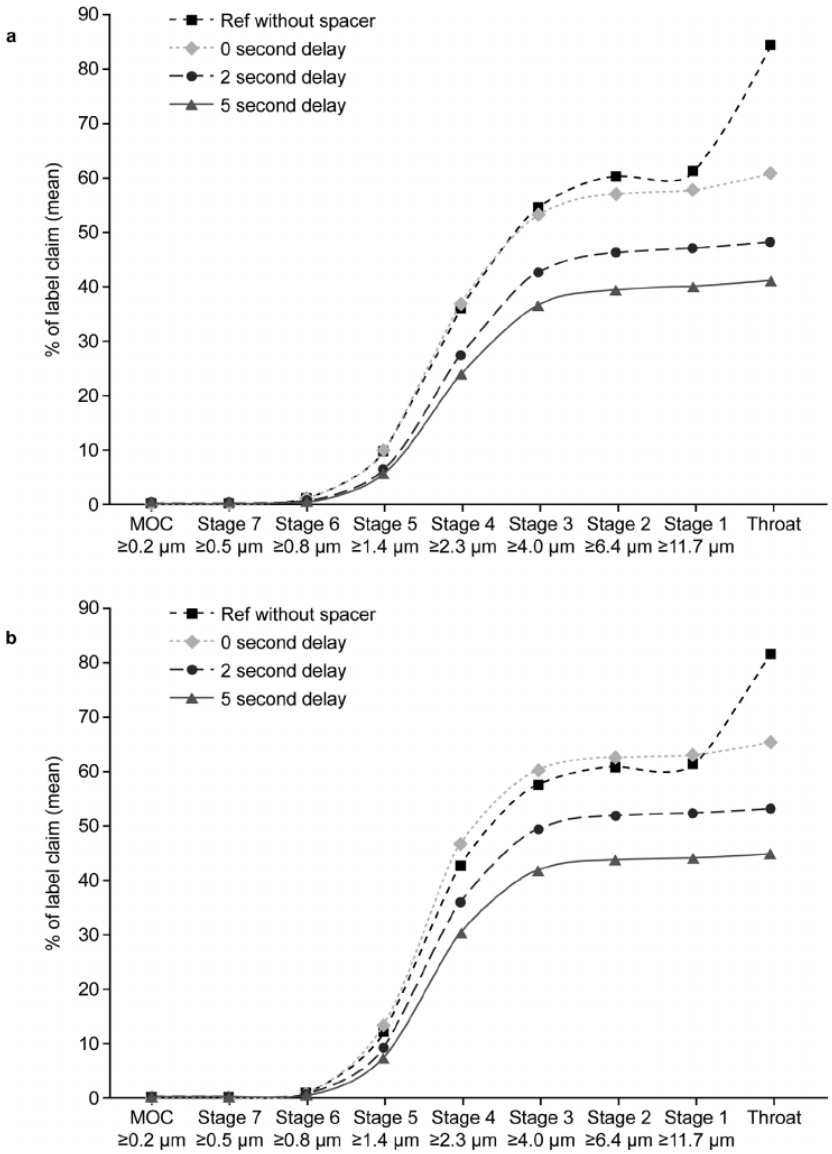

Fig. 6. Cumulative distribution normalized to label claim for a) budesonide and b) formoterol. MOC, micro-orifice collector.

exhalation valves of appropriate material not adding to significant deposition of the drug. Since the breathing profiles used were adult profiles with rather large inhalation volumes, a large part of the aerosol was expected to be withdrawn from the spacer during the immediate inhalation part of the first breathing cycle, thus leading to a very short aerosol residence time in the spacer with low deposition losses inside the spacer.

The slightly lower doses found when the pMDI was actuated immediately before the exhalation part of the breathing cycle (similar to doses withdrawn with constant flow after a 2- to 5-second delay) was attributed to an increased residence time in the spacer before the aerosol was withdrawn by the inhalation part of the next breathing cycle. No obvious malfunction of the exhalation valve was identified, since this would have resulted in lower doses.

This study has some limitations. Firstly, healthy subjects were used throughout the study. This approach was taken to avoid the potential for day-to-day variability in lung delivery in patient populations due to their disease state that could affect the PK data and enabled testing of the effect of the spacer without other interfering factors. However, PK data with the Symbicort® pMDI in adults with COPD, and adults and children with asthma show generally comparable PK profiles across the populations [24]. The second limitation is the fact that this study only used one spacer. The findings of this study may therefore not be universally applicable to other spacers that have different geometry and are constructed of different materials, since these factors can influence drug delivery. The AeroChamber Plus® Flow-Vu® spacer was chosen as it is commonly used and widely available in the regions where Symbicort® is marketed. The third limitation is the lack of spacer data in pediatrics.

This study was conducted to support approval for a COPD indication for Symbicort® pMDI in Europe, so pediatric data were not of concern. In other regions, Symbicort® is approved for asthma patients as young as 6 years of age and in this age range an inspiratory volume to easily empty a 150 mL spacer would not be of concern.

5. Conclusions

The results observed in this clinical study clearly demonstrate the benefit of the AeroChamber Plus® Flow-Vu® spacer in terms of addressing poor inhalation technique with Symbicort® (budesonide/formoterol) pMDI 160/4.5 μg/actuation (2 actuations). The use of the spacer improved exposures in these subjects to a similar level as those with a good inhalation technique without a spacer. The findings from the *in vitro* study support the fine particle dose characteristics of Symbicort® pMDI with the AeroChamber Plus® Flow-Vu® spacer.

Author disclosures

This study was supported by AstraZeneca. M Gillen, JO Svensson, R Lamarca, K Rask, U Larsdotter Nilsson, and G Eckerwall are employees of AstraZeneca. J Burke is an employee of JB Stats Ltd, Reading, UK, and provided statistical assistance in the preparation of this manuscript. P Forte is an employee of PAREXEL International Limited.

