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Particle size matters: diagnostics and treatment of small airways involvement in asthma

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ABSTRACT: Small airways are an important site of inflammation and obstruction in asthma, which contributes to the severity of airway hyperresponsiveness (AHR) that is usually measured by nebulisation of large-particle stimuli. We investigated whether small and large particle sizes of aerosolised adenosine monophospate (AMP) provoke similar severity of AHR. Additionally, effects of the small-particle inhaled corticosteroid (ICS) ciclesonide and large-particle ICS fluticasone on AHR to large- and small-particle size AMP were assessed.

After a 4-week run-in period using open-label fluticasone (100 μg *b.i.d.*), 37 mild-to-moderate asthmatics underwent provocations with standard-size (3.7 μm), large-particle (9.9 μm) and small-particle (1.06 μm) AMP. Subjects received 4-week ciclesonide (160 μg *s.i.d.*) or fluticasone (100 μg *b.i.d.*) treatment (double-blind and double-dummy) followed by large- and small-particle AMP provocation.

Small-particle AMP induced a 20% decrease in forced expiratory volume in 1 s (FEV_1) at a significantly higher dose than large-particle AMP. Ciclesonide and fluticasone had comparable effects on AMP provocations. Not all subjects reached the provocative concentration causing a 20% fall in FEV_1 (PC_{20}) at the highest AMP dose. In those who did, ciclesonide improved small-particle AMP PC_{20} by 1.74 doubling doses (DD) ($p=0.03$), whereas fluticasone did not. Conversely, fluticasone improved large-particle AMP PC_{20} significantly (1.32 DD; $p=0.03$), whereas ciclesonide did not.

Small-particle AMP provocation appears to be a promising tool to assess changes in small airway inflammation. Future adjustments are necessary taking into account the very small particle size used, with large exhaled fractions. In asthmatics reaching a PC_{20} with small- and large-particle AMP provocations, ciclesonide improves hyperresponsiveness to small particle size AMP, and fluticasone to large particle size. This warrants further research to target provocations and treatment to specific airway sizes.

KEYWORDS: Asthma, bronchoprovocation, nebuliser, small airways

Asthma is an inflammatory disease affecting large, intermediate and small airways [1, 2]. In the past, involvement of the small airways (*i.e.* airways with an internal diameter <2 mm) could only be assessed poorly, due to limitations of *in vivo* sampling techniques and a lack of specificity of physiological measurements to assess peripheral airway obstruction. However, analyses of *post mortem* and resected lung tissue samples have demonstrated that considerable small airway disease exists in asthma [3, 4]. More recently, advances in less invasive techniques, such as measurement of alveolar and bronchial exhaled nitric oxide (eNO), the multibreath washout technique [5] and volumetric measurement of air trapping, have confirmed the important role of small airway inflammation in mild-to-moderate asthma [6, 7].

Since small airways are an important therapeutic target, novel formulations of inhaled corticosteroids (ICS) have been developed. These newer formulations use hydrofluoroalkane (HFA) propellants and some yield smaller particle sizes that allow better access to the distal airways. Imaging studies suggest that these new formulations produce beneficial changes in the distal airways as well [6, 8]. For example, the HFA formulation of beclomethasone dipropionate (BDP) enabled effective asthma control at much lower doses

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than CFC-propelled BDP [9]. Ciclesonide is a novel small-particle ICS that has shown clinical efficacy in asthma in low doses [10–13].

In both clinical and research settings, methacholine challenge tests are being used to quantify airway hyperresponsiveness (AHR), a core feature of asthma. Another provocative stimulus, adenosine monophosphate (AMP), reflects eosinophilic airway inflammation better than methacholine [14]. Research groups have managed to assess small airway changes by means of pulmonary function tests and high-resolution computed tomography after methacholine bronchoprovocation [6, 7, 15]. However, it remains unclear whether methacholine has actually reached the small airways to cause these effects. Deposition in the small airways of inhaled particles is dependent on the inhalation manoeuvre and particle size, which is determined by the type and output rate of the nebuliser, as well as other factors. For methacholine provocation tests, the DeVilbiss 646 is the nebuliser of recommendation [16]. This nebuliser can yield variable particle sizes depending mainly on its output flow rate [17] and at a flow rate of 12 $L \cdot min^{-1}$, methacholine is aerosolised to particles of 3.7 μm [18]. Particles of 5–7 μm in mass mean aerodynamic diameter (MMAD), as produced by an ultrasonic nebuliser, do not reach the lower airways, in contrast to particles of 2–3 μm MMAD produced by a jet nebuliser [19]. Theoretically, nebulisers producing particles that may reach smaller airways can be used in bronchoprovocation tests, which then may reflect the hyperresponsiveness of the small airways in asthma.

The aim of the present study was two-fold. First, we evaluated the use of small-particle and large-particle AMP bronchoprovocation tests as markers of small and large airways involvement in asthma. Secondly, we assessed the differential effects of small-particle *versus* large-particle ICS on the outcome of these bronchoprovocation tests. For small-particle ICS, we used ciclesonide and for large-particle ICS we used fluticasone.

