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Ciclesonide improves measures of small airway involvement in asthma

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ABSTRACT

Rationale

Ciclesonide is delivered as a small-particle inhaled corticosteroid and improves lung function and airway hyperresponsiveness.

Objective

To assess whether ciclesonide can specifically improve small airway function in asthma.

Methods

Sixteen mild-to-moderate asthma patients (7 males, median age 39 (range 19-56) years, FEV_1%predicted 89% (range 62-120)) were randomized to 5-week treatment with placebo or 320 µg ciclesonide once daily. The following small airway parameters were assessed: $FEF_{25-75\%}$, percentage fall in FVC at PC_{20}adenosine-'5-monophosphate (AMP) and at PC_{20}methacholine (MCh), expiratory lung volume after MCh challenge on Computed Tomography (CT) scan, Single Breath N_2 closing volume, and alveolar exhaled Nitric Oxide (eNO).

Results

Seven subjects received placebo, nine ciclesonide. Both CT measurements of expiratory lung volume after MCh challenge and alveolar eNO decreased significantly more with ciclesonide, median (range) 4.4 ppb (1.4-54.8) and 59 mL (1569 to -117) respectively, than with placebo, -0.4 ppb (7.3 to -3.4) and -121 mL (20 to -236) respectively ($p<0.05$). Ciclesonide did not significantly improve other small airways parameters.

Conclusions

Inflammation and patency of small airways, reflected by alveolar eNO and air trapping on CT scan, both improve with ciclesonide even in this small number of patients. This indicates that ciclesonide exerts anti-inflammatory effects on small airways.

Key words: asthma, ciclesonide, small airways

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways and anti-inflammatory treatment with inhaled corticosteroids (ICS) constitutes the cornerstone of asthma management. Nevertheless, a considerable subset of asthma patients does not benefit from ICS or does not gain optimal asthma control [1-3]. It can be speculated that inflammation of the small airways contributes to the poor asthma control observed, since small airways are not directly reached by conventional ICS [4].

The small airways, i.e. airways with an internal diameter < 2 mm, have not always been considered important in asthma. After having been dubbed 'the quiet zone' by Mead in 1970 because they merely contributed 10% to total airway resistance [5], the small airways have regained attention over the past fifteen years as to their role in asthma. At present, it is acknowledged that increasing physiological and pathological evidence exists that inflammation of the small airways is similarly and often even more pronounced than in larger airways in severe asthma [6,7]. This new insight in the importance of small airway inflammation in asthma has led to the introduction of ICS with small-particle formulations that target this site of inflammation. Ciclesonide (Alvesco®) is such an ICS, as it is formulated as a solution delivered via a hydrofluoroalkane-134a (HFA) metered-dose inhaler (MDI). A labelling study showed that a high fraction of ciclesonide (52%) is deposited in the lung. Additionally, 3D SPECT analysis revealed that the highest ciclesonide deposition was found in peripheral regions of the lung, i.e. the zones with small airways and alveoli [8].

Ciclesonide has been demonstrated to maintain asthma control [9] and improve lung function (Forced Expiratory Volume in 1 second [FEV_1], Peak Expiratory Flow [PEF] and Forced Vital Capacity [FVC]) in both mild-to-moderate and

moderate-to-severe asthma [10]. Furthermore, ciclesonide reduces symptoms and airway hyperresponsiveness assessed with both Methacholine (MCh) [11] and Adenosine-‘5-monophosphate (AMP) [12,13].

Although it is known that ciclesonide improves lung function and inflammation [14-17], it is still unknown whether ciclesonide specifically improves small airway function and inflammation. It has been demonstrated that ciclesonide reaches the small airways [8], therefore it can be hypothesized that ciclesonide improves small airway parameters in asthma. To determine the efficacy of ciclesonide, we evaluated different parameters of small airway function and inflammation in 16 mild-to-moderate asthma patients in a double-blind randomized, placebo-controlled pilot trial with ciclesonide 320 µg once daily.

MATERIALS AND METHODS

Subjects

Subjects were recruited from the out-patient clinic of the Department of Pulmonology of the University Medical Center Groningen and with advertisements in local papers. The local Medical Ethics Committee reviewed and approved the study protocol, and the study was registered in a public trial database (clinicaltrials.gov identifier NCT00163345). All subjects gave their written informed consent.

Inclusion Criteria

Subjects of either gender, between 18 and 60 years of age, with a history of asthma according to GINA criteria [18] and using $\leq$ 800 µg budesonide/day or its equivalent were eligible for study participation. In addition, subjects were required to have a baseline $FEV_1 \geq 60$ % of predicted reference value [19], bronchial responsiveness to

both MCh and AMP, defined as a provocative concentration causing a fall in FEV_1 from baseline $\geq$ 20%, PC_{20}MCh ≤ 4.9 mg/mL and PC_{20}AMP ≤ 40 mg/mL, and proven atopy defined by at least 1 positive skin prick test to 18 common aero-allergens.

