Universiteit Leiden
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Non-invasive sampling methods of inflammatory biomarkers in asthma and allergic rhinitis

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CHAPTER 3

Effect of an NK1/NK2 receptor antagonist on airway responses and inflammation to allergen in asthma

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Abstract

Rationale: The tachykinins substance P and neurokinin A (NKA) are implicated in the pathophysiology of asthma.
Objective: We tested the safety, tolerability, pharmacological and biological efficacy of a tachykinin NK1/NK2 receptor antagonist, AVE5883, in asthmatics in 2 double-blind, placebo-controlled, cross-over studies.
Methods: The pharmacological efficacy of a single inhaled dose (4.8 mg) of AVE5883 was tested against inhaled NKA in 20 asthmatics. Subsequently, we studied the biological efficacy of the pharmacologically effective dose on inhaled allergen in a multiple dose trial (4.8 mg TID, 9 days) in 12 asthmatics with dual responses to inhaled house dust mite. On day 8, an allergen challenge was conducted and airway response was measured by FEV_1 until 9 h post-allergen. Exhaled NO, $PC_{20}FEV_1$(Methacholine) and induced sputum were performed on days 1,7 and 9.
Results: AVE5883 had a bad taste and transient bronchospasm occurred in some subjects. A single inhaled dose shifted the dose-response to NKA by 1.2 doubling doses. Unexpectedly, pretreatment with multiple doses of AVE5883 enhanced the allergen-induced early and late airway responses. However, there were no significant differences in the allergen-induced changes in exhaled NO, $PC_{20}FEV_1$(Methacholine) and sputum cell differentials between placebo and AVE5883.
Conclusions: Despite its demonstrated pharmacological activity against inhaled NKA, multiple doses of AVE5883 increased the allergen-induced airway responses without affecting markers of airway hyperresponsiveness and airway inflammation. Our data question the prominent role of neurogenic inflammation in asthma and, consequently, the therapeutic potential of dual tachykinin antagonists.

Introduction

Asthma is a chronic inflammatory disease of the lower airways associated with various comorbidities and characterized by variable, often reversible, airflow obstruction (1). Pathophysiologically, airway hyperresponsiveness (AHR) to various bronchoconstrictor stimuli is the hallmark of asthma, which appears to be related to chronic airway inflammation (2). Hence, anti-inflammatory therapy with inhaled corticosteroids is the cornerstone of pharmacotherapy of persistent asthma (1). However, longterm use of (high doses of) inhaled corticosteroids may induce troublesome local and/or systemic side effects (3). Furthermore, despite a good, overall clinical efficacy, even high doses of inhaled corticosteroids do not fully suppress the airway inflammation in all asthmatic patients (4-6). Therefore, novel therapeutic options are being explored targeting various aspects of airway inflammation.

Within human airways, the tachykinins Substance P (SP) and Neurokinin A (NKA) are the predominant neuropeptides released from the nonadrenergic-noncholinergic (NANC) system by mechanical, thermal, chemical or inflammatory stimuli (7;8). It appears, that SP exerts its pro-inflammatory effects mainly by stimulation of the tachykinin NK1 receptors, whereas NKA mainly causes tachykinin NK2 receptor-mediated effects (9). Upon inhalation, SP induces AHR and the so-called 'neurogenic inflammation' within the airways of both non-asthmatic and asthmatic individuals, characterized by microvascular leakage, mucus secretion, and inflammatory cell responses (9-11), whereas inhaled NKA mainly causes bronchoconstriction (12,13). Furthermore, in contrast with non-asthmatic controls, increased tachykinin NK1 and NK2 receptor mRNA expression has been demonstrated within the airways of asthmatic patients (14,15). Correspondingly, in subjects with allergic asthma, increased concentrations of SP have been found in sputum and bronchoalveolar lavage (BAL) at baseline, with further increase following segmental allergen challenge (8,16). Similarly, in still another study in allergic asthma, increased NKA levels have been detected 4 h following an allergen bronchoprovocation test (7).

These observations provided evidence that the tachykinins SP and NKA may contribute to airway inflammation and hence may be implicated in the pathophysiology of asthma. Therefore, several tachykinin NK1 and/or NK2 receptor antagonists have been developed and tested against indirect challenges including NKA, adenosine monophosphate and hypertonic saline in patients with asthma (17-21). However, until recently, there are no published studies on clinical efficacy of dual tachykinin NK1/NK2 receptor antagonists in asthma nor on their biological efficacy in allergen challenge, being the most representative model of asthma.

AVE5883 is a non-peptidyl, dual tachykinin NK1/NK2 receptor antagonist with high specificity and affinity for tachykinin NK1 and NK2 receptors (Ki = 5.6 and 3.1 nM for the NK1 and NK2 receptor respectively, and >10 μM for a variety of other physiologically important receptors). In sensitized guinea-pigs, intra-peritoneally administered AVE5883 has been shown to reduce ovalbumin-induced airway hyperreactivity and eosinophil influx in the BAL [data on file]. Similarly, intratracheally administered AVE5883 protected against capsaicin-induced bronchoconstriction in sensitized guinea-pigs and aerosolised AVE5883 inhibited the NKA-induced increase in airway resistance in dogs [data on file].