MATERIALS AND METHODS

Study design

This study was performed using a double-blind, double-dummy, randomised parallel-group design, and was registered in a public database prior to execution (clinicaltrials.gov identifier NCT00306163). Subjects were randomised for treatment with large-particle (fluticasone) or small-particle (ciclesonide) ICS. The flow chart of the study is presented in figure 1.

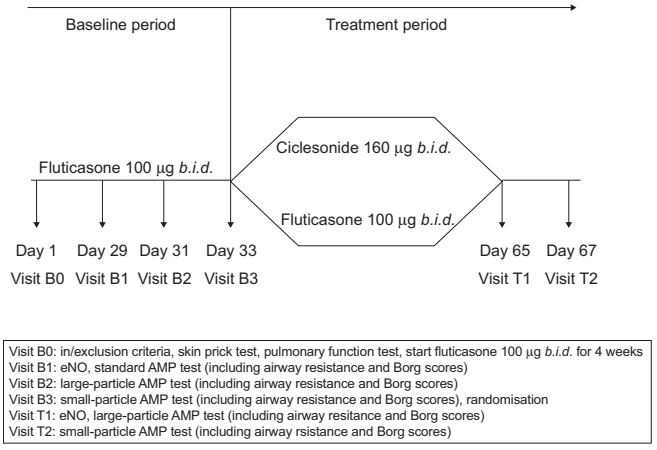

FIGURE 1. Flow chart of study design. eNO: exhaled nitric oxide; AMP: adenosine monophosphate.

Subjects

Subjects were recruited from the outpatient clinics of the pulmonology departments of hospitals in the northern provinces of The Netherlands and with advertisements in local papers. The local Medical Ethics Committee (University Medical Center, Groningen, The Netherlands) approved the study protocol and all subjects gave written informed consent. Subjects between 18 and 60 yrs of age were included with a history of asthma according to the Global Initiative for Asthma (GINA) criteria [20], either steroid-naïve, or using $\leqslant$500 μg fluticasone propionate or equivalent, a pre-bronchodilator forced expiratory volume in 1 s (FEV_1) >1.20 L, and atopy (*i.e.* at least one positive skin prick test to 18 common aeroallergens).

Current or ex-smokers who stopped smoking <1 yr prior to the study and those with $\geqslant$10 pack-yrs exposure were excluded, as were those with a history of chronic obstructive pulmonary disease or other diseases likely interfering with the study, unstable asthma (more than three exacerbations in the previous year or one exacerbation in the past 2 months), concomitant medication that was not allowed (*e.g.* oral corticosteroids <4 weeks prior to participation), intolerance of short-acting β_2-agonists, and females who were pregnant, lactating or lacking adequate methods of contraception. Subjects were excluded during study participation if their asthma became unstable.

Bronchoprovocation tests with aerosolised AMP

Standard AMP test

The standard AMP test was performed conforming to the American Thoracic Society (ATS) protocol (2-min tidal breathing protocol) using a DeVilbiss 646 nebuliser with a calibrated output rate of 0.13 $mL \cdot min^{-1}$ [16]. The MMAD with this method is 3.9 μm (range 3.1–5.5 μm) at an inspiratory flow rate of 10 $L \cdot min^{-1}$ [21]. Spirometry was measured with a dry wedge spirometer (Jaeger Masterscope, Hoechberg, Germany), which was calibrated daily. Baseline FEV_1, forced vital capacity (FVC) and synchronised vital capacity (SVC) were measured. Thereafter, 3 mL saline, followed by doubling doses of aerosolised AMP (0.04–320 $mg \cdot mL^{-1}$) were administered until FEV_1 dropped $\geqslant$20% from baseline. If FEV_1 did not drop $\geqslant$20% from baseline, the test was terminated after administration of the maximum dose of 320 $mg \cdot mL^{-1}$. The provocative concentration causing a 20% fall in FEV_1 (PC_{20}) was calculated according to standardised procedures [16]. At any given dose of AMP causing a $\geqslant$20% fall in FEV_1 from baseline or after administration of the maximum AMP dose for the standard-size AMP test (160 $mg \cdot mL^{-1}$), all subjects were given 400 μg salbutamol per spacer.

