Pharmacokinetics of inhaled monodisperse beclomethasone as a function of particle size

J. E. Esposito-Festen,^{1,*} P. Zanen,^{2,*} H. A. W. M. Tiddens¹ & J.-W. J. Lammers²

¹Department of Pediatrics, Division of Respiratory Medicine, Erasmus University Medical Center Rotterdam-Sophia Children's Hospital and ²Heart Lung Center Utrecht, University Medical Center Utrecht, the Netherlands

What is already known about this subject

- For asthmatic adults, bronchodilators with a MMAD between 3 and 6 μm were shown to give the best improvement in lung function and the least systemic side-effects.
- It is not known, however, what is the most efficacious particle size for inhaled steroids in asthmatic adults. Clinical efficacy and systemic side-effects of inhaled steroids should be measured to define the optimal particle size.

What this study adds

- Our study investigated the systemic absorption of inhaled steroids. We found that a particle size of 2.5 μm and 4.5 μm gave a higher pulmonary bioavailability compared with the 1.5 μm monodisperse aerosols in adults with mild asthma and therefore were more likely to elicit systemic adverse effects.

Correspondence

P. Zanen M.D. Ph.D, Heart Lung Center Utrecht, University Hospital Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands.

Fax: +31 (0) 30 250 5421/605 2001

E-mail: p.zanen@umcutrecht.nl

* Both authors contributed equally

Keywords

asthma, monodisperse aerosols, steroids

Received

6 June 2006

Accepted

18 December 2006

Published OnlineEarly

18 April 2007

Aims

For optimal efficacy, antiasthma drugs should be delivered to the desired region in the airways. To date, the optimal particle size for steroids in adults is not known. The aim of the study was to evaluate the pulmonary bioavailability for inhaled beclomethasone dipropionate (BDP) aerosols of different particle sizes.

Methods

In a randomized single-blind crossover trial, 10 mild asthmatic patients inhaled monodisperse BDP aerosols with mass median aerodynamic diameters (MMADs) of 1.5, 2.5 and 4.5 μm . Gastrointestinal absorption was blocked by activated charcoal. Plasma concentrations of 17-beclomethasone monopropionate (17-BMP) were measured by liquid chromatography plus mass spectrometry.

Results

Aerosols with MMADs of 1.5 μm , 2.5 μm , and 4.5 μm gave mean maximum concentrations (C_{max}) of 17-BMP of 475 pg ml^{-1} , 1300 pg ml^{-1} , and 1161 pg ml^{-1} , respectively. The area under the curve (AUC) values of 17-BMP for MMADs of 1.5 μm , 2.5 μm , and 4.5 μm were 825 $\text{pg ml}^{-1} \text{ h}$, 2629 $\text{pg ml}^{-1} \text{ h}$, and 2276 $\text{pg ml}^{-1} \text{ h}$, respectively. The mean terminal half-time of 17-BMP for all three aerosol sizes was around 1.5 h.

Conclusions

Monodisperse BDP aerosols with a MMAD of 1.5 μm gave two-three fold lower values for C_{max} and AUC than those with MMADs of 2.5 and 4.5 μm .

Introduction

For decades, the administration of medication via the inhaled route has been the mainstay of therapy for respiratory diseases such as asthma and cystic fibrosis [1–3]. More recently, inhalation therapy was also introduced for diseases such as diabetes mellitus, where insulin is delivered via inhalation to lower blood glucose concentrations [4–6].

The efficacy of inhalation therapy, i.e. greater beneficial effects and a lower incidence of systemic side-effects, largely depends on targeting a drug to the relevant region in the lungs. Targeting is influenced by factors such as the physiology, anatomy and pathology of the airways, breathing patterns, and the characteristics of the inhaled drug [7–13]. Of these, the physiological, anatomical and pathological factors are hard to control. Breathing patterns can be managed in theory, but this is difficult in daily practice. However, it is the characteristics of the inhaled drug, such as particle size, that can be controlled effectively to enhance lung deposition.