We tested the safety and tolerability, in combination with the pharmacological and biological efficacy and the pharmacokinetics, of inhaled AVE5883 in clinically stable patients with mild to moderate persistent asthma not on maintenance anti-inflammatory therapy. In the first study, we tested the pharmacological efficacy of a single inhaled dose of AVE5883 against NKA-induced bronchoconstriction. Subsequently, in another study in patients with similar asthma characteristics, we tested the biological efficacy of multiple inhaled doses of AVE5883 against allergen-induced airway responses and markers of airway inflammation.

Some of the results of both studies have previously been reported in the form of abstracts (22,23).

Methods

SUBJECTS

NKA challenge study: 20 non-smoking, patients with clinically stable, mild to moderate persistent asthma participated in the NKA-challenge study (Table 1). All patients had a history of persistent asthma for at least 1 year (according to Global Initiative for Asthma (GINA) criteria (1)), without any other clinically relevant disorders. Except for inhaled short-acting β2-agonists prn, no one was using concomitant anti-asthma or anti-allergy medication for at least 6 weeks prior to and during the study. Patients had no history of viral infections of the lower airways for at least 6 weeks before enrollment. Caffeine-containing beverages and short-acting inhaled β2-agonists were withheld at least 8 h before each visit. Baseline forced expiratory volume in one second (FEV_1) had to be ≥75% of predicted. Patients were hyperresponsive to both inhaled methacholine bromide (MBR) and inhaled NKA, showing a 20% fall in FEV_1 ($PC_{20}FEV_1$(MBR)) of lower than 19.6 mg/mL (= 16 mg/mL methacholine chloride, equals 80 μmol/mL) and a $PC_{20}FEV_1$(NKA) of lower than 441.2 x10^{-3} μmol/mL (equals 500 μg/mL), respectively, at screening.

Allergen challenge study: 12 patients participated in the allergen challenge study (Table 1). Four of them have previously participated in the NKA challenge study (>6 months ago). All patients met the same aforementioned criteria, with a maximum $PC_{20}FEV_1$(NKA) of ≤ 882.4 x10^{-6} μmol/mL (equals ≤1000 μg/mL) at screening. In addition, patients had a positive skin prick test to house dust mite (HDM, a positive response was defined as a mean wheal diameter ≥3mm) and a documented late asthmatic response (LAR) to inhaled HDM extract, i.e. fall in FEV_1 >15% from baseline between 3 and 9 h post-allergen.
Both study protocols were approved by the Leiden University Medical Centre Ethics Committee, and all participants gave written informed consent.

TABLE 1 PATIENTS' BASELINE CHARACTERISTICS.

CHARACTERISTICS		NKA CHALLENGE	ALLERGEN CHALLENGE
Number of patients		20[a]	12[b]
Gender	Male	5	6
	Female	15	6
Atopy status	Yes	18	12
	No	2	0
Age (years)	Mean [Range]	29.6 [19 – 47]	24.4 [20 – 39]
FEV_1 (L)	Mean [Range]	3.7 [2.6 – 5.7]	3.8 [3.1 – 5.9]
FEV_1 (% predicted)	Mean [Range]	102.4 [80.3 – 125.5]	91.6 [73.6 – 121.4[c]]
$PC_{20}FEV_1$ (MBr) (mg/mL)	Mean [Range]	2.2 [0.1 – 8.5]	2.6 [0.1 – 14.4]
$PC_{20}FEV_1$ (NKA) (*10^{-3} μmol/mL)	Mean [Range]	143.2 [5.9 – 593.8[d]]	357.9 [5.9 – 1115.7[e]]

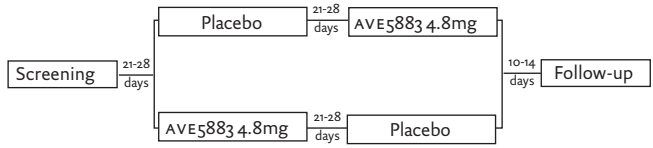
[a] One subject did not complete the NKA challenge study, [b] One subject did not complete the Allergen challenge study, [c] One subject with a lower baseline FEV_1 (73.6% predicted) was included, [d] One subject with a $PC_{20}FEV_1$(NKA) of 593.8 *10^{-3} μmol/mL was included, [e] One subject with a $PC_{20}FEV_1$(NKA) of 1115.7 *10^{-3} μmol/mL was included.

STUDY DESIGN

NKA challenge study: This was a single-center, randomized, double-blind, placebo-controlled, single-dose, cross-over study. Before entering the study, selection criteria were examined on 2 screening visits, 24 h apart. Fourteen to 28 days after screening, eligible patients were randomized into the study.

