

# Effect of an NK<sub>1</sub>/NK<sub>2</sub> Receptor Antagonist on Airway Responses and Inflammation to Allergen in Asthma

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**Rationale:** The tachykinins substance P and neurokinin A (NKA) are implicated in the pathophysiology of asthma.

**Objective:** We tested the safety, tolerability, and pharmacologic and biological efficacy of a tachykinin NK<sub>1</sub>/NK<sub>2</sub> receptor antagonist, AVE5883, in patients with asthma in two double-blind, placebo-controlled crossover studies.

**Methods:** The pharmacologic efficacy of a single inhaled dose (4.8 mg) of AVE5883 was tested against inhaled NKA in 20 patients with asthma. Subsequently, we studied the biological efficacy of the pharmacologically effective dose on inhaled allergen in a multiple-dose trial (4.8 mg three times per day, 9 d) in 12 patients with asthma with dual responses to inhaled house dust mite. On Day 8, an allergen challenge was conducted, and airway response was measured by FEV<sub>1</sub> until 9 hours postallergen. Exhaled NO, provocative concentration of methacholine bromide causing a 20% fall in FEV<sub>1</sub>, 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. Pretreatment with multiple doses of AVE5883 enhanced the allergen-induced early and late airway responses. There were no significant differences in the allergen-induced changes in exhaled NO, provocative concentration of methacholine bromide causing a 20% fall in FEV<sub>1</sub>, and sputum cell differentials between placebo and AVE5883.

**Conclusions:** Despite its demonstrated pharmacologic 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.

**Keywords:** tachykinins; neurokinin A bronchoprovocation test; allergen bronchoprovocation test; asthma; AVE5883

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 seems to be related to chronic airway inflammation (2). Hence, antiinflammatory therapy with inhaled corticosteroids is the cornerstone of pharmacotherapy of persistent asthma (1). However, long-term use of high doses of inhaled corticosteroids may induce

## AT A GLANCE COMMENTARY

### Scientific Knowledge on the Subject

The tachykinins substance P and neurokinin A are implicated in the pathophysiology of asthma. There are no published studies on the efficacy of dual tachykinin NK<sub>1</sub>/NK<sub>2</sub> receptor antagonists against allergen challenge or on their clinical efficacy in asthma.

### What This Study Adds to the Field

A single inhaled dose of AVE5883 provided protection against neurokinin A–induced bronchoconstriction in patients with asthma, whereas 7 days' pretreatment with multiple daily doses failed to reduce allergen-induced airway responses and markers of airway hyperresponsiveness/inflammation.

troublesome local 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 patients with asthma (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 system by mechanical, thermal, chemical, or inflammatory stimuli (7, 8). It seems that SP exerts its proinflammatory effects mainly by stimulation of the tachykinin NK<sub>1</sub> receptors, whereas NKA mainly causes tachykinin NK<sub>2</sub> receptor–mediated effects (9). On inhalation, SP induces AHR and the so-called “neurogenic inflammation” within the airways of individuals with and without asthma, characterized by microvascular leakage, mucus secretion, and inflammatory cell responses (9–11), whereas inhaled NKA mainly causes bronchoconstriction (12, 13). Furthermore, in contrast with nonasthmatic control subjects, increased tachykinin NK<sub>1</sub> and NK<sub>2</sub> receptor mRNA expression has been demonstrated within the airways of patients with asthma (14, 15). In subjects with allergic asthma, increased concentrations of SP have been found in sputum and bronchoalveolar lavage (BAL) at baseline, with further increase after segmental allergen challenge (8, 16). In another study in allergic asthma, increased NKA levels have been detected 4 hours after 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 NK<sub>1</sub> or NK<sub>2</sub> receptor antagonists have been

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developed and tested against indirect challenges, including NKA, adenosine monophosphate, and hypertonic saline in patients with asthma (17–21). Until recently, there have been no published studies on the clinical efficacy of dual tachykinin NK<sub>1</sub>/NK<sub>2</sub> receptor antagonists in asthma or on their biological efficacy in allergen challenge, which is the most representative model of asthma.

AVE5883 is a nonpeptidyl, dual tachykinin NK<sub>1</sub>/NK<sub>2</sub> receptor antagonist with high specificity and affinity for tachykinin NK<sub>1</sub> and NK<sub>2</sub> receptors ( $K_i = 5.6$  and  $3.1$  nM for the NK<sub>1</sub> and NK<sub>2</sub> receptor, respectively, and  $> 10$   $\mu$ M for a variety of other physiologically important receptors). In sensitized guinea pigs, intraperitoneally administered AVE5883 has been shown to reduce ovalbumin-induced airway hyperreactivity and eosinophil influx in the BAL fluid. Similarly, intratracheally administered AVE5883 protected against capsaicin-induced bronchoconstriction in sensitized guinea pigs, and aerosolized AVE5883 inhibited the NKA-induced increase in airway resistance in dogs.