Exclusion criteria

Current smokers or ex-smokers who quitted smoking < 1 year prior to study participation or with $\geq$ 10 packyears were excluded. In addition, subjects were not eligible if they had 1) a history of COPD or other pulmonary or concomitant diseases expected to interfere with the study, 2) unstable asthma (defined as more than 3 exacerbations in the past year or 1 exacerbation in the past 2 months), 3) concomitant medication that was not allowed (e.g. oral corticosteroids within 4 weeks prior to study participation), 4) intolerance for short-acting β_2-agonists (SABA) or suspected hypersensitivity for ICS, 5) or were females who were pregnant or lactating or lacking an effective method of contraception.

Study Design

This pilot study was designed as a double-blind, randomized, placebo-controlled, parallel-group trial (Flow Chart; Figure 1). The study consisted of a 4-week pre-baseline period, for those pre-treated with ICS with or without a long-acting β_2-agonist (LABA), a 2 to 3-week baseline period, and a 5 to 6-week treatment period, depending on whether a bronchoscopy was performed or not. Treatment with ICS or LABA was withdrawn during the pre-baseline period and substituted with SABA only as rescue medication. Subjects who were treated with SABA only as rescue medication and who had an FEV_1 ≥ 60% of predicted entered the study in the baseline period. After the 2 to 3-week baseline period, subjects demonstrating

bronchial responsiveness to both MCh and AMP were randomized to receive either ciclesonide 320 µg once daily or placebo in the morning for 5-6 weeks. Randomization was stratified for pre-treatment with or without ICS.

Small airways parameters

Alveolar exhaled Nitric Oxide fraction

Endogenous Nitric Oxide production is increased in asthma due to inflammation of airway epithelium [20]. Measurement of alveolar exhaled Nitric Oxide (eNO) reflects inflammation in small airways [21].

At 2 baseline visits, 1 week apart, and after treatment an eNO measurement was performed at multiple flow rates (30 mL/s, 50 mL/s, 100 mL/s and 200 mL/s) on a NIOX (Aerocrine, Stockholm, Sweden). The mean eNO value (ppb) of three technically acceptable attempts per flow rate was used for analysis. Alveolar eNO fraction (ppb) as well as the bronchial NO flux (nL/s) were calculated with a modification of the two-compartment model of nitric oxide exchange by Tsoukias and George [22]. The test was performed on two occasions to train subjects in performing eNO tests correctly. eNO values acquired during the third baseline visit (day 9) were used to analyze treatment effects.

Air trapping on expiratory CT scan

Quantitative image analysis of CT scans have been performed at end-expiration (near Residual Volume (RV) level), both before and after bronchoprovocation, which reflects regional air trapping due to small airways obstruction [23,24].

An inspiratory CT scan was acquired during a 10-second breathhold at full inspiration (near Total Lung Capacity) at baseline. This was followed by a CT scan

during a 10-second breathhold at end-expiration, which approximates RV. Subsequently, a methacholine provocation was performed on site, again followed by an end-expiratory scan immediately after PC_{20}MCh had been reached. After 5 weeks of treatment, an end-expiratory scan after reaching PC_{20}MCh was acquired again. Inspiratory and expiratory manoeuvres were practiced twice before the procedure and subjects were coached by a trained technician during scanning in supine position. All scans were performed on a 16-slice MultiDetector CT (MDCT) scanner (Siemens Somatom Sensation 16, Siemens AG Medical Solutions, Erlangen, Germany) at 120 kVp, 25 mAs (inspiration) and 30 mAs (expiration), 0.5 second rotation time. A table feed of 18 mm per rotation, and a 1 mm slice thickness with 0.6 mm increment were used. The estimated effective radiation dose was 0.78 mSv for inspiratory scans and 0.94 mSv for expiratory scans.

Anonymized MDCT data were sent to an analyst at MeVis (Center for Medical Diagnostic Systems and Visualization, Bremen, Germany) who was blinded to the intervention. Scan data were analyzed by the advanced image analysis software MeVisPULMO3D. A detailed description of the lung segmentation with MeVisPULMO3D software is provided in the online repository. Based on the segmentation, quantitative volumetric and densitometric analyses were performed of total lung, right and left lung separately and of each individual lung lobe. The parameters used in this study were volume (mL), mean lung density (MLD; in Hounsfield Units [HU]), 15^{th} percentile density (HU), and percentage of low attenuation areas (LAA; %). LAA were defined at a cut-off point of -950 HU. Methacholine-induced air trapping on CT was defined at baseline as the absolute change in MLD, 15^{th} percentile density and LAA between the two expiratory scans before and after methacholine. The change in volume between the two expiratory

scans before and after Methacholine was also corrected for inspiratory lung volume by using the following equation:

$$\% \text{ Volume Change} = \frac{((\text{Inspiration-Expiration}) - (\text{Inspiration-Expiration postMCh})) * 100\%}{(\text{Inspiration-Expiration})}$$

Closing volume with Single-Breath N_2 test

Closing volume measured with a Single-Breath Nitrogen (SBN_2) test reflects air trapping due to small airways obstruction [25-27].