Each treatment period consisted of two study days, separated by a washout period of 12-16 days. Clinically stable subjects inhaled either AVE5883 (cumulative dose of 4.8 mg) or placebo, 30 minutes before an NKA-challenge. Blood samples for pharmacokinetics were collected from a venous cannula inserted in a forearm vein (pre-dose, 10, 30 and 45 minutes and 1, 2, 4, 6 and 8 h post-dose). A standardized NKA challenge was performed 30 minutes after the last study drug inhalation. In principle, pre-challenge FEV_1 had to return within 10% of baseline. However, in order to keep the time-interval between dosing and NKA challenge constant in all patients and to allow also more hyperresponsive asthmatics in the study, in the case of an unexpected drop in FEV_1 following study medication, decreases in pre-NKA FEV_1 were accepted up to 20% from baseline, provided within a safe range (FEV_1 >2.3 L, allowing a safe $PC_{20}FEV_1$(NKA)). Ten to 14 days post-study, there was a follow-up visit.
Allergen challenge study: This was a single-center, randomized, multiple-dose, placebo-controlled, double-blind, cross-over study. At least 3 weeks before the study, selection criteria were examined. Eligible patients with a documented $PC_{20}FEV_1$(NKA) and a demonstrated LAR to inhaled HDM at screening were randomized into the study. On Day 1, exhaled NO (eNO) measurement, followed by a $PC_{20}FEV_1$(MBR), and 1 h later, a sputum induction, were performed. To ensure asthma stability, mean baseline FEV_1 had to be within 10% and the $PC_{20}FEV_1$(MBR) had to remain within 1 doubling dose on Day 1 of both treatment periods. Clinically stable patients inhaled the first dose of study medication at 2:00 pm (± 2 h) followed by an evening dose at approx. 8:00 pm, followed by FEV_1 measurements. On Days 2-7, study medication was inhaled three times daily at 8:00 am, 2:00 pm and 8:00 pm (± 2 h). Subjects were discharged from the unit 1 h after the morning dose on Day 2 and returned on Day 7 (intake was monitored by telephone contact). On Day 8, the afternoon dose was skipped and on Day 9 patients only inhaled the morning dose. On Day 8, a standardized allergen bronchoprovocation test was conducted approximately 45 min post-dosing (provided pre-allergen FEV_1 returned within 10% of the predose value), and the airway response was recorded by FEV_1 until 9 h post-allergen. Blood samples were collected for pharmacokinetics from a venous cannula inserted in a forearm vein on Day 8 (pre-dose, 15 and 30 minutes and 1, 1.5, 2, 3, 4, 5, 6, 7, 8 and 9 h post-dose) and on Day 7 and 9 (pre-dose, and 1, 2, 4, and 6 h post-dose). The pro-inflammatory effects of inhaled allergen were monitored by eNO, sputum cell differentials and $PC_{20}FEV_1$(MBR) on Days 7 and 9, 24 h pre- and post-allergen, respectively. Ten to 14 days post-study, a follow-up visit was scheduled (Figure 1). Throughout the study, patients recorded any symptoms and signs of asthma, medication use, and adverse events into a diary.

All bronchoprovocation tests were performed at the same time of the day (±2 h). After all bronchoprovocation tests, patients received salbutamol ≥2x100 μg through Volumatic (GlaxoSmithKline, Zeist, The Netherlands), until the FEV_1 returned within 10% from baseline.



	Scr.1	Scr.2	Scr.3	Day 1	Day 7	Day 8	Day 9	Day 1	Day 7	Day 8	Day 9	Follow-up
Medicalexam.	X											X
$PC_{20}FEV_1$(NKA)	X											
Exhaled NO		X		X	X		X	X	X		X	
$PC_{20}FEV_1$(MBr)		X		X	X		X	X	X		X	
Sputum		X		X	X		X	X	X		X	
Allergen			X			X				X		

FIGURE 1 Study flowchart - allergen challenge study. there was at least a one-week washout between screening visit 1 and 2. SCR = screening, $PC_{20}FEV_1$(NKA) = provocative concentration of neurokinin a causing a 20% fall in FEV_1, $PC_{20}FEV_1$(MBR) = provocative concentration of methacholine bromide causing a 20% fall in FEV_1, sputum = sputum induction, allergen = allergen challenge.

STUDY MEDICATION

For both study protocols AVE5883 ([4-(1-{2-3-(3,4-Dichloro-phenyl)-1-(3,4,5-trimethoxy-benzoyl)-pyrrolidin-3-yl]-ethyl}-4-phenyl-piperidine-4-carbonyl)-piperazin-1-yl]-acetic acid) was supplied by sanofi-aventis pharmaceuticals Inc. (Cheshire, UK) as a sterile solution or matching placebo in an alcohol/propellant (HFA-227) mixture, both delivered in a pressurized metered dose inhaler (pMDI; 300 μg/actuation). Each administration consisted of 16 actuations in 8 min yielding a cumulative dose of 4.8 mg AVE5883. Post-dosing subjects were required to rinse their mouth.

INHALATION CHALLENGES & RESPONSE MEASUREMENTS

The airway response to the inhaled aerosols was measured by FEV_1 according to standardized lung function techniques and recorded by a spirometer connected to a personal computer (Vmax Spectra, Sensor Medics, Bilthoven, The Netherlands) (24). MBR, NKA and allergen bronchoprovocation challenge tests were performed by tidal breathing method according to validated techniques as described previously (25-27). A detailed description of methods used is presented in the online supplement.

SPUTUM INDUCTION AND ANALYSIS

Sputum induction and analysis were performed according to the entire expectorate ('full sample') method, that has been previously validated (26). Hypertonic saline aerosols (NaCl 4.5%) were generated at room temperature by a DeVilbiss Ultraneb 2000 ultrasonic nebulizer (Tefa Portanje, Woerden, The Netherlands) and inhaled for 3x5 min at 15 min intervals (26). Sputum samples were processed according to a previously validated method after adding DTT 0.1% (28). Subsequently, cytospins were made (50 μL/cytospin; Shandon Cytospin 4, Thermon Electron Corporation, Runcorn, UK) and differential cell counts performed on May-Grünwald-Giemsa-stained cytospins; cells were expressed as a percentage of 500 nucleated cells excluding squamous cells as described previously (26). Samples with > 80% squamous cells were excluded from analysis. A detailed description of methods used is presented in the online supplement.