Large-particle AMP test

The large-particle AMP test was modeled on the design of a large-particle cat allergen challenge test by LIEUTIER-COLAS *et al.* [22]. The test was performed with a Pari TIA nebuliser (Pari, Starberg, Germany) attached to a pressure reducer set at

1.2 bar. As the generated output was far larger than with standard AMP protocol, nebulisation time was set to 30 s tidal breathing, in order to achieve administration of similar dose of AMP as with the standard AMP set-up. Particle size distributions (psd) in the aerosols from the Pari TIA were measured 10 s from the start of nebulisation and lasted 10 s, after it was checked that no changes occured within the first 30 s of nebulisation. For the measurements at room temperature and moderate relative air humidity using the laser diffraction technique (Sympatec HELOS BF MAGIC) in combination with an INHALER 2000 adapter, a 100 mm lens was used (measuring range 0.9–175 μm). Calculations were made using the Fraunhofer theory, after it was checked that the Mie theory with the correct optical parameters yielded the same result. For the Pari TIA, a MMAD of 9.88 μm (range 9.84–9.91 μm) was obtained (mean and spread of three measurements at 20 $L \cdot min^{-1}$ suction rate). The droplet size distribution ranged from 3.37 μm (3.04–3.60 μm) for X_{10} to 19.03 μm (18.21–19.61 μm) for X_{90}, in which X_{10} and X_{90} are characteristic values from the culmulative volume undersize curve, representing the diameters for which 10% respectively 90% of the total volume of the aerosol is in smaller particle diameters, respectively. Administration of saline followed by increasing doubling doses of AMP and measurement of spirometry was similar to the standard AMP protocol. A PC_{20} of 640 $mg \cdot mL^{-1}$ was assigned to those subjects in whom FEV_1 did not fall $\geqslant$20% with the maximum dose of AMP (320 $mg \cdot mL^{-1}$).

Small-particle AMP test

The small-particle AMP test was modelled on the design of a small-particle cat allergen challenge test developed by LIEUTIER-COLAS *et al.* [22]. The test was performed with a Microcirrus nebuliser (Intersurgical BV, Uden, The Netherlands) attached to a pressure reducer set at 2.5 bar. The generated output was similar to the output of the standard AMP protocol; therefore, the 2-min tidal breathing method was used. psd from the Microcirrus were measured using the same equipment and conditions as described for the Pari TIA. Because AMP administration with the Microcirrus was over a longer period (2 min) than with the Pari TIA (30 s), measurements of 10 s with the Microcurrus were repeated four times during nebulization of the same 3-mL volume, with interval times of 20 s. The first measurement was started after a lag time of 10 s. Only measurements with sufficient optical concentration were used for computation of the mean for a total nebulisation time of 2 min. No changes in psd as a function of the nebulisation time were observed. Mean MMAD of four nebulizations with the Microcirrus is 1.06 μm (range 1.04–1.08 μm). The droplet size distribution ranges from 0.60 μm (0.60–0.61 μm) for X_{10} to 2.18 μm (2.17–2.19 μm) for X_{90}. Administration of saline, followed by increasing doubling doses of AMP and measurement of spirometry was similar to the standard AMP protocol. A PC_{20} of 640 $mg \cdot mL^{-1}$ was assigned to those subjects in whom FEV_1 did not fall $\geqslant$20% with the maximum dose of AMP (320 $mg \cdot mL^{-1}$).

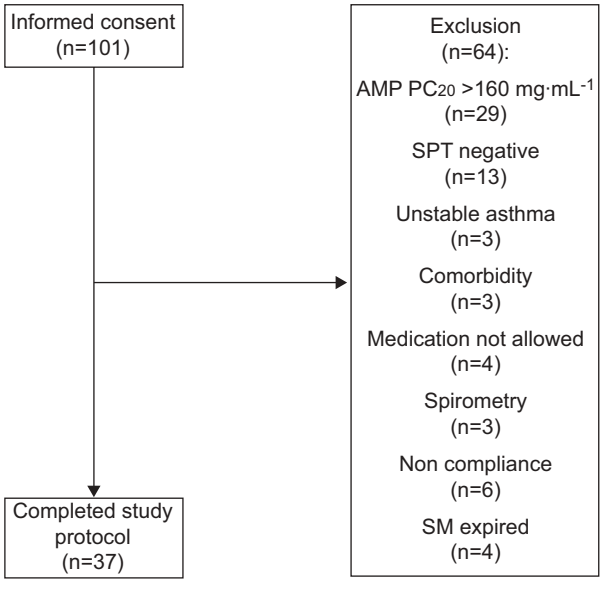

FIGURE 2. Flow chart of inclusion and exclusion criteria. AMP: adenosine monophosphate; PC_{20}: provocative concentration causing a 20% fall in forced expiratory volume in 1 s; SPT: skin prick test; SM: study medication.

Bronchial and alveolar eNO

Bronchial and alveolar eNO were calculated after eNO measurement with a NIOX instrument (Aerocrine, Stockholm, Sweden) at multiple flow rates (30, 50, 100 and 200 $mL \cdot s^{-1}$). Per flow rate, the mean of three technically acceptable attempts was used for analysis. Bronchial and alveolar fractions were calculated with a modification of the mathematical model of Tsoukias and George [23].

Borg dyspnoea score

Dyspnoea was scored according to the modified Borg scale, which ranges from 0 (no dyspnoea) to 10 (maximal breathlessness) [24], before and after each administration of either saline, AMP or salbutamol at the end of each AMP test.