At 2 baseline visits, 1 week apart, and after treatment a SBN_2 test was performed (Quark PFT®, Cosmed, Rome, Italy). Subjects were coached into tidal breathing, after which they slowly inspired pure oxygen to total lung capacity. Hereafter, they slowly exhaled to residual volume (RV) level, during which the N_2 concentration was measured and plotted against lung volume. The slope of the alveolar N_2 plateau was calculated by one investigator (JC) by drawing the best-fit line through Phase III of the expiratory volume-concentration curve. To minimize intra-observer variability, one reader measured closing volume (mL) and slope of the alveolar N_2 plateau (dN_2 in %/mL) on one day after all subjects had completed the study. Two measurements were selected for analysis when closing volume differed less than 20% or 100 mL. The mean of both measurements was used for analysis. The SBN_2 test was performed on two occasions, to train subjects in performing the closing volume manoeuvre correctly. Closing volume and dN_2 values acquired during the second baseline visit (day 9) were used to analyze treatment effects.

Δ FVC % and Δ SVC % at PC_{20}Methacholine and at PC_{20}AMP

The percentage fall from baseline in FVC and Slow inspiratory Vital Capacity (SVC)

at the time 20% fall in FEV_1 occurred during bronchial hyperresponsiveness testing (ΔFVC% at PC_{20} and ΔSVC% at PC_{20} respectively), may reflect air trapping due to excessive bronchoconstriction or small airways closure [28,29].

MCh and AMP challenge testing was performed using the standardized 2-minute tidal breathing protocol [30]. Additionally, FVC and SVC were measured in a combined manoeuvre (see online depository) at 30 and 90 seconds after each inhaled dose of either MCh or AMP. Spirometry was measured with a daily-calibrated dry wedge spirometer (Jaeger Masterscope, Hoechberg, Germany). Subjects received doubling doses of Methacholine bromide (0.038 - 19.6 mg/mL) at 2 baseline visits, 1 week apart, and after treatment (Figure 1). They received doubling doses of AMP (0.04 - 320 mg/mL) at baseline and after treatment. The fall in FVC and SVC (ΔFVC% and ΔSVC%) at PC_{20} was calculated using log-linear interpolation.

Cytokines measured in epithelial lining fluid (ELF) in peripheral airways

The most direct method to assess airway inflammation is *via* bronchoscopy. The diameter of a bronchoscope is too large to reach the small airways, but microsampling probes may reach the peripheral airways [31,32]. The technique and cytokine measurements are described in the online data repository.

Statistical Analysis

A Wilcoxon signed rank test was used to assess within treatment differences, a Mann-Whitney U test for between treatment differences (the difference between changes with ciclesonide and placebo treatment). All analyses were considered to be explorative in the absence of a statistical power calculation given the pilot nature of

the study. Analyses were performed with SPSS 12.0.2 for Windows (SPSS INC. Chicago, IL, USA).

RESULTS

Study Population

16 subjects were randomized to treatment and completed the study, seven subjects receiving placebo and 9 ciclesonide 320 µg once daily in the morning. Demographics and lung function at baseline of both groups were not significantly different (Table 1).

Table 1 Patient characteristics and lung function at baseline and after treatment

	Placebo (n=7)		Ciclesonide (n=9)	
	Baseline	**Post-treatment**	**Baseline**	**Post-treatment**
Male gender, n (%)	2 (29)		5 (56)	
Age, yrs	44 (21-53)		36 (19-56)	
BMI, kg/m^2	24 (19-30)		24 (20-28)	
FEV_1 % pred	97 (76-120)	91 (76-118)	88 (62-109)	98 (79-116) *†
FEV_1/FVC, %	77 (68-88)	78 (66-83)	67 (52-79)	70 (60-83)
FVC % pred	101 (84-144)	105 (82-141)	115 (93-122)	117 (96-136) *†
SVC % pred	103 (82-145)	109 (81-145)	112 (94-131)	122 (98-135) *†
PC_{20}MCh, mg/mL	0.4 (0.2-4.2)	0.3 (0.1-3.6)	0.5 (0.1-2.0)	1.3 (0.2-39.2) *†
PC_{20}AMP, mg/mL	4.8 (0.2-23.1)	3.8 (0.7-23.4)	4.0 (0.2-36.2)	35.1 (1.2-640.0) *†
eNO at 50 mL/s, ppb	65 (34-204)	83 (28-222)	99 (33-281)	36 (17-59) *†