EXHALED NITRIC OXIDE

Exhaled NO measurements were performed in triplicate within 10% at a exhalation flow of 50 mL/s according to current ATS recommendations (29), by a chemoluminescence analyzer (Ecomedics CLD88sp, Duernten, Switzerland). A detailed description of methods used is presented in the online supplement.

PLASMA CONCENTRATION OF AVE5883

Blood samples (7 mL) were collected into sodium heparinate vacutainers and centrifuged at 3000 rpm for 10 minutes at 4 °C. AVE5883 was determined in plasma samples of subjects treated with AVE5883 by Bioanalytics, DMPK, sanofi-aventis pharmaceuticals Inc, Bridgewater, NJ, using a validated LC/MS/MS method with a lower limit of quantification of 0.1 ng/mL.

ANALYSIS

NKA challenge study: The protective effect of AVE5883 against NKA-induced bronchoconstriction was assessed by comparison of the $PC_{20}FEV_1$(NKA) (log10 transformed) after AVE5883 and placebo pre-treatment. $PC_{20}FEV_1$(NKA) was calculated by linear interpolation of the inhaled dose of NKA below and above a 20% fall in FEV_1 (27). If no fall in $FEV_1 \geq 20\%$ was reached after inhalation of the highest NKA-dose, $PC_{20}FEV_1$ (NKA) was set at 1.76 μmol/mL (=2000 μg/mL; i.e. one doubling dose higher than the highest dose tested) (27). The difference in $PC_{20}FEV_1$ (NKA) between AVE5883 and placebo was tested using an analysis of variance with sequence, subject (within sequence), and treatment as factors. The safety and tolerability was primarily assessed through examination of treatment-emergent adverse

events. Treatment-emergent adverse events consisted of all on-treatment adverse events and any pre-treatment adverse events that worsened in intensity (severity or frequency) after the start of study medication. Descriptive statistics were provided for plasma concentration-time data and plasma pharmacokinetic parameters.

Allergen challenge study: The effect of AVE5883 on the allergen-induced airway responses was determined by comparing the absolute corresponding area under the time-response curve (AUC) for both the EAR (0-3 h post-allergen), and the LAR (3-9 h post-allergen) between AVE5883 and placebo. The trapezoidal rule was applied for the calculation of the AUCs (30). Similarly, the differences in the maximal percent fall in FEV_1 during the EAR and LAR were compared between the two treatments.

In addition, the differences in allergen-induced changes in eNO, $PC_{20}FEV_1$ (MBR) and the sputum differential cell counts (mast cells, eosinophils, neutrophils, lymphocytes, macrophages, epithelial and squamous cells) were assessed by comparing the (changes of the) corresponding values 24 h before and 24 h after allergen challenge between the two treatments. $PC_{20}FEV_1$ (MBR) was calculated by linear interpolation of the airway responses below and above a 20% fall in FEV_1 (27). The airway responses to inhaled allergen were expressed as percentage fall in FEV_1 from post-diluent baseline and plotted as time-response curves during both treatment periods. For the assessment of treatment differences (AVE5883 vs placebo) in these outcome parameters, an analysis of variance (ANOVA) appropriate to the 2-period, 2-sequence, 2-treatment cross-over design was used. The ANOVA model contained factors of treatment, sequence, and subject within sequence. Carry-over effects were examined for the LAR AUC analysis. P-values <0.05 were considered statistically significant.

The sample size of 12 evaluable patients for this study was based upon the simplifying assumption for a comparison of the two treatments using a paired t-test. Given this assumption, the calculated sample size required to detect a 30% mean difference in the LAR was 10 subjects (α = 0.05 (two-tailed), β = 0.10 (one-tailed), power = 90%; within subject standard deviation = 25%) (31).

Results

SAFETY & TOLERABILITY

NKA challenge study: A total of 20 patients were randomized and 19 patients completed the study. One subject was withdrawn after the first study day because of a moderate bronchoconstriction (i.e. a fall in FEV_1 of 34% from

baseline) within 5 min of inhalation of AVE5883. Overall, the study medication was well-tolerated, although all patients on AVE5883 vs. none on placebo (P) reported bad taste. The most commonly occurring adverse event was transient, self-limiting bronchospasm starting within 12 minutes after study drug inhalation reported by 8 patients on AVE5883 and 4 on P. Other reported adverse events were headache (5 patients on AVE5883 and 3 on P) and self-limiting dyspnoea (2 patients on AVE5883 and 5 on P).