We tested the safety and tolerability, in combination with the pharmacologic and biological efficacy and the pharmacokinetics, of inhaled AVE5883 in clinically stable patients with mild to moderate persistent asthma who were not on maintenance antiinflammatory therapy. In the first study, we tested the pharmacologic efficacy of a single inhaled dose of AVE5883 against NKA-induced bronchoconstriction. 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.** Twenty nonsmoking 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 criteria (1), without any other clinically relevant disorders. Except for inhaled short-acting  $\beta_2$ -agonists as needed, no subjects had used concomitant antiasthma or antiallergy medication for at least 6 weeks before 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  $\beta_2$ -agonists were withheld at least 8 hours before each visit. Baseline FEV<sub>1</sub> had to be greater than or equal to 75% of predicted. Patients were hyperresponsive to inhaled methacholine bromide (MBr) and inhaled NKA, showing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>FEV<sub>1</sub>[MBr]) of less than 19.6 mg/ml (= 16 mg/ml methacholine chloride, equals 80  $\mu$ mol/ml) and a PC<sub>20</sub>FEV<sub>1</sub>(NKA) of less than  $441.2 \times 10^{-3}$   $\mu$ mol/ml (equals 500  $\mu$ g/ml), respectively, at screening.

**Allergen challenge study.** Twelve patients participated in the allergen challenge study (Table 1). Four patients had previously participated in the NKA challenge study ( $> 6$  mo ago). All patients met the same aforementioned criteria, with a maximum PC<sub>20</sub>FEV<sub>1</sub>(NKA) of less than or equal to  $882.4 \times 10^{-6}$   $\mu$ mol/ml (equals  $\leq 1,000$   $\mu$ g/ml) at screening. Patients had a positive skin prick test to house dust mite (HDM) (a positive response was defined as a mean wheal diameter  $\geq 3$  mm) and a documented late asthmatic response (LAR) to inhaled HDM extract (i.e., a fall in FEV<sub>1</sub>  $\geq 15\%$  from baseline between 3 and 9 h postallergen). Both study protocols were approved by the Leiden University Medical Centre Ethics Committee, and all participants gave written informed consent.

### Study Design

**NKA challenge study.** This was a single-center, randomized, double-blind, placebo-controlled, single-dose crossover study. Before subjects were entered into the study, selection criteria were examined on two screening visits, 24 hours apart. Fourteen to 28 days after screening, eligible patients were randomized into the study. Each treatment period consisted of 2 study days, separated by a washout period of 12 to 16 days. Clinically stable subjects inhaled 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 (predose, 10, 30, and 45 min; and 1, 2, 4, 6, and 8 h postdose). A standardized NKA challenge was performed 30 minutes after the last study drug inhalation. Prechallenge FEV<sub>1</sub> had to return to within 10% of baseline. To keep the time interval between dosing and NKA challenge constant in all patients and to allow more hyperresponsive asthmatics in the study, in the case of an unexpected drop in FEV<sub>1</sub> after study medication, decreases in pre-NKA FEV<sub>1</sub> were accepted up to 20% from baseline, provided they were within a safe range (FEV<sub>1</sub>  $> 2.3$  L, allowing a safe PC<sub>20</sub>FEV<sub>1</sub>[NKA]). There was a follow-up visit 10 to 14 days poststudy.

**Allergen challenge study.** This was a single-center, randomized, multiple-dose, placebo-controlled, double-blind crossover study. At least 3 weeks before the study, selection criteria were examined. The washout between both study periods was at least 3 weeks. Eligible patients with a documented PC<sub>20</sub>FEV<sub>1</sub>(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<sub>20</sub>FEV<sub>1</sub>(MBr) and, 1 hour later, a sputum induction were performed. To ensure asthma stability, mean baseline FEV<sub>1</sub> had to be within 10%, and the PC<sub>20</sub>FEV<sub>1</sub>(MBr) had to remain within one doubling dose on Day 1 of both treatment periods. Clinically stable patients inhaled the first dose of study medication at 2 P.M. ( $\pm 2$  h) followed by an evening dose at approximately 8 P.M. and FEV<sub>1</sub> measurements. On Days 2 through 7, study medication was inhaled three times daily at 8 A.M., 2 P.M., and 8 P.M. ( $\pm 2$  h). Subjects were discharged from the unit 1 hour 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 minutes after dosing (provided preallergen FEV<sub>1</sub> returned within 10% of the predose value), and the airway response was recorded by FEV<sub>1</sub> until 9 h postallergen. Blood samples were collected for pharmacokinetics from a venous cannula inserted in a forearm vein on Day 8 (predose, 15 and 30 min, and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, and 9 h postdose) and on Days 7 and 9 (predose and 1, 2, 4, and 6 h postdose). The proinflammatory effects of inhaled allergen were monitored by eNO,