Values are presented as medians (ranges), unless stated otherwise. * Between treatment difference statistically significant ($p<0.05$). † Within treatment difference in ciclesonide group statistically significant ($p<0.05$)
BMI: Body mass Index, MCh: methacholine, AMP: adenosine-‘5-monophosphate, eNO: exhaled nitric oxide

Small airways parameters measured at baseline were also not statistically different between treatment groups (Table 2), although higher values were observed in the ciclesonide group as a result of the randomization of more males to this group.

Table 2 Small airway parameters at baseline and after treatment

	Placebo (n=7)		Ciclesonide (n=9)	
	Baseline	**Post-treatment**	**Baseline**	**Post-treatment**
Alveolar eNO, ppb	14.7 (8.5-39.2)	16.5 (5.6-39.6)	17.3 (6.9-67.3)	8.5 (3.7-12.5) *†
$FEF_{25-75\%}$ % predicted	63 (34-87)	61 (54-86)	52 (29-66)	63 (30-97)‡
Closing volume (SBN_2), mL	140 (95-495)	105 (60-430)	230 (60-820)	115 (35-975)
ΔFVC% at PC_{20}MCh	13.6 (4.9-15.3)	13.2 (2.5-19.4)	12.4 (6.1-16.8)	12.7 (5.6-19.7)
ΔFVC% at PC_{20}AMP	12.2 (5.4-14.3)	14.1 (9.0-18.9)	12.0 (3.5-17.2)	12.3 (4.1-15.9)
Total expiratory lung volume on CT after methacholine, mL	2993 (2158-4636)	2973 (2368-4916)	4165 (2262-5576)	3831 (2338-5166) *

Values are presented as medians (range). * Between treatment difference statistically significant ($p<0.05$). † Within treatment difference in ciclesonide group statistically significant ($p<0.05$). ‡ Within treatment difference in ciclesonide group: $p=0.051$
eNO: exhaled nitric oxide, SBN_2: Single Breath Nitrogen test, MCh: methacholine, AMP: adenosine-‘5-monophosphate

Treatment Effects

Alveolar eNO

Median (range) alveolar eNO values were significantly lower after ciclesonide (8.5 ppb (3.7-12.5 ppb)) than after placebo (16.5 ppb (5.6-39.6 ppb)), p=0.012. The decrease in alveolar eNO from baseline with ciclesonide (median 4.4 ppb) was significantly different from the change from baseline with placebo (median -0.4 ppb)(p=0.006; Figure 2).

Air trapping on expiratory CT scan

Methacholine-induced air trapping at baseline is presented in Table E1 in the online data repository.

Median (range) expiratory lung volume after MCh decreased by 59 mL (1569 to -117 mL) with ciclesonide and increased by 121 mL (-20 to 236 mL) with placebo, though these within-treatment differences were not statistically significantly. The changes in expiratory lung volume, MLD, and 15^{th} percentile density on expiratory CT scan after MCh challenge testing differed significantly between the ciclesonide and placebo group (between-treatment difference), p=0.042, p=0.016, p=0.023 respectively (Figure 3a-c), whereas the change in percentage LAA was of borderline significance (p=0.055, Figure 3d).

Closing volume with SBN_2 test

Closing volume decreased in both the placebo (median 140 to 105 mL) and ciclesonide group (230 to 115 mL). These decreases were not statistically significant, both within and between treatment groups (Figure 4).

ΔFVC% and ΔSVC% at PC_{20}methacholine and at PC_{20}AMP

ΔFVC% and ΔSVC% at PC_{20}methacholine and at PC_{20}AMP did not change significantly with either treatment.

$FEF_{25-75\%}$ % predicted

Mean forced expiratory flow between 25 and 75% of forced vital capacity ($FEF_{25-75\%}$) was measured with spirometry. Median $FEF_{25-75\%}$ % predicted increased in the ciclesonide-treated group from 52% to 63%, which was of borderline significance (p=0.051). The change with ciclesonide was not significantly different from the change with placebo (table 2).