Allergen challenge study: A total of 12 patients were randomized and 11 patients completed the study. One subject was withdrawn because of a viral exacerbation requiring oral prednisone between treatment period I and II. Similar to the NKA challenge study, AVE5883 was generally well-tolerated although all patients reported a bad taste after AVE5883 inhalation. The most common adverse event was self-limiting dyspnoea occuring within 30 minutes of study drug inhalation (in 5 patients on AVE5883 vs. 2 on P). However, in all patients pre-allergen FEV_1 was within 10% of predose value (see table E2 of individual pre-dose and pre-allergen FEV_1 in online supplement). In addition, headache was reported by 3 patients on AVE5883 vs. 3 on P. Based on observations in the unit, diaries and canisters' weight, all patients were compliant with the dosing regime. There was no difference in asthma control, including short-acting β2-agonist usage and PEF-measurements between the two treatment periods.

In both studies, no serious adverse events occurred and there were no clinically significant changes in physical examination, vital signs, laboratory parameters and baseline spirometry values.

PHARMACOKINETICS

The pharmacokinetics of AVE5883 after single dose (NKA challenge study) and multiple dose (allergen challenge study) administration are shown in Table 2. AVE5883 plasma concentrations showed considerable inter-subject variability and were similar in the two studies.

EFFECT OF AVE5883 ON AIRWAY CALIBER

In the NKA challenge study, pre-NKA FEV_1 was between 80% and 90% of baseline in 5 out of 19 patients. However, in the allergen challenge study multiple drug dosings did not significantly affect the pre-allergen airway caliber. In both studies, pre-challenge FEV_1 was not significantly different between the two treatments (NKA study: (mean ± SEM) 3.40 ± 0.23 L (AVE5883); 3.46 ± 0.20 L (P); p=0.40) and allergen challenge study: (mean ± SEM): 3.82 ± 0.3 L (AVE5883); 3.76 ± 0.3 L (P); p=0.52). Individual pre-dose and pre-challenge FEV_1 data for both studies are provided in the online supplement (Table E1 and E2).

TABLE 2 MAIN PHARMACOKINETIC PARAMETERS OF INHALED AVE5883

PARAMETER	NKA CHALLENGE STUDY	ALLERGEN CHALLENGE STUDY
Dose (mg)	4.8 single dose	4.8 three times a day
Number of patients	19	11
AUClast (ng x h/mL)	11.1 ± 6.0*	9.8 ± 4.9*
Cmax (ng/mL)	5.4 ± 3.7*	3.6 ± 1.8*
Tmax (h)	0.30 [0.28 – 1.12]**	0.35 [0.25 - 1.00]**
T 1/2 (h)	ND	6.93 ± 2.11 *

ND = Not determined, auclast = AUC from time zero to time of last measured concentration, 8 h in the NKA challenge study and 9 h in the allergen challenge study (*mean ±SD, **median [range])

EFFECT OF AVE5883 ON NKA-CHALLENGE

A single inhaled dose of AVE5883 reduced the NKA-induced bronchoconstriction in 16 out of 19 patients. In 5 of these 16 patients there was an increase in $PC_{20}FEV_1$(NKA) of at least 2 doubling doses. In addition, 7 patients did not reach a $PC_{20}FEV_1$ (NKA) following inhalation of the highest dose of NKA versus 2 subjects in the placebo group.

On average, AVE5883 caused a rightward shift of the dose-response curve to inhaled NKA of at least 1.2 doubling doses as compared to P pretreatment (mean difference in log10 $PC_{20}FEV_1$ (NKA) ± SD: 0.35 ± 0.10; 90% CI: 0.17-0.53; p=0.004). Excluding those 5 subjects with pre-NKA FEV_1 FEV_1 between 80-90% of baseline, a subgroup analysis still showed a significant effect of AVE5883 compared to P (see online supplement, Table E3).

EFFECT OF AVE5883 ON ALLERGEN-INDUCED AIRWAY RESPONSES

In all patients, inhaled HDM induced an EAR and a LAR at screening. Pretreatment with AVE5883 (4.8 mg TID, 9 days) did not protect against the allergen-induced airway responses (Figure 2). Conversely, as compared with P, there was a slightly greater fall in FEV_1 from baseline following AVE5883 inhalations in terms of area under the curve (AUC) during both the EAR: mean AUC (0-3 h) ± SEM (%fall*h): 23.7 ± 3.0 (AVE5883) and 18.0 ± 3.0 (P) (p=0.02); and the LAR: mean AUC (3-9 h) ± SEM (%fall*h): 145.5 ± 11.7 (AVE5883) and 116.2 ± 11.7 (P), (p=0.01). Although the maximal percentage fall (max%fall) from baseline FEV_1 during EAR was comparable between the two treatments: max%fall ± SEM: -19.9 ± 2.2 (AVE5883) and -18.0 ± 2.2 (P), (p=0.29), during LAR it was more pronounced following AVE5883: (max %fall ± SEM) -38.7 ± 2.9 as compared to -33.6 ± 2.9 (P); (p<0.01). At 24 h post-allergen (Day 9), allergen challenge caused a significant decrease in baseline FEV_1 in both treatment groups. However, the changes in FEV_1 were not significantly different between the two treatments (p=0.77).