Airway resistance

Hypothetically, if only small airways become obstructed, FEV_1 may not be reduced to a large extent. Therefore, we additionally measured airway resistance at three time-points: before each AMP test, at the moment of bronchoconstriction (*i.e.* immediately after PC_{20} was reached or in the absence of a PC_{20} after administration of the maximum dose) and 10 min after administration of 400 μg salbutamol. Airway resistance was measured with a shutter-interrupter technique using the measurement program ROcclusion (Jaeger Masterscope, Würzburg, Germany).

Randomisation

Subjects were randomised to treatment with either small corticosteroid particles (ciclesonide 160 μg *s.i.d.*) or large corticosteroid particles (fluticasone 100 μg *b.i.d.*). Both investigators and patients were blinded to treatment. A double-dummy procedure was adhered to in order to guarantee blinding, as ciclesonide was administered as an metered-dose inhaler and fluticasone as a dry-powder inhaler. Randomisation codes were broken after the last patient had completed the study.

Statistical analysis

Data were entered by double-entry protocol into a database. A power calculation was performed prior to study execution.

It was assumed that the treatment effect on the difference between large- and small-particle PC_{20} values would amount to approximately 1–1.5 DD AMP. Based on experience with trials using AMP bronchoprovocations, the standard deviation of the AMP PC_{20} was assumed to amount to 1 DD. Using a subject size of 40, a power calculation yielded a minimal detectable difference of 0.44 DD with a power of 80% and an α of 5%

Data are presented as median (interquartile range (IQR)). A p-value <0.05 was considered to indicate statistical significance. Differences between large- and small-particle PC_{20} values were tested with Wilcoxon signed rank tests, and differences between groups with Mann–Whitney U-tests. Within- and between-treatment differences in lung function were tested by means of a paired and independent t-test. Spearman's nonparametric rank correlation was calculated to assess the relationship between airway resistance and PC_{20} with the three particle sizes.

RESULTS

Study population

A total of 101 subjects gave informed consent; 64 subjects did not enter or discontinued the study due to various reasons and 37 asthmatics completed the study protocol (fig. 2). Characteristics of the latter subjects are shown in table 1. 19 subjects were randomised to ciclesonide treatment and 18 subjects to fluticasone. After a 4-week run-in period with fluticasone, there were no statistically significant differences in clinical characteristics between the treatment groups (table 1). Side-effects were comparable between fluticasone and ciclesonide (individual data not shown).

AMP PC_{20}

AMP PC_{20} tests according to the standard-, large- and small-particle protocols did not differ significantly between treatment groups at randomisation or after the treatment period (table 2 and fig. 3).

TABLE 1 Baseline characteristics

	Ciclesonide	Fluticasone	p-value
Subjects n	19	18	
Males	6 (31.6)	10 (55.6)	0.14
Ex-smokers	2 (10.5)	5 (27.8)	0.23
Age yrs	39.6 (32.5–46.6)	46.0 (37.9–55.5)	0.10
Lung function after run-in#			
FEV_1 % pred	91.3 (76.7–109.3)	86.8 (81.6–100.1)	0.69
FVC % pred	110.2 (92.9–119.7)	104.2 (90.5–118.9)	0.72
SVC % pred	104.9 (94.6–122.0)	106.1 (91.7–118.3)	0.83
FVC/SVC %	97.9 (95.3–100.5)	95.5 (92.3–100.3)	0.20
FEV_1/FVC % pred	92.9 (85.0–102.0)	90.1 (83.6–99.4)	0.67
$FEF_{50\%}$ % pred	68.1 (48.6–83.2)	53.6 (46.8–70.9)	0.52
$FEF_{25–75\%}$ % pred	65.3 (45.7–86.2)	58.4 (47.5–76.9)	0.58

Data are presented as n (%) or median (interquartile range), unless otherwise stated. n=37. Between-group differences are tested with the Mann–Whitney U-test. FEV_1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; SVC: synchronised vital capacity; $FEF_{50\%}$: forced expiratory flow at 50% of FVC; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of FVC. #: 4 weeks fluticasone 100 μg *b.i.d.* (day 33).

In the total study population, PC_{20} measured with standard-particle AMP did not differ significantly from the large-particle AMP PC_{20}, but both were significantly smaller than the PC_{20} value obtained with small-particle AMP (median PC_{20} 12.0 $mg \cdot mL^{-1}$ (IQR 6.6–59.4 $mg \cdot mL^{-1}$), 9.6 $mg \cdot mL^{-1}$ (IQR 4.5–109.7 $mg \cdot mL^{-1}$) and 225.8 $mg \cdot mL^{-1}$ (IQR 32.1–640.0 $mg \cdot mL^{-1}$), respectively; $p<0.001$).

There were no significant differences in treatment effect on change in (Δ)FVC % predicted at PC_{20}, ΔSVC % pred at PC_{20} or ΔFVC/SVC % pred at PC_{20} measured with either small or large particle sizes of AMP.