For mild and severely affected asthmatic adults bronchodilators with a MMAD between 2.8 and 6 μm were shown to give the best improvement in lung function and the least systemic side-effects [14–16]. It is not known, however, whether a MMAD in this range is also the most efficacious particle size for inhaled steroids in asthmatic adults. As the corticosteroid receptor distribution is different from that of the β_2 -receptors, inhaled corticosteroids are likely to require a different deposition pattern [8]. Clinical efficacy and systemic side effects of inhaled steroids should be measured to define the optimal particle size. Hence, we conducted a pharmacokinetic study using monodisperse beclomethasone dipropionate (BDP) aerosols to investigate their pulmonary bioavailability as a function of aerosol size in adults with mild asthma. Since BDP is rapidly and completely converted to 17-beclomethasone monopropionate (17-BMP), which is more potent than BDP, we focussed on the pharmacokinetics of 17-BMP.

Methods

Study population

Non-smoking men and women with stable mild asthma, between the ages of 18–60 years, were invited to participate. Subjects had to comply with the ATS-criteria for asthma [17], and their baseline forced expiratory volume in one second (FEV_1) had to exceed 70% of the predicted value. Subjects had to show a reversibility of at least 9% of the predicted FEV_1 value after administration of 200 μg salbutamol. Subjects were excluded if they had a secondary illness, pregnancy, and/or treatment that might interfere with a reaction to bronchodi-

Figure 1

Schematic representation of the experimental set-up

lators. All patients gave their written informed consent before entry into the trial, which was approved by the hospital Ethics Committee.

Study design

The study was designed as a randomized single-blind crossover trial. Patients were studied at the lung function laboratory at the University Hospital Utrecht on 3 separate days at intervals of 1 week. Oral medication for asthma was not allowed. Maintenance treatment with inhaled corticosteroids was discontinued 3 days prior to each study day. Long- and short-acting β_2 -adrenoceptor agonists were stopped 15 and 8 h prior to the start of the trial, respectively. The baseline FEV_1 during each following session was not allowed to deviate by more than 10% from that on day 1. Patients were first trained to inhale correctly. The inhalation manoeuvre consisted of a single inhalation from residual volume to total lung capacity with a constant flow of 30–60 l min^{-1} , followed by a 10 s breath holding period and subsequently a slow exhalation. A hot wire anemometer placed close to the patient's mouth was used to measure inhaled volume and flow velocity. An indicator connected to the anemometer facilitated the patients to inhale at a constant flow. The amounts of aerosol deposited in the anemometer were negligible.

Aerosol generation

In our experimental set-up (Figure 1), we used the electrohydrodynamic atomization (EHDA)-technique to generate monodisperse aerosols. This EHDA-technique has been extensively described by Ijsebaert *et al.* [18]. Monodisperse BDP aerosols (geometric SD < 1.2) of different MMADs, 1.5 μm , 2.5 μm and 4.5 μm , were generated from a BDP-ethanol solution (4% BDP in

97% ethanol). The generated monodisperse aerosols were collected in a large glass reservoir (20 l). Through an outlet at the end of the reservoir, part of the generated aerosols was sampled by an aerodynamic particle sizer 33 (APS) (TSI, St. Paul, MN). The APS continuously measured particle size distribution and aerosol concentration ($\mu\text{g l}^{-1}$ air) in the reservoir. Through another outlet patients inhaled 100 μg of the generated BDP aerosols. The volume of inhaled air containing a dose of 100 μg BDP aerosol was calculated by dividing this dose by the concentration of BDP aerosol in the reservoir. As soon as the subjects had inhaled a dose of 100 μg BDP, they were switched over to ambient air to stop further inhalation of BDP aerosol.

Blood sampling

In order to evaluate pulmonary bioavailability, gastrointestinal absorption was blocked by 5 g activated charcoal prior to inhalation of the BDP aerosols and 1, 2 and 4 h after inhalation [19]. An indwelling catheter (Becket Dickinson, Insythe W 18 gauge) was inserted into a forearm vein and a baseline blood sample was obtained. Consecutive blood samples were collected at 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360 and 420 min after inhalation. The first 2 ml of each sample was discarded. After collection of the sample, the indwelling catheter was flushed with 4 ml of 0.9% saline. The blood samples were collected in lithium-heparin tubes and immediately centrifuged in cooled centrifuges (7°C) for 10 min at 2200 g. Plasma from the samples was frozen at -70°C within 1 min after harvesting. This protocol was designed to ensure correct 17-BMP measurements, as any conversion from BDP to 17-BMP was instantly stopped.

Pharmacokinetic analysis

Plasma concentrations of 17-BMP were analyzed using high performance liquid chromatography coupled simultaneously with mass spectrometry (HPLC-MS-MS).