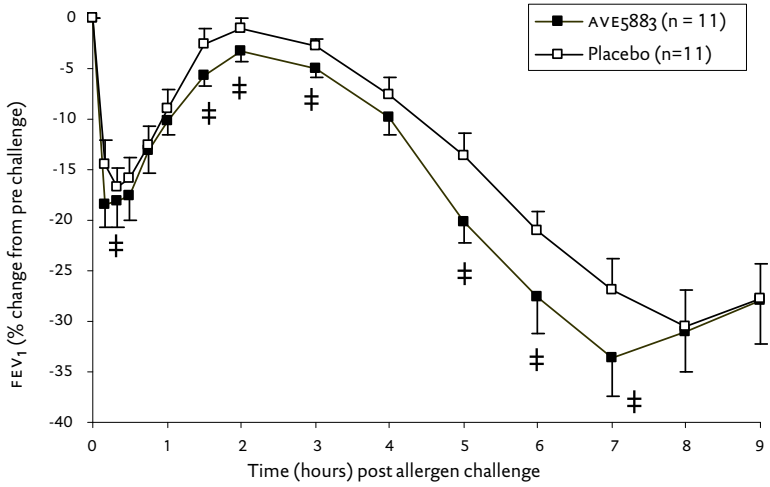


FIGURE 2 Allergen-induced airway responses in percentage change from pre-allergen FEV_1 (mean ± sem) 0-9 h following allergen challenge during AVE5883 (closed circles) and P (open squares) treatment. There was a significant difference in both the EAR (AUC 0-3 h) and LAR (AUC 3-9 h) between the two treatments, ‡ = P<0.05.

EFFECT OF AVE5883 ON ALLERGEN-INDUCED CHANGES IN AIRWAY RESPONSIVENESS TO METHACHOLINE

Allergen challenge caused a significant increase in AHR to methacholine (24 h pre- vs. 24 h post-challenge) during AVE5883 treatment (mean change in $PC_{20}FEV_1$(MBR) ± SEM: -0.23 ± 0.08 mg/mL; doubling concentrations; p=0.02). Similarly, there was an allergen-induced decrease in $PC_{20}FEV_1$ (MBR) following placebo (mean change in $PC_{20}FEV_1$ (MBR) ± SEM: -0.32 ± 0.08 mg/mL; doubling concentrations; p=0.003). However, these allergen-induced changes in airway responsiveness to MBR were not significantly different between the two treatments (p=0.21) (Figure 3).

EFFECT OF AVE5883 ON ALLERGEN-INDUCED CHANGES IN eNO

Multiple doses of AVE5883 did not affect baseline eNO values as compared to placebo treatment (Day 1 vs Day 7, p=0.28). The allergen challenge induced a significant increase in eNO (Day 7 vs Day 9) in both treatment periods (p <0.001). However, the changes in eNO were not statistically different between the two treatments (mean change ± SEM (Day 7 vs Day 9) 37.64 ± 6.40 ppb (AVE5883) and 43.44 ± 6.57 ppb (P), p=0.32) (Figure 4).

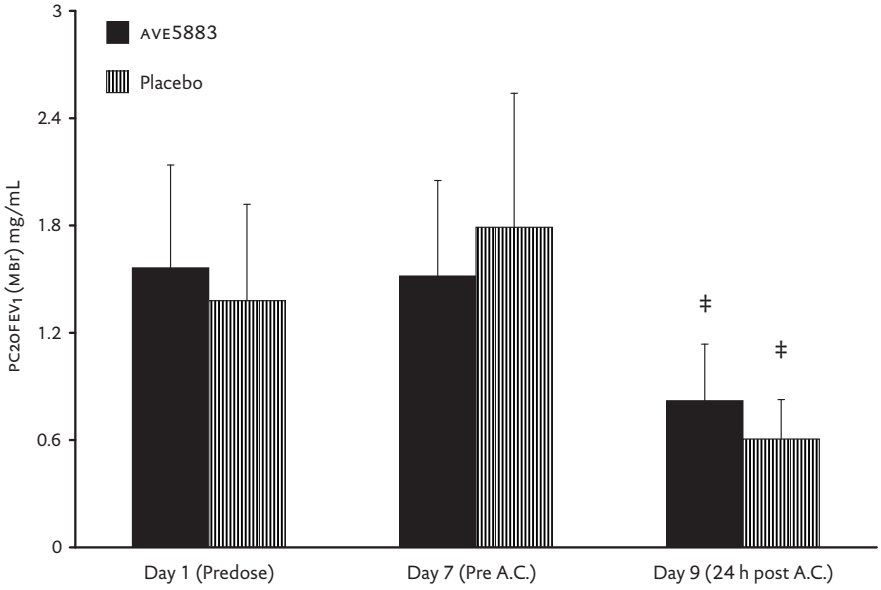


FIGURE 3 Airway responsiveness to mbr expressed as $PC_{20}FEV_1$(MBR) on days 1, 7 and 9 during both AVE5883 (filled bars) and P (striped bars) treatment period. Allergen challenge decreased $PC_{20}FEV_1$(MBR) during both treatment periods (day 7 vs. 9), ‡ = $P<0.05$. However, there was no significant difference in the changes in allergen-induced airway responsiveness to MBR between the two treatments ($p=0.21$).