Not all subjects reached a PC_{20} with the small- and large-particle AMP protocol at baseline (subjects with baseline PC_{20} <640 $mg \cdot mL^{-1}$ in table 2). The 23 subjects with a small-particle AMP PC_{20} <640 $mg \cdot mL^{-1}$ had significantly lower forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$) % pred than the 14 subjects with PC_{20} >640 $mg \cdot mL^{-1}$ (median 56.3% (IQR 36.1–72.7%) *versus* 67.6% (IQR 58.2–98.0), respectively; $p=0.04$). Consistent with this, forced expiratory flow at 50% of FVC ($FEF_{50\%}$) % pred was significantly smaller in those with small-particle AMP PC_{20} <640 $mg \cdot mL^{-1}$ (median 55.4% (IQR 38.9–70.6%) *versus* 69.1% (IQR 59.3–102.1%), respectively; $p=0.012$).

The following sub-analyses on treatment effects were performed on all subjects with PC_{20} <640 $mg \cdot mL^{-1}$ (n=23).

Large-particle protocol

15 out of 19 subjects in the ciclesonide group and 16 out of 18 subjects in the fluticasone group had a baseline PC_{20} <640 $mg \cdot mL^{-1}$. Within the fluticasone group, there was a significant treatment effect on improvement of ^{2}log PC_{20} of 1.32 DD ($p=0.03$). In the ciclesonide group, there was an improvement of ^{2}log PC_{20} of 0.74 DD ($p=0.17$) (table 2 and fig. 3).

Small-particle protocol

11 out of 19 subjects in the ciclesonide group and 12 out of 18 subjects in the fluticasone group had a baseline PC_{20} <640 $mg \cdot mL^{-1}$. Within the ciclesonide group, there was a significant treatment effect on improvement of ^{2}log PC_{20} of 1.74 DD ($p=0.03$). In the fluticasone group there was an improvement of ^{2}log PC_{20} of 0.8 DD ($p=0.17$) (table 2 and fig. 3).

eNO

A small number of subjects in the study could not perform eNO tests due to technical problems with the NIOX equipment. Fluticasone changed alveolar eNO values significantly more than ciclesonide, by a median of -1.1 (IQR -2.1–0.1) *versus* 0.4 (IQR -0.8–3.9), respectively; $p=0.03$) (table 3), whereas changes in bronchial eNO values were comparable.

Borg dyspnoea score

The change in Borg dyspnoea score from before to after the last dose of AMP was significantly higher with the standard-particle AMP test than with small-particle AMP (median 3 (IQR 2–5) *versus* 2 (IQR 1–4), respectively; $p=0.043$).

TABLE 2 Adenosine monophosphate (AMP) provocative concentration causing a 20% fall in forced expiratory volume in 1 s (PC_{20}) for the different particle sizes, measuremed at baseline and after treatment with ciclesonide or fluticasone

	All subjects			Subjects with baseline PC_{20} <640 mg·mL^{-1}		
	Ciclesonide	Fluticasone	p-value	Ciclesonide	Fluticasone	p-value
Subjects n	19	18		11	12	
Baseline PC_{20} DD						
Standard-particle AMP	4.2±2.8	3.5±2.1	0.41			
Large-particle AMP	4.9±3.3	3.8±3.2	0.30	3.7±2.6	3.0±2.4	0.44
Small-particle AMP	6.8±3.6	6.6±2.7	0.87	4.9±3.7	5.2±2.1	0.81
Post-treatment PC_{20} DD						
Large-particle AMP	4.8±2.7	4.5±2.3	0.77	4.5±2.3#	4.3±2.4¶	0.89
Small-particle AMP	7.4±2.0	6.7±2.8	0.42	6.6±2.0¶	6.0±2.7#	0.53

Data are presented as mean±SD, unless otherwise stated. Values are ^{2}log PC_{20} values. Between-group differences were tested with t-test. The 23 subjects at the right part of the table are those subjects who reach a PC_{20} <640 mg·mL^{-1} with the the small- and large-particle AMP protocol at baseline. DD: doubling dose. # p=0.17 within group; ¶: p=0.03 within group.

There were no significant differences in change in Borg dyspnoea score between standard- and large-particle AMP, or between large- and small-particle AMP. There were no significant between-treatment differences in change in dyspnoea score.

Airway resistance

Airway resistance was significantly higher immediately after compared with before each bronchoprovocation test (p<0.001; table 4). There were no significant differences between changes in airway resistance with AMP provocation tests with the three different particle sizes. Also, there were no significant differences in treatment effects on the change in airway resistance after AMP provocation tests.

There was a significant association between the severity of hyperresponsiveness to small-particle AMP and the change in airway resistance after provocation with small-particle AMP: a higher PC_{20} (thus, less hyperreponsiveness) was significantly correlated with a smaller change in airway resistance after the small-particle AMP test (Spearman's rank correlation test r= -0.59; p<0.001). There were no significant associations between PC_{20} values obtained with large- or standard-particle AMP and changes in airway resistance.