Funding

The *in vitro* characterization and statistical assistance in the preparation of this manuscript were funded by AstraZeneca.

Acknowledgements

The authors would like to thank Emmace Consulting AB who performed the *in vitro* characterization under guidance from JO Svensson.The authors would also like to thank Matthew Bexon and Clair Clowes of inScience Communications, Springer Healthcare, UK, for providing medical writing support, which was funded by AstraZeneca in accordance with Good Publication Practice (GPP3) guidelines (http://www.ismpp.org/gpp3).

References

[1] A.S. Melani, et al., Inhaler mishandling remains common in real life and is associated with reduced disease control, Respir. Med. 105 (6) (2011) 930 938.
[2] C. Pothirat, et al., Evaluating inhaler use technique in COPD patients, Int. J. Chronic Obstr. Pulm. Dis. 10 (2015) 1291 1298.
[3] V. Giraud, N. Roche, Misuse of corticosteroid metered-dose inhaler is associated with decreased asthma stability, Eur. Respir. J. 19 (2) (2002) 246 251.
[4] Global Initiative for Asthma, Pocket Guide for Asthma Management and Prevention (For Adults and Children Older than 5 Years): a Pocket Guide for Health Professionals, (2017) Updated 2017. Available from: http://www.ginasthma.org/.
[5] N. Roche, P.N. Dekhuijzen, The evolution of pressurized metered-dose inhalers from early to modern devices, J. Aerosol Med. Pulm. Drug Deliv. 29 (4) (2016) 311 327.
[6] M.G. Cochrane, et al., Inhaled corticosteroids for asthma therapy: patient compliance, devices, and inhalation technique, Chest 117 (2) (2000) 542 550.
[7] Global Initiative for Asthma, Global Strategy for Asthma Management and Prevention, (2017) Available from: www.ginasthma.org.
[8] M. Hindle, H. Chrystyn, Relative bioavailability of salbutamol to the lung following inhalation using metered dose inhalation methods and spacer devices, Thorax 49 (6) (1994) 549 553.
[9] P.M. Calverley, et al., Maintenance therapy with budesonide and formoterol in chronic obstructive pulmonary disease, Eur. Respir. J. 22 (6) (2003) 912 919.
[10] W. Szafranski, et al., Efficacy and safety of budesonide/formoterol in the management of chronic obstructive pulmonary disease, Eur. Respir. J. 21 (1) (2003) 74 81.
[11] A. Sharafkhaneh, et al., Effect of budesonide/formoterol pMDI on COPD exacerbations: a double-blind, randomized study, Respir. Med. 106 (2) (2012) 257 268.
[12] P. Kuna, et al., Effect of budesonide/formoterol maintenance and reliever therapy on asthma exacerbations, Int. J. Clin. Pract. 61 (5) (2007) 725 736.
[13] J. Corren, et al., Twelve-week, randomized, placebo-controlled, multicenter study of the efficacy and tolerability of budesonide and formoterol in one metered-dose inhaler compared with budesonide alone and formoterol alone in adolescents and adults with asthma, Clin. Therapeut. 29 (5) (2007) 823 843.
[14] D.P. Tashkin, et al., Efficacy and safety of budesonide and formoterol in one pressurized metered-dose inhaler in patients with moderate to very severe chronic obstructive pulmonary disease: results of a 6-month randomized clinical trial, Drugs 68 (14) (2008) 1975 2000.
[15] P. Kuna, I. Kuprys, Symbicort Turbuhaler: a new concept in asthma management, Int. J. Clin. Pract. 56 (10) (2002) 797 803.
[16] AstraZeneca, Symbicort Turbohaler 100/6, Inhalation Powder Summary of Product Characteristics, (2017).
[17] (CHMP), C.f.M.P.f.H.U, EMEA (Ed.), Guideline on the Pharmaceutical Quality of Inhalation and Nasal Products, European Medicines Agency London, 2006, pp. 1 27.
[18] L. Thorsson, S. Edsbacker, T.B. Conradson, Lung deposition of budesonide from Turbuhaler is twice that from a pressurized metered-dose inhaler P-MDI, Eur. Respir. J. 7 (10) (1994) 1839 1844.
[19] C. Reisner, et al., Pearl's PT010 triple combination provides comparable budesonide exposure to symbicort and comparable glycopyrronium and formoterol exposure to PT003, Eur. Respir. J. 44 (Suppl 58) (2014) 1891.
[20] M.B. Dolovich, J.P. Mitchell, Canadian Standards Association standard CAN/CSA/Z264.1-02:2002: a new voluntary standard for spacers and holding chambers used with pressurized metered-dose inhalers, Canc. Res. J. 11 (7) (2004) 489 495.
[21] A. Eklund, et al., Pharmacokinetics of budesonide and formoterol administered via a series of single-drug and combination inhalers: four open-label, randomized, crossover studies in healthy adults, Biopharm Drug Dispos. 29 (7) (2008) 382 395.
[22] D. Singh, et al., Effect of AeroChamber Plus on the lung and systemic bioavailability of beclometasone dipropionate/formoterol pMDI, Br. J. Clin. Pharmacol. 72 (6) (2011) 932 939.
[23] M.B. Dolovich, Characterization of medical aerosols: physical and clinical requirements for new inhalers, Aerosol. Sci. Technol. 22 (4) (1995) 392 399.
[24] A. Tronde, et al., Pharmacokinetics of budesonide and formoterol administered via 1 pressurized metered-dose inhaler in patients with asthma and COPD, J. Clin. Pharmacol. 48 (11) (2008) 1300 1308.