Calibration standards, study and control samples were thawed at room temperature, mixed thoroughly and centrifuged again for 10 min at 2200 g. Subsequently, the samples were extracted over a 50 mg C18 solid phase extraction (SPE) column. The SPE column was conditioned with 200 μl methanol (HPLC grade) followed by 400 μl water (Milli Q). Next, the column was loaded with a mix of 600 μl sample and 350 μl internal standard working solution. The eluate was dried under a stream of nitrogen at 40°C and reconstituted in 50 μl 40 : 60 (v : v) acetonitrile : 25 mM ammonium formate pH 5 buffer. An aliquot (40 μl) of the above-prepared sample

was analyzed using a gradient elution on a 50×2.1 mm ODS3 5 μm column. The temperature of the column was kept at 40°C at a flow rate of 0.8 ml min^{-1} .

The conditions for the MS-MS were: split ratio 1 in 4, ionization/interface TurboIonspray at 450°C, scan mode selected reaction monitoring (SRM) and cycle time 3.5 min.

The following transitions were monitored for BDP and 17-BMP, respectively: m/z 521 $\rightarrow$ m/z 319 and m/z 527 $\rightarrow$ m/z 319.

Data were processed using the software package *Analyst*. LC-MS peaks were integrated and peak area ratios of 17-BMP were calculated with respect to their internal standards. The limit of quantification was 50 pg ml^{-1} for both BMP and 17-BMP.

Statistics

From the plasma concentration curves the following parameters were calculated: $\text{AUC}(0,t)$ the area under the plasma concentration–time curve from zero to the last measured time point (t) via the linear trapezoidal rule; $\text{AUC}(0, \infty)$ the area under the plasma concentration–time curve from time zero extrapolated to infinity, calculated as $\text{AUC}(0,t) + C_{\text{last}}/\lambda_z$; With the sampling scheme of this study being rather long, the plasma concentrations at the last measured points were frequently below the limit of quantification and these concentrations were set to 0. If this was the case, the $\text{AUC}(0, \infty)$ was not estimated and set equal to $\text{AUC}(0,t)$; C_{max} the maximal concentration after inhalation; λ_z the apparent terminal elimination rate constant and $t_{1/2}$ the apparent terminal half-life.

The statistical analysis was carried out according to current bioequivalence guidelines and assessed possible differences due to particle size in $\text{AUC}(0,t)$, $\text{AUC}(0, \infty)$ and C_{max} [20]. Hence, the individual $\text{AUC}(0,t)$, $\text{AUC}(0, \infty)$ and C_{max} data were log-transformed (natural logarithm) and subsequently analyzed using analysis of variance (ANOVA). The ANOVA model included subjects and aerosol sizes as main factors. The mean square error of the ANOVA was used to calculate the 90% confidence intervals of the $\text{AUC}(0,t)$, $\text{AUC}(0, \infty)$ and C_{max} ratios.

Results

Study population

Ten mild asthmatic patients (of whom nine were women) completed the trial. The average age (SD) was 46.6 (10.7) years. Mean FEV_1 (SD) was 83.2 (15.9) % of the predicted value. No adverse events were reported. During the test the inhaled volume (SD) was 4.9 (1.3) l, the inhalation flow (SD) 36.6 (1.9) l min^{-1} and subjects took a median of 5.1 breaths during the 1.5 μm , 3.2

Figure 2

Mean plasma concentrations of 17-beclomethasone monopropionate (17-BMP) after inhalation of monodisperse aerosols with mass median aerodynamic diameters (MMADs) of 1.5 μm (◆), 2.5 μm (■), and 4.5 μm (▲). Gastrointestinal drug absorption was blocked by ingestion of activated charcoal

breaths during the 2.5 μm and 1.4 breaths during the 4.5 μm aerosol administration (with increasing particle size the mass per litre increases, requiring less time to obtain the target dose).

Pharmacokinetics

The mean plasma concentration–time profiles of 17-BMP for aerosols with MMADs of 1.5 μm, 2.5 μm, and 4.5 μm are shown in Figure 2. The derived pharmacokinetic parameters, i.e. log-transformed AUC(0, *t*), AUC(0, ∞) and C_{max} and the geometric mean bioavailabilities following all three inhalations are presented in Table 1. The pharmacokinetic comparison of the three monodisperse aerosols is shown in Table 2. AUC and C_{max} were significantly different between the 1.5 μm and the 2.5 μm aerosols ($P < 0.003$) and between the 1.5 μm and the 4.5 μm aerosols ($P < 0.009$). There was no significant difference in AUC and C_{max} between the 2.5 μm and the 4.5 μm aerosols. The 2.5 μm and 4.5 μm aerosols produced two–three fold higher serum concentrations at all sampling times compared with the 1.5 μm aerosols.