Secondary parameters

The mean increase in ^{2}log PC_{20}MCh was significantly larger with ciclesonide than placebo, 1.4 versus 0.2 doubling doses respectively (p=0.031). The mean increase in ^{2}log PC_{20}AMP was also significantly larger with ciclesonide than placebo, 2.9 versus 0.3 doubling doses respectively (p=0.029). The change in FEV_1 % predicted, FVC % predicted and SVC % predicted was also significantly larger with ciclesonide (median increase of 6%, 6% and 4% predicted, respectively) than placebo (median decrease of 2%, 2% and 1% predicted, respectively), p=0.003, p=0.003 and p=0.023. Bronchial eNO decreased significantly with ciclesonide by a median of 1.4 nL/s (0.2 - 5.6), p=0.016, the change being significantly larger with ciclesonide than placebo (p=0.004).

Cytokines measured in epithelial lining fluid (ELF) in peripheral airways

Bronchoscopy was performed in 7 subjects (2 placebo, 5 ciclesonide). Due to blood contamination in 21 out of a total of 42 (50%) peripherally placed probes, cytokine measurements of peripherally-sampled ELF were only possible in 1 patient in the placebo and 2 patients in the ciclesonide group both at baseline and post-treatment. TARC was not detectable in any of the probes. Other cytokine concentrations in ELF are presented in Table E2 in the online repository. Statistical analysis was not performed due to the small sample size.

DISCUSSION

This pilot study demonstrates that treatment with ciclesonide 320 µg once daily can specifically improve parameters reflecting inflammation and patency of the small airways, even in a small sample of 16 patients with mild-to-moderate asthma. Earlier studies already showed the efficacy of ciclesonide in maintaining asthma control and in reducing symptoms and airway hyperresponsiveness [9-13,16,17,33-36]. Our study confirms and extends these observations in that ciclesonide exerts anti-inflammatory effects on small airways as well. We demonstrated beneficial effects of the small-particle ICS ciclesonide on small airway involvement in asthma compared to placebo. Further studies have to evaluate whether treatment with this small-particle ICS is superior to treatment with a large-particle ICS, with respect to small airway involvement, symptoms, and control of asthma. If so, the importance of ICS distribution throughout the whole lung must be kept in mind when treating patients who do not gain optimal asthma control with conventional large-particle ICS.

This study is the first to assess the effects of ciclesonide on small airway parameters. Verbanck *et al* demonstrated beneficial effects of small-particle ICS on acinar lung zone abnormalities in asthma, but did not compare these effects with

placebo [37]. Hauber *et al* previously demonstrated beneficial effects of a small-particle ICS on peripheral airways by a reduction of eosinophilic inflammation in transbronchial biopsies and an increase in $FEF_{25-75\%}$ % predicted [38]. In the same study, signs of airway remodelling were reduced [39], thus demonstrating direct effects of a small-particle ICS on peripheral airway inflammation, remodelling and airway function. Nevertheless, transbronchial biopsies are not easily applicable in clinical practice due to their invasive nature. In a less invasive manner, a large-particle and a small-particle ICS were investigated for effects on small airways by assessing methacholine-induced air trapping on expiratory high-resolution CT scans in mild-to-moderate asthma. The small-particle ICS improved methacholine-induced air trapping significantly more than the large-particle ICS, indicating a direct effect of the former on the small airways [40]. Consistent with these results, Zeidler *et al* demonstrated that montelukast (leukotriene receptor antagonist) reduced methacholine-induced air trapping on expiratory CT scans in mild-to-moderate asthma, in association with improved quality of life. However, other parameters of small airway dysfunction, such as closing volume (SBN_2 test) were not related to the montelukast-induced reduction of air trapping [23]. Our study confirms that treatment with a small-particle ICS significantly reduces methacholine-induced air trapping due to small airway closure. Additionally, we found the effects of the small-particle ICS ciclesonide on an even less invasive marker of small airway inflammation, i.e. alveolar eNO. This is an interesting finding since alveolar eNO is an easy, non-invasive measure that is preferable to CT scanning or transbronchial biopsies for clinical follow-up of small airway inflammation.

A possible limitation of our CT methodology may be the lack of spirometric gating. Therefore, theoretically, we can never be entirely sure that end-expiratory

lung volume is not affected by e.g. an incomplete expiration, and thus the lack of spirometric gating may affect reproducibility of the measurements. Nevertheless, other studies that also do not apply spirometric gating demonstrated that air trapping on HRCT is significantly associated with spirometric indices of global and peripheral airway obstruction [24]. This indicates that even without spirometric gating CT scanning is still very sensitive to assess small airways disease in asthma. Although spirometric gating is of value when measuring air trapping on a baseline expiratory scan, one could question the use of spirometric gating in assessing methacholine-induced air trapping in asthma. Due to methacholine-induced air trapping lung inflation may occur and residual volume may increase, thereby affecting the trigger to scan. The subjects in our study were trained to perform maximal inspiration and maximal expiration correctly and the inspiratory/expiratory manoeuvres were closely observed during scanning. Scans were only acquired when inspiratory/expiratory manoeuvres were technically satisfactory.