Spirometry

Fluticasone improved mean±SD FEV_1 % pred significantly more than ciclesonide (0.5±4.3 *versus* -3.0±4.6%, respectively; p=0.021). There were no significant differences in treatment effects of ciclesonide and fluticasone on FVC, SVC, FVC/SVC or $FEF_{50\%}$. $FEF_{25-75\%}$, however, improved more with fluticasone than with ciclesonide (0.6±5.6 *versus* -3.6±6.0% pred; p=0.034).

DISCUSSION

This study renews the discussion on the role of particle size in the deposition of inhaled substances. Particle size appears to be important both with respect to diagnostic tools, such as bronchoprovocation tests, and the inhaled therapeutics used in asthma. First, we found that small-particle AMP induced a 20% fall in FEV_1 after a significantly higher dose than a larger particle size AMP provocation. Secondly, our double-blind study with large- and small-particle ICSs in mild-to-moderate asthma showed that there were no significant differences in clinical effects of ICSs with a small and a large particle size (*i.e.* ciclesonide and fluticasone, respectively). Not all subjects reached PC_{20} at the highest applied AMP dose (320 mg·mL^{-1}). In the group of asthmatics who did achieve this, ciclesonide improved ^{2}log PC_{20} AMP with small particle size by 1.74 DD (p=0.03), whereas fluticasone did not. Conversely, fluticasone improved ^{2}log PC_{20} with large-particle AMP significantly (1.32 DD; p=0.03), whereas ciclesonide did not.

We demonstrated that PC_{20} values differ significantly when they are calculated from tests with standard, small or large particle sizes. Surprisingly, large-particle PC_{20} values were smaller than those of standard particles, which in turn were smaller than small-particle PC_{20} values. When designing this study, we hypothesised that the opposite would occur, *i.e.* the lowest PC_{20} was to be expected with small-particle nebulisation, as the small airways account for >50% of total airway surface [25], and a larger response at a lower dose of AMP was expected. How can we explain this intriguing finding? First, it may be that, due to the very small size of the small-particle AMP (MMAD 1.04 μm), a considerable fraction of particles may not have been deposited onto the airway surface and were subsequently exhaled. This will then cause a smaller bronchoconstrictive response than with larger particles that tend to deposit onto the airway surface far more easily. An alternative explanation may be the properties of the airway structure of small and large airways. Although small airways are involved in the inflammatory process of mild-to-moderate asthma, this does not automatically imply that small airways also show a similar severity of hyperresponsiveness compared to intermediate or large airways, as there are also marked structural differences between airways of different size and generation [26]. Furthermore, PC_{20} is defined by the 20% fall in FEV_1, which may also explain why small-particle PC_{20} is larger than that with larger particle sizes. FEV_1 is thought to be generated, to a large extent, by the airway diameter of the first four generations of the bronchial tree [27] and, therefore, may not