In most patients, the highest concentrations of 17-BMP for the 1.5 μm aerosols were seen at 10 min after inhalation and for the 2.5 μm and 4.5 μm aerosols at 20 min after inhalation. Six hours after inhalation 17-BMP concentrations were below the limit of quantification in most patients. The median elimination half-

life of 17-BMP was 1.5 h for the 1.5 μm aerosols, 1.6 h for the 2.5 μm aerosols and 1.4 h for the 4.5 μm aerosols.

Discussion

The aim of this study was to investigate the pulmonary bioavailability of different monodisperse BDP aerosol sizes in adults with mild asthma. We found that AUC and C_{max} were approximately two–three times lower for the 1.5 μm aerosols compared with the 2.5 μm and 4.5 μm aerosols.

Monodisperse aerosols are useful to investigate the relationship between aerosol particle size and systemic availability, as aerosol distribution curves show minimal overlap. The drawback of this approach is the great effort required to generate monodisperse aerosols. In the present study we used the EHDA method, which is a validated and consistent method to produce monodisperse BDP aerosols [18]. It is also a relatively easy method compared with the spinning top generator, vibrating orifice or Sinclair-LaMer generators.

For accurate charting of the systemic availability of monodisperse steroid aerosols as a function of particle size, we controlled various factors that could affect lung deposition and lung dose. Firstly, patients inhaled the aerosol at a constant flow from a large volume glass reservoir, which acted like a spacer. Inhalation was followed by a 10 s breath holding period. This procedure minimized oropharyngeal deposition and increased residence time, which in its turn enhanced deposition by sedimentation [21–24]. Secondly, the concentration of aerosolized drug and the aerosol particle size in the glass reservoir were kept constant and were measured continuously by an aerodynamic particle sizer (APS). This minimized variations in inhaled dose between and within our patients, and between and within the different particle sizes. Thirdly, we controlled potential patient factors by ensuring that the FEV₁ at start of each visit was within 10% of the patients screening value. Fourthly, the gastrointestinal absorption was blocked by activated charcoal to ensure that the systemic availability represented only the drug absorption through the lung. The method of assessing the systemic bioavailability of the active metabolite 17-BMP, after inhalation of BDP with the charcoal block method, has been described and validated previously [19, 25]. The kinetic parameters for 17-BMP found in our study, such as t_{max} and $t_{1/2}$, were within the range found in previous pharmacokinetic studies [19, 25, 26]. With the methodology used in our study, differences in the measured kinetic parameters were unlikely to be biased by the factors discussed above.

Table 1

Log-transformed pharmacokinetic data for beclometasone 17-monodipropionate following inhalation of monodisperse aerosols with MMADs of 1.5 μm , 2.5 μm and 4.5 μm

Aerosol size		log AUC(0,t)	log AUC(0, ∞)	log C_{max}
1.5 μm	Mean (SD)	6.38 (1.03)	6.39 (1.04)	5.97 (0.72)
2.5 μm	Mean (SD)	7.33 (1.05)	7.38 (1.10)	6.81 (0.86)
4.5 μm	Mean (SD)	7.10 (1.47)	7.10 (1.47)	6.60 (1.07)

log AUC log-transformed area under the plasma concentration-time curve, log C_{max} log-transformed maximum plasma concentration.

Table 2

Comparisons between the pharmacokinetic parameters of beclomethasone 17-monodipropionate for aerosols with MMADs of 1.5 μm , 2.5 μm and 4.5 μm

	1.5 μm vs. 2.5 μm	Comparison (A vs. B) 2.5 μm vs. 4.5 μm	1.5 μm vs. 4.5 μm
log AUC ($\text{pg ml}^{-1} \text{ h}$)	-2.933	0.223	-2.710
90% CI for difference ($\text{pg ml}^{-1} \text{ h}$)	-4.415, -1.451	-1.201, 1.647	-4.192, -1.227
P value	0.003	0.788	0.005
log C_{max} (pg ml^{-1}) Mean difference (A-B)	-0.779	0.208	-0.570
90% CI for difference (pg ml^{-1})	-1.116, -0.441	-0.116, 0.533	-0.908, -0.233
P value	0.001	0.279	0.009

log AUC log-transformed area under the plasma concentration-time curve, log C_{max} log-transformed maximum plasma concentration, CI confidence interval.