EFFECT OF AVE5883 ON ALLERGEN-INDUCED CHANGES IN SPUTUM CELL DIFFERENTIALS

On pre-allergen Day 7, there was no difference in the % sputum eosinophils between both treatment periods (mean ± SEM: 4.86 ± 1.75 % (AVE5883) and 3.33 ± 1.58 % (P)). The allergen challenge induced a rise in sputum eosinophils during both treatment periods (Day 7 vs. Day 9; mean change ± SEM 8.09 ± 3.018; (AVE5883)) and mean change ± SEM 7.08 ± 2.972; (P)). Since only 3 patients managed to expectorate evaluable sputum samples on both Days 7 and 9 of the two treatment periods, no adequate power analysis could be performed on the allergen-induced changes in sputum cell count between the two treatments. However, based on evaluable samples, there was a clear trend towards increase in sputum eosinophils following allergen challenge. This is in agreement with the allergen-induced changes in ENO and $PC_{20}FEV_1$(MBR).

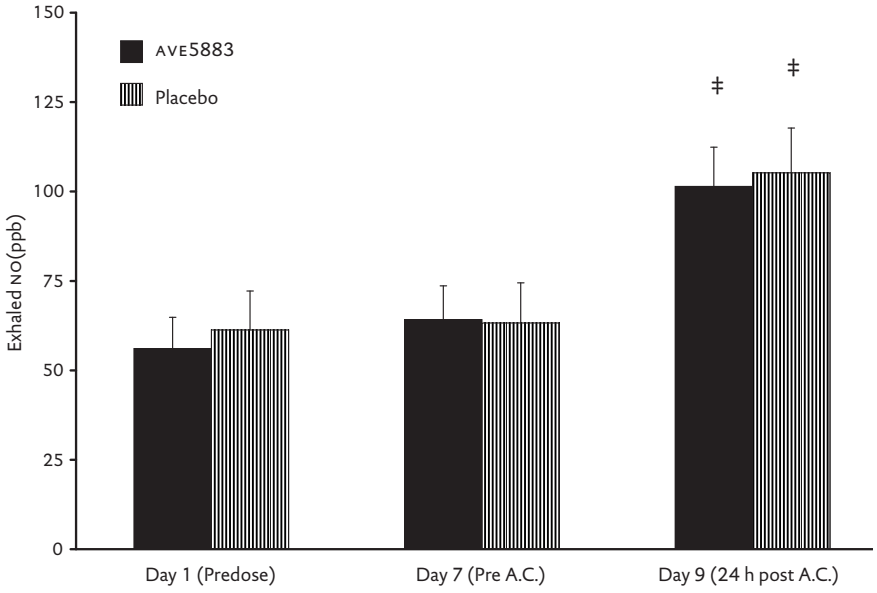


FIGURE 4 Exhaled NO as mean (± SEM) on days 1, 7 and 9 during both AVE5883 (filled bars) and P (striped bars) treatment period. As compared with pre-allergen (day 7), there was a significant increase in eNO 24 h post-allergen (day 9) during both treatments, ‡ = ($P<0.05$). However, the changes in eNO were not significantly different between the two treatments ($P=0.32$).

Discussion

We report combined study data on the safety, tolerability, pharmacological and biological efficacy and pharmacokinetics of a single and multiple inhaled doses of AVE5883, a novel dual tachykinin NK1/NK2 receptor antagonist, in patients with mild to moderate persistent asthma. In all patients, both dosing regimens of inhaled AVE5883 were safe and generally well-tolerated. and no clinically relevant adverse effects occurred. However, the substantial number of 16 actuations in combination with repeated deep inhalations from the pMDI device may have induced self-limiting dyspnoea accompanied by a transient drop in FEV_1 in some patients. As dyspnoea and FEV_1 were recorded at 12 minutes post-dosing in the single dose study and at 30 minutes in the multiple dose study, respectively, this may explain the higher occurrence of dyspnoea and/or drop in FEV_1 in the single dose (NKA-challenge) study. Despite pharmacological activity of a single inhaled dose (4.8 mg) against NKA-induced bronchoconstriction, multiple inhaled doses of AVE5883 (4.8

mg TID for 7 days) increased the allergen-induced airway responses and failed to reduce allergen-induced markers of airway inflammation and airway hyperresponsiveness in patients with similar asthma characteristics.
In a comparable proof of concept study in patients with similar asthma characteristics, a single inhaled dose of a less specific tachykinin NK1/NK2 receptor antagonist, FK224, failed to protect against NKA-induced bronchoconstriction (32). In contrast, a single oral dose of another dual tachykinin NK1/NK2 receptor antagonist, DNK333, provided significant protection against NKA-induced bronchoconstriction in patients with mild persistent asthma, causing a rightward shift of the dose-response curve to inhaled NKA by on mean 4.08 doubling doses (18). Moreover, in patients with similar asthma characteristics, an oral triple tachykinin receptor antagonist, CS-003, has been shown to produce a potent and long-lasting rightward shift of the NKA-dose-response curve (21). Our study results confirm and extend previous findings, showing that an inhaled dual tachykinin NK1/NK2 receptor antagonist is also capable of inhibiting NKA-induced bronchoconstriction in asthma, albeit to a lesser extent than the more potent oral compounds (18;21). Alternatively, an inhaled formula may offer the benefit of targeted therapy with possibly fewer systemic side effects.
In correspondence with several animal studies with other tachykinin NK1/NK2 receptor antagonists (33-35), aerosolized AVE5883 not only provided protection against both NKA-induced bronchoconstriction, but also against other (tachykinin-driven) bronchoconstrictor stimuli, including capsaicin and ovalbumin in sensitized guinea-pigs and dogs [data on file]. To our knowledge, this is the first study reporting on the effects of a dual tachykinin NK1/NK2 receptor antagonist on allergen-induced airway responses and markers of AHR/inflammation in asthmatic subjects *in vivo*. Despite a partial antagonistic effect of a single inhaled dose (4.8 mg) against exogenous NKA in patients with asthma, unexpectedly, pretreatment with multiple inhaled doses of AVE5883 (4.8 mg TID for 9 days) enhanced the allergen-induced airway responses without affecting the markers of airway AHR/inflammation. Furthermore, since AVE5883 did not affect baseline FEV_1 throughout the treatment period and since pre-allergen FEV_1 was not different between both treatments, this argues against a clear-cut mechanistic explanation for this phenomenon. In conclusion, while animal studies have produced a large body of evidence warranting efficacy of dual tachykinin NK1/NK2 receptor antagonists in asthmatic patients in vivo, we were unable to substantiate this hypothesis in the present study.
We do not believe that the lack of efficacy of AVE5883 against allergen-induced airway and inflammatory responses has been caused by methodological or dosing errors. First, we applied previously validated methods