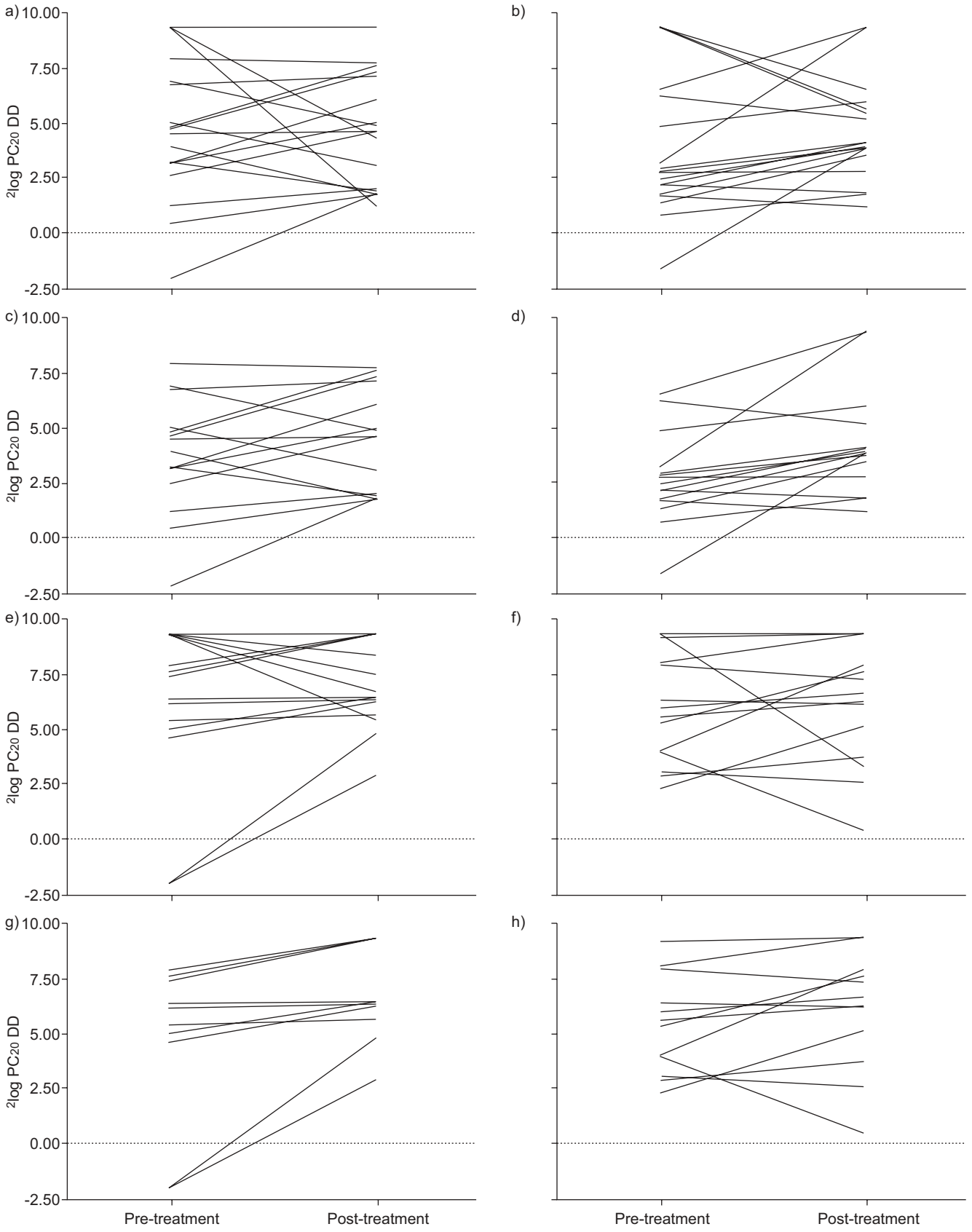

FIGURE 3. Provocative concentration causing a 20% fall in forced expiratory volume in 1 s (PC_{20}) of a–d) large- and e–h) small-particle adenosine monophosphate before and after treatment with a, c, e, g) ciclesonide and b, d, f, h) fluticasone in a, b, e, f) all subjects and c, d, g, h) subjects with baseline PC_{20} <640 mg·mL^{-1}.

TABLE 3 Exhaled nitric oxide (eNO)

	Ciclesonide#		Fluticasone¶		p-value
	Subjects	eNO	Subjects	eNO	
Baseline					
Alveolar	18	4.78 (3.52–8.12)	17	5.52 (4.72–6.83)	0.51
Bronchial	18	0.66 (0.49–1.39)	17	0.79 (0.67–1.12)	0.41
Post-treatment					
Alveolar	16	4.61 (3.29–6.91)	16	4.73 (3.69–5.69)	0.88
Bronchial	16	0.67 (0.42–1.44)	16	0.82 (0.46–1.33)	0.90
Differences					
Alveolar	15	0.40 (-0.82–3.85)	15	-1.06 (-2.41–0.07)	0.03
Bronchial	15	0.17 (-0.11–0.70)	15	0.02 (-0.15–0.09)	0.22

Data are presented as n or median (interquartile range), unless otherwise stated. Between-group differences were tested with Mann–Whitney U-test. Alveolar eNO is expressed in ppb and bronical eNO in $nL \cdot s^{-1}$. #: n=19; ¶: n=18.

TABLE 4 Airway resistance

	Airway resistance	p-value
Standard-particle AMP		
R_{OCC} before AMP	0.43 (0.34–0.55)	
R_{OCC} after AMP	0.59 (0.45–0.89)	<0.001¶
R_{OCC} after salbutamol#	0.33 (0.27–0.43)	<0.001+
Large-particle AMP		
R_{OCC} before AMP	0.40 (0.36–0.54)	
R_{OCC} after AMP	0.56 (0.45–0.84)	<0.001¶
R_{OCC} after salbutamol#	0.33 (0.28–0.42)	<0.001+
Small-particle AMP		
R_{OCC} before AMP	0.41 (0.35–0.48)	
R_{OCC} after AMP	0.50 (0.43–0.76)	<0.001¶
R_{OCC} after salbutamol#	0.31 (0.26–0.39)	<0.001+

Data are presented as median (interquartile range). AMP: adenosine monophosphate; R_{OCC}: occlusion resistance. #: 400 μg per spacer; ¶: after AMP *versus* before AMP; +: after salbutamol *versus* after AMP.

be much reduced in the case where small airways are even extensively obstructed. All of the above-mentioned explanations may be applicable and are in accordance with findings in an earlier study by CASSET *et al.* [28] on the effects of particle sizes of inhaled allergens, which demonstrated lower provocative doses causing a 20% fall in FEV_1 with larger particle sizes too. It would have been advantageous if we had had access to better tools to evaluate small airway bronchoconstriction than the interrupter resistance technique used in this study. Forced oscillation or impulse oscillometry possibly allow for a much better analysis of AMP-induced changes in the small airways. In addition, the multibreath washout test with determination of proximal ventilation index (S_{cond}) and peripheral ventilation index (S_{acin}) is a promising and highly sensitive tool, as confirmed by a recent paper by VERBANCK *et al.* [29]. Unfortunately, at the time of the study, these techniques were not available to the study site.