**TABLE 1. PATIENTS' BASELINE CHARACTERISTICS**

| Characteristics                                                        | NKA Challenge | Allergen Challenge |
|------------------------------------------------------------------------|---------------|--------------------|
| Number of patients                                                     | 20*           | 12†                |
| Sex                                                                    |               |                    |
| Male                                                                   | 5             | 6                  |
| Female                                                                 | 15            | 6                  |
| Atopy status                                                           |               |                    |
| Yes                                                                    | 18            | 12                 |
| No                                                                     | 2             | 0                  |
| Age, yr                                                                |               |                    |
| Mean                                                                   | 29.6          | 24.4               |
| Range                                                                  | 19–47         | 20–39              |
| FEV <sub>1</sub> , L                                                   |               |                    |
| Mean                                                                   | 3.7           | 3.8                |
| Range                                                                  | 2.6–5.7       | 3.1–5.9            |
| FEV <sub>1</sub> , % predicted                                         |               |                    |
| Mean                                                                   | 102.4         | 91.6               |
| Range                                                                  | 80.3–125.5    | 73.6–121.4‡        |
| PC <sub>20</sub> FEV <sub>1</sub> (MBr), mg/ml                         |               |                    |
| Mean                                                                   | 2.2           | 2.6                |
| Range                                                                  | 0.1–8.5       | 0.1–14.4           |
| PC <sub>20</sub> FEV <sub>1</sub> (NKA), $\times 10^{-3}$ $\mu$ mol/ml |               |                    |
| Mean                                                                   | 143.2         | 357.9              |
| Range                                                                  | 5.9–593.8§    | 5.9–1,115.7        |

*Definition of abbreviations:* NKA = neurokinin A; PC<sub>20</sub>FEV<sub>1</sub>(MBr) = provocative concentration of methacholine bromide causing a 20% fall in FEV<sub>1</sub>; PC<sub>20</sub>FEV<sub>1</sub>(NKA) = provocative concentration of neurokinin A causing a 20% fall in FEV<sub>1</sub>.

\* One subject did not complete the NKA challenge study.

† One subject did not complete the allergen challenge study.

‡ One subject with a lower baseline FEV<sub>1</sub> (73.6% predicted) was included.

§ One subject with a PC<sub>20</sub>FEV<sub>1</sub>(NKA) of  $593.8 \times 10^{-3}$   $\mu$ mol/ml was included.

|| One subject with a PC<sub>20</sub>FEV<sub>1</sub>(NKA) of  $1,115.7 \times 10^{-3}$   $\mu$ mol/ml was included.

sputum cell differentials, and PC<sub>20</sub>FEV<sub>1</sub>(MBr) on Days 7 and 9, 24 hours pre- and postallergen, respectively. Ten to 14 days poststudy, a follow-up visit was scheduled (Figure 1). Throughout the study, patients recorded 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 (≥ 2 × 100 µg) through Volumatic (GlaxoSmithKline, Zeist, The Netherlands) until the FEV<sub>1</sub> returned to within 10% of baseline.

### 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) (Sanofi-Aventis Pharmaceuticals, Inc., Cheshire, UK) was used as a sterile solution or matching placebo in an alcohol/propellant (HFA-227) mixture delivered in a pressurized metered dose inhaler (300 µg/actuation). Each administration consisted of 16 actuations in 8 minutes, yielding a cumulative dose of 4.8 mg AVE5883. Postdosing, subjects were required to rinse their mouth.

### Inhalation Challenges and Response Measurements

The airway response to the inhaled aerosols was measured by FEV<sub>1</sub> according to standardized lung function techniques and recorded by a spirometer connected to a personal computer (V<sub>max</sub> Spectra; Sensor Medics, Bithoven, 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 the 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, which 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 3 × 5 minutes at 15-minute intervals (26). Sputum samples were processed according to a previously validated method after adding dithiothreitol 0.1% (28). Subsequently, cytopins were made (50 µl/cytospin) (Shandon Cytospin 4; Thermo Electron Corporation, Runcorn, UK), and differential cell counts were performed on May-Grünwald-Giemsa-stained cytopins. Cells were expressed as a percentage of 500 nucleated cells excluding squamous cells as described previously (26). Samples with more than 80% squamous cells were excluded from analysis. A detailed description of the methods used is presented in the online supplement.