and all participating patients had both an airway responsiveness to inhaled NKA and an allergen-induced late asthmatic response (25;26). In addition, pre-allergen FEV_1, recorded at approx. 1 h post-dosing, was not affected by inhalation of the study medication, nor were there any significant differences in baseline data between both treatment groups. Second, we based our dosing regimen on the same dose and mode of administration that effectively reduced the NKA-induced bronchoconstriction in the first part of the study in patients with similar asthma characteristics. Since a steady state plasma concentration was expected within three days, 7 days treatment with AVE5883 pre-allergen was deemed sufficient to demonstrate biological efficacy. In a similar study protocol, inhaled corticosteroids have been shown to provide a significant reduction of allergen-induced airway responses and markers of airway inflammation following 7 to 8 days pretreatment (36;37).

What could possibly account for the lack of effect of AVE5883 against allergen challenge? First, it may be possible that while NKA and SP play an important role in the allergen-driven airway inflammation in several animal models of asthma (38), this may not similarly apply to asthmatic patients due to species-related differences. For instance, Bowden *et al* (39) reported that in guinea-pigs, the most commonly applied laboratory species, approximately 60% of intra-epithelial fibres within the trachea constitute of SP nerve fibres, while in humans this is only 1% (40). Furthermore, in asthmatic airways the number of the SP fibres was not found to be increased as compared to nonasthmatic controls (41). Correspondingly, up to now reported (single) tachykinin NK1 or NK2 receptor antagonists have shown little if any efficacy against (tachykinin-driven) bronchoconstrictor stimuli in asthmatic subjects, despite previously shown pharmacological efficacy against the respective agonist (NKA or SP) (20;42). In the first study, multiple oral doses of the specific NK2 receptor antagonist, SR 48968, failed to protect against adenosine-induced bronchoconstriction in subjects with allergic asthma (20). In another study, CP-99,994, an NK1 receptor antagonist, did not inhibit hypertonic saline-induced bronchoconstriction and cough in patients with mild persistent asthma (42).

Still another possibility could be that SP, being a major pro-inflammatory tachykinin (8), is likely to play a more important role in allergen-induced airway inflammation than NKA, which appears to possess more direct bronchoconstrictor properties (13). And although AVE5883, being a dual NK1/NK2 antagonist was expected to inhibit the effects of both tachykinins, we only tested its protective properties against NKA, and are hence not fully informed about its pharmacological efficacy against inhaled SP in asthma in vivo. In line with this and based on its modest antagonistic properties against inhaled NKA, it may be that AVE5883 is not potent enough to offer protection against allergen-induced airway response and inflammation. Comparable findings

were reported in clinical trials with early leukotriene (LT) receptor antagonists in asthma. Despite a 3.8-fold rightward shift in the dose response curve to inhaled LTD4 in mild asthmatics, the oral LT receptor antagonist L-649,923 failed to protect against allergen-induced bronchoconstriction. However, the more potent LT antagonist zafirlukast, causing an approx. 10-fold shift in the dose-response curve to inhaled LTD4, significantly protected against allergen-induced airway responses and the associated AHR (43-46). Finally, although similar plasma concentrations of AVE5883 were observed in both studies, higher and longer lasting plasma exposure of AVE5883 may have been required to warrant adequate drug concentrations within the airways, before and during the allergen-induced late asthmatic response. Therefore, considering the relatively short terminal elimination half-life of the drug (T1/2 = 6.93 h), higher doses and/or a more frequent dosing of AVE5883 may have been required to achieve any protective effect.

In conclusion, a single inhaled dose of AVE5883 provided a modest protection against NKA-induced bronchoconstriction in patients with mild to moderate persistent asthma, whereas 7 days pretreatment with multiple daily doses of this dual tachykinin NK1/NK2 receptor antagonist paradoxically enhanced the allergen-induced airway responses without affecting the markers of airway inflammation/hyperrresponsiveness in a patient population with similar asthma characteristics. Therefore, these findings question the prominent role of neurogenic inflammation in asthma and consequently, the therapeutic potential of dual tachykinin antagonists. Hence, more research is required to determine the precise role of tachykinins and their receptors in the allergic airway inflammation that will help to establish the position of potent combined tachykinin receptor antagonists in the treatment of asthma.

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