One of the pitfalls of small airway research in asthma is the absence of a gold standard to assess small airway function and or inflammation. Nevertheless, all tests used in our study have been extensively investigated and suggested by other research groups as adequate parameters to reflect functioning of the small airways, which justifies our choice of these tests [21,25,29,30,42]. Furthermore, the finding in our study that a small-particle ICS improves small airway parameters in contrast to placebo in a small number of asthmatics provides a sound basis for the validity of these parameters.

Why would ciclesonide significantly improve alveolar eNO and methacholine-induced air trapping on expiratory CT, but not the other small airway parameters evaluated? First, some of the small airway parameters tested had a large variability

(see table 2), thus reducing statistical power. A formal power calculation was not performed in this pilot study as effect sizes of small airway parameters were unknown when designing the study. Nevertheless, it is reassuring that other intervention studies providing positive effects of small-particles ICS have used similar sample sizes [39,43, 44]. Second, the lack of improvement in closing volume after ciclesonide treatment does not rule out a beneficial effect on small airway closure, as SVC improved in our patients. Therefore closing capacity may have been a better measure than closing volume, however this could not be examined due to the lack of lung volume measurements. Another explanation for the results may be that the tested small airway parameters do not all measure the same aspects of small airway disease. King *et al* described that closing volumes measured with SBN_2 test can differ greatly between two individuals who have a similar extent of airway closure and air trapping on a CT scan, because the SBN_2 test detects airway closure during expiration at a lung volume that is different from the end-expiratory lung volume [45,46]. Van Veen *et al* described that ΔFVC at PC_{20}MCh was not associated with alveolar eNO in contrast to other measures of peripheral airway dysfunction [21]. We conclude that future studies are needed to determine which is the best tool to monitor small airway improvements; for the time being eNO and expiratory CT scanning are promising.

Seven subjects underwent a bronchoscopy at baseline and after treatment during which ELF from central and peripheral airways was sampled with microsampling probes. Half of all sampled probes were contaminated with blood from bronchial mucosa. We conclude that it is not feasible to sample ELF from peripheral airways in clinical studies investigating asthma patients, in contrast to successful application in acute respiratory distress syndrome and COPD [32,33].

In summary, we have demonstrated that treatment with the small-particle ICS ciclesonide 320 μg once daily improves alveolar eNO and methacholine-induced air trapping on expiratory CT scan in patients with mild-to-moderate asthma. Our findings suggest that alveolar eNO is a useful tool to aid in diagnosing and monitoring small airway pathology in asthma since it is already sensitive to changes in a small number of patients. It has already been demonstrated that ciclesonide reaches the small airways [8], and our study provides evidence for the first time that ciclesonide exerts anti-inflammatory effects at this site.

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COMPETING INTERESTS

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FIGURE LEGENDS

Figure 1 Flow chart

Footnote: Procedures were performed in the same order (top to bottom) as in the the flow chart.

ICS: inhaled corticosteroid; eNO: exhaled Nitric Oxide; SBN_2: single breath nitrogen test; MCh: methacholine; AMP: adenosine-'5-monophosphate; CT: computed tomography