The results of our study can be explained by adopting the deposition mechanisms as presented by Heyder *et al.* [27]. They found that the alveolar deposition of monodisperse particles between 2 and 6 μm in size was substantially higher compared with that of particles smaller than 2 μm .

The greater systemic availability of the 2.5 μm and 4.5 μm particles compared with the 1.5 μm particles can be explained by the inhalation technique employed in our study. The constant and deep low-flow inhalation followed by a breath holding period, presumably allowed the 2.5 μm and 4.5 μm particles to pass the extrathoracic airways without any deposition and to deposit in the conducting and peripheral airways in high doses. The deposition of these particles was favoured by sedimentation during the relatively long inhalation and breath-holding period. The 1.5 μm particles are typically able to pass through the extrathoracic and bronchial airways and reach and deposit in the alveolar region.

However, a high proportion of these small particles will be exhaled again due to the lack of efficient deposition mechanisms [27].

The results of our study seem to conflict with the results of previous pharmacokinetic studies comparing hydrofluoroalkane-134 (HFA)-BDP aerosols (MMAD 1.1 μm) with chlorofluorocarbon (CFC)-BDP aerosols (MMAD 3 μm). These studies showed that HFA-BDP yielded a two times greater systemic availability compared with the same dose of CFC-BDP [28, 29]. This difference in systemic availability was explained by the smaller MMAD of the HFA-BDP compared with the CFC-BDP. However, the known differences in spray characteristics (low vs. high velocity) of the HFA- and CFC-pMDIs, as well as the differences in inhalation techniques, were not considered to be a possible source of the noted differences. A head-to-head comparison between these studies and our study cannot be made, because HFA-BDP and CFC-BDP aerosols are poly-

disperse aerosols and we used monodisperse aerosols. Polydisperse aerosols have a large particle size distribution. Therefore, it is not possible to assess the contribution of the various particle sizes on the systemic availability. Hence, the increased systemic availability found for the HFA-BDP aerosols might well be due to the increased deposition of both small and large particles in the conducting and peripheral airways.

Type of dispersity of the aerosol cannot solely explain the discrepancies in systemic availability between our study and the HFAs *vs.* CFC studies [26, 28, 29]. The HFA *vs.* CFC studies followed a different study design. First, the HFA *vs.* CFC studies did not use activated charcoal to block gastrointestinal absorption and the systemic availability found in these studies is therefore a mixture of lung and GI tract absorption. As we used charcoal, our data solely reflect lung absorption [26, 28, 29]. Second, the HFA *vs.* CFC studies measured the first plasma concentration of 17-BMP 0.5–1 h after inhalation [26, 28, 29]. However, as C_{max} can be reached 10 min after inhalation [19], the maximum concentration and a considerable part of the AUC might have been missed. Finally, one of the HFA-CFC studies measured systemic availability from beclomethasone plasma concentrations rather than from 17-BMP concentrations [29]. Beclomethasone, however, is only a small component of the systemically available drug compared with 17-BMP [19, 28].

The aim of our study was to determine the systemic availability of inhaled corticosteroids with different aerosol particle sizes. The 1.5 μm aerosols gave a significantly lower systemic availability compared with the 2.5 μm and 4.5 μm aerosols. However, our data give only an indication of total lung deposition. They do not allow conclusions to be drawn about the distribution of deposition in the lung or about clinical efficacy. Furthermore, these results obtained in adults are probably not applicable to young children. As children have narrower airways, the distribution of deposition in the airways, total deposition, and systemic availability will be different. To complete the definition of optimal aerosol particle size for inhaled corticosteroids in adults, clinical efficacy studies with monodisperse aerosols should be carried out.

The authors wish to thank the patients who participated in this study.

GlaxoSmithKline is gratefully acknowledged for the pharmacokinetic analysis of the blood samples and for their financial support.

J-W.J.L. has received from GlaxoSmithKline (the Netherlands), a travelgrant of €500 to attend an inter-

national symposium (ERS) and a research grant of €150 000. This article is part of the Ph.D. thesis of J.E.E-F. This Ph.D. was financed by AstraZeneca, the Netherlands.

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