A small number of subjects did not have a >20% decrease in FEV_1 after the highest administered concentration of AMP with either the small- and/or large-particle protocol. This may be the reason why there were no significant treatment effects on PC_{20} when analysed in the full group of participants. Subanalyses within individuals who demonstrated a PC_{20} <640 $mg \cdot mL^{-1}$ revealed that ciclesonide, the small-particle ICS, significantly improved small airway obstruction, as reflected by small-particle PC_{20}. Furthermore, fluticasone, the large-particle ICS, improved large airway obstruction, which was, similarly, reflected by improvements in large-particle PC_{20}. These results do meet the predefined hypothesis that small-particle treatment has a larger effect on small airway obstruction than large-particle treatment. Interestingly, subjects who did respond to the small-particle AMP challenge tests with PC_{20} <640 $mg \cdot mL^{-1}$ differed from subjects without such a response by demonstrating more small airway obstruction, as reflected by $FEF_{25-75\%}$ and $FEF_{50\%}$. This finding is in support of the hypothesis that in patients with more pronounced small airway involvement in asthma, there is more small airway hyperresponsiveness, as demonstrated by small-particle AMP PC_{20}, and it is this specific subgroup that appears to benefit most from small-particle ICS treatment with ciclesonide. Nevertheless, ciclesonide is known to improve both large and small airway parameters [30, 31] and, therefore, the finding in this study that fluticasone improves large-particle PC_{20} more than ciclesonide is subject to speculation is surprising; more so, as ciclesonide was equivalent to fluticasone in improving AMP PC_{20} in a previous study [32]. One may argue whether the duration of the treatment period in our study was sufficiently long to detect the hypothesised treatment effects. Longer treatment periods may offer larger effect sizes of PC_{20} values by increasing power. Nevertheless, both ciclesonide and fluticasone have been demonstrated to significantly improve bronchial hyperresponsiveness (BHR) to either methacholine or AMP after a period of 4 weeks [30–32]. Moreover, a shorter duration of the treatment period has a lower chance of intercurrent spontaneous changes in the clinical expression of asthma and dropping out of patients. Therefore, we believe that the investigated study periods were sufficiently long to enable adequate detection of improvement in BHR.

Unexpectedly, fluticasone improved FEV_1, $FEF_{25-75\%}$ and alveolar eNO better than ciclesonide did. In other trials, ciclesonide has been shown to be equivalent to fluticasone in increasing FEV_1 [33]. The smaller effect of ciclesonide on small airway parameters, such as $FEF_{25-75\%}$ and alveolar eNO, compared with fluticasone is even more surprising. Whether these results are true or merely reflect noise remains unclear: it is possible that pre-treatment differences have influenced the results by regression to the mean. However, if these results are true, a few factors may be recognised to have influenced these effects. First of all, the difference in dosing regimen may account for the difference in treatment effect, as fluticasone was dosed twice-daily as opposed to the once-daily dosing of ciclesonide. Furthermore, in sharp contrast to our study, earlier studies demonstrating equivalent efficacy of ciclesonide to fluticasone were performed without run-in periods in which

ICSs were being used. The use of fluticasone in the run-in period may have provided additive effects to the fluticasone that was used during the treatment period, which is when effects on lung function were evaluated. In contrast, the switch from fluticasone in the run-in period to ciclesonide during the treatment period may have caused a smaller beneficial effect on conventional lung function parameters.

In summary, the currently investigated small- and large-particle AMP challenge tests appear to be promising tools for differentiating which patient will benefit most from either large- or small-particle ICS. Some adjustments may improve this. Possibly, the use of a different parameter than FEV_1 to establish small airway obstruction will aid in improving the small airway bronchoprovocation test. Furthermore, a slightly larger small-particle AMP (*e.g.* 1.4–2.0 μm) will possibly prove to be a better indicator of small airways involvement in asthma than the extremely small particles that were applied in this study. The use of slightly larger small-particle AMP may then overcome the issue of large exhaled fractions of AMP due to insufficient deposition in peripheral airways of the extreme small particles. When slightly increasing the size of the particles, this may result in a larger number of respondents to the small-particle bronchoprovocation test, which in turn could lead to better identification of subjects with more pronounced small airway involvement in asthma. This study has demonstrated that within the group of responders to small- and large-particle AMP bronchoprovocation tests, ciclesonide is more effective than fluticasone in treating hyperresponsiveness, as tested with small particle size. More investigations with larger study populations are necessary in order to establish a reliable test reflecting small and large airway inflammation. Hopefully, this will enable us to create patient-adjusted treatment regimens, with treatment specifically targeted to the most prevalent site of inflammation.

SUPPORT STATEMENT

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CLINICAL TRIAL

This study is registered at www.clinicaltrials.gov with clinical trial identifier number NCT00306163.

STATEMENT OF INTEREST

A statement of interest for D.S. Postma and the study itself can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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