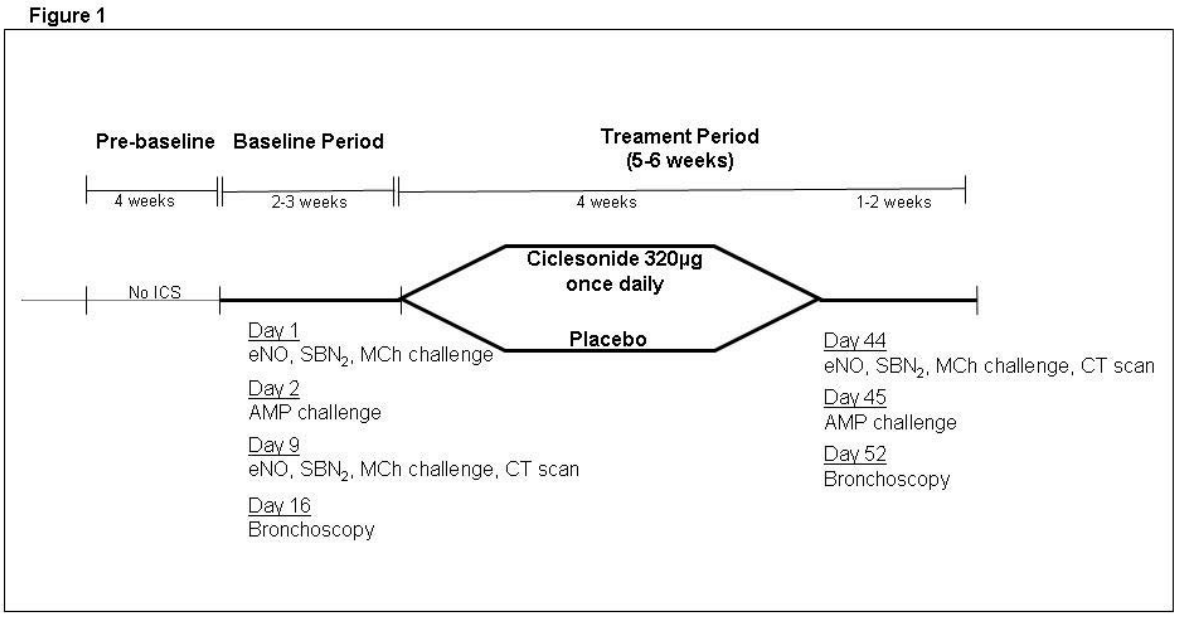

Figure 2 Alveolar exhaled Nitric Oxide

Alveolar eNO before and after treatment with placebo and ciclesonide

Footnote: Within treatment difference in ciclesonide-group with Wilcoxon signed rank test: p=0.012. Between treatment difference with Mann-Whitney U test: p=0.006. ns = not significant

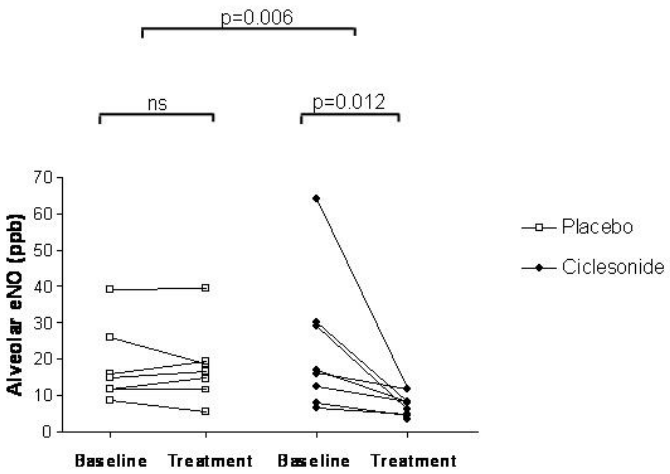

Figure 2

Figure 3 Methacholine-induced air trapping on CT

Methacholine-induced air trapping on CT before and after treatment with placebo and ciclesonide **a** Total expiratory lung volume after methacholine, **b** Mean lung density after methacholine, **c** 15th percentile density after methacholine, **d** Percentage LAA after methacholine

Footnote: Between treatment differences with Mann-Whitney U test: **a** p=0.042, **b** p=0.016, **c** p=0.023, **d** p=0.055. ns = not significant

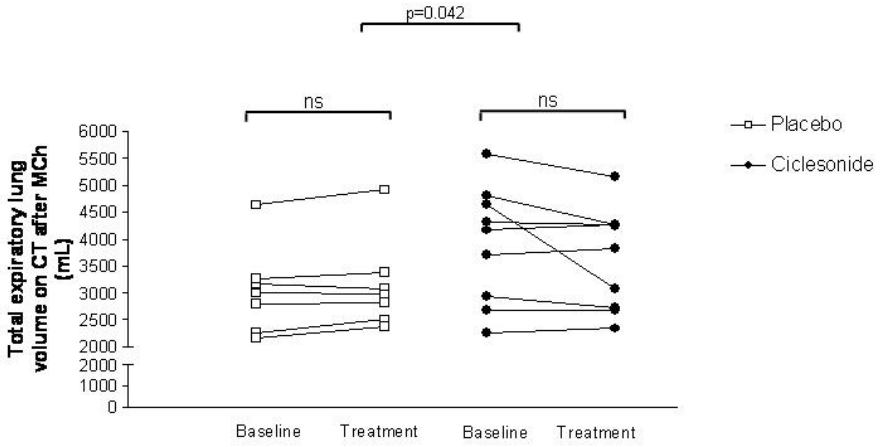

Figure 3a

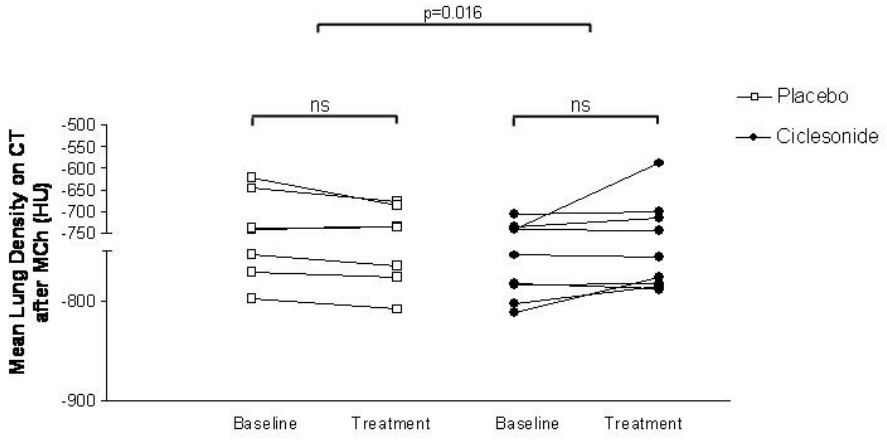

Figure 3b

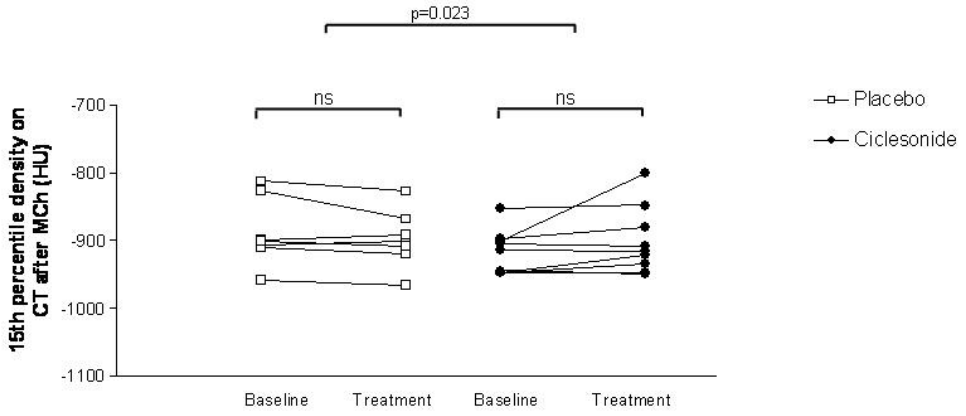

Figure 3c

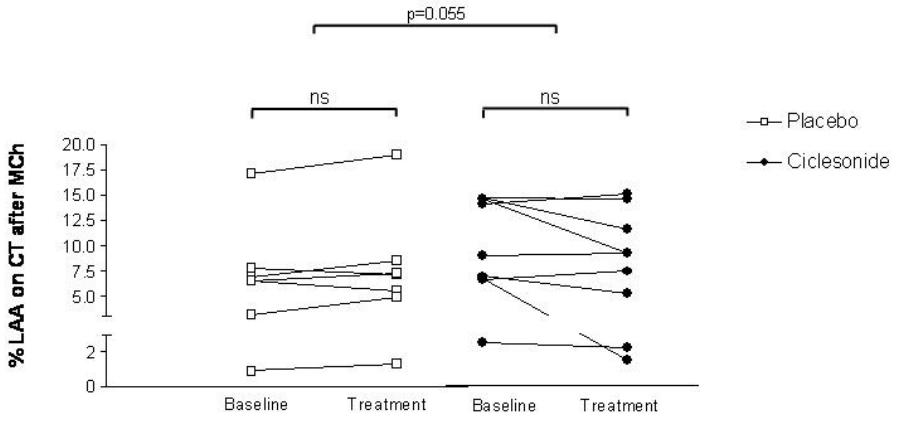

Figure 3d

Figure 4 Closing volume at SBN_2 test

Footnote: ns = not significant

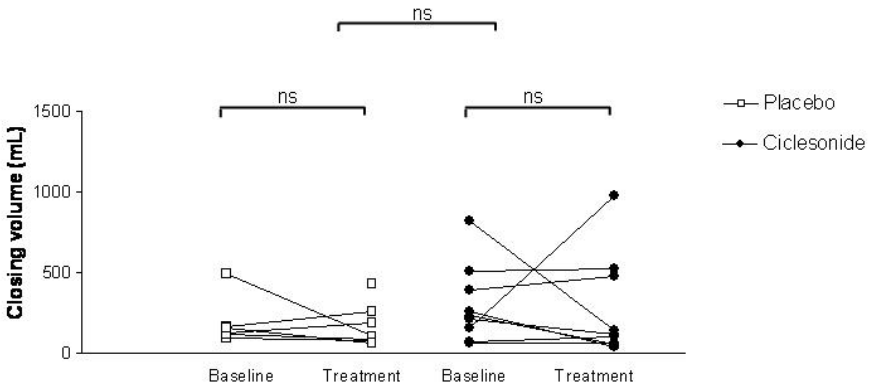

Figure 4