### eNO

eNO measurements were performed in triplicate within 10% at a exhalation flow of 50 ml/second according to current American Thoracic Society recommendations (29) by a chemoluminescence analyzer (Ecomedics CLD88sp; Ecomedics, Duernten, Switzerland). A detailed description of the 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 3,000 rpm for 10 minutes at 4°C. AVE5883 was determined in plasma samples of subjects treated with AVE5883 (Bioanalytics, DMPK; Sanofi-Aventis Pharmaceuticals, Inc., Bridgewater, NJ) using a validated liquid chromatography (LC)/mass spectrometry (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<sub>20</sub>FEV<sub>1</sub>(NKA) (log<sub>10</sub> transformed) after AVE5883 and placebo pretreatment. PC<sub>20</sub>FEV<sub>1</sub>(NKA) was calculated by linear interpolation of the inhaled dose of NKA below and above a 20% fall in FEV<sub>1</sub> (27). If no fall in FEV<sub>1</sub> greater than or equal to 20% was reached after inhalation of the highest NKA dose, PC<sub>20</sub>FEV<sub>1</sub>(NKA) was set at 1.76 µmol/ml (= 2,000 µg/ml; i.e., one doubling dose higher than the highest dose tested) (27). The difference in PC<sub>20</sub>FEV<sub>1</sub>(NKA) between AVE5883 and placebo was tested using analysis of variance (ANOVA) with sequence, subject (within sequence), and treatment as factors. The safety and tolerability were primarily assessed through examination of treatment-emergent adverse events. Treatment-emergent adverse events consisted of all on-treatment adverse events and any pretreatment 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 the early asthmatic response (EAR) (0–3 h postallergen) and the LAR (3–9 h postallergen) between AVE5883 and placebo. The trapezoidal rule was applied for the calculation of the AUCs (30). The differences in the maximal percent fall in FEV<sub>1</sub> during the EAR and LAR were compared between the two treatments.

The differences in allergen-induced changes in eNO, PC<sub>20</sub>FEV<sub>1</sub>(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 hours before and 24 hours after allergen challenge between the two treatments. PC<sub>20</sub>FEV<sub>1</sub>(MBr) was calculated by linear interpolation of the airway

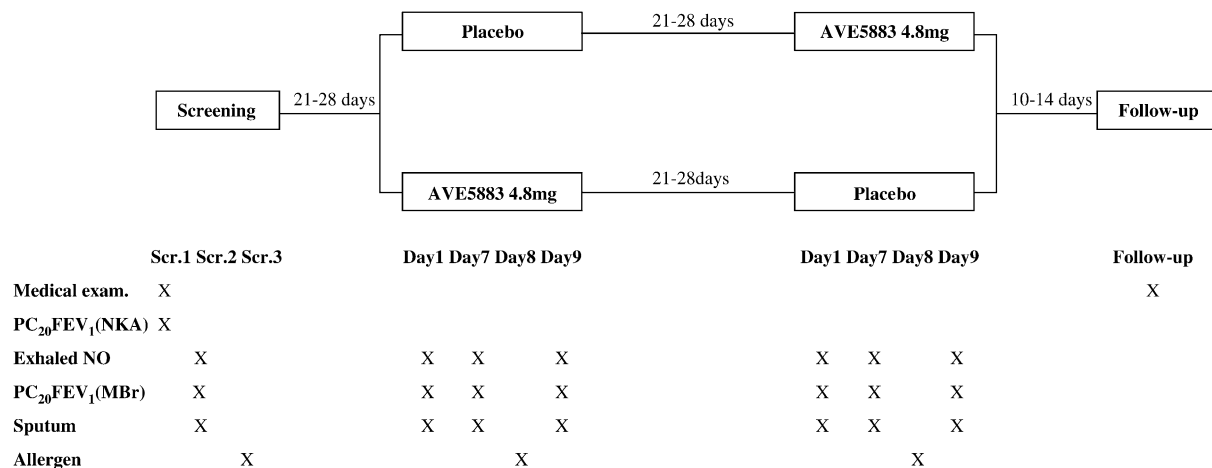


**Figure 1.** Study design: allergen challenge study. There was at least a 1-week washout between screening visits 1 and 2. Allergen = allergen challenge; PC<sub>20</sub>FEV<sub>1</sub>(MBr) = provocative concentration of methacholine bromide causing a 20% fall in FEV<sub>1</sub>; PC<sub>20</sub>FEV<sub>1</sub>(NKA) = provocative concentration of neurokinin A causing a 20% fall in FEV<sub>1</sub>; Scr = screening; Sputum = sputum induction.

responses below and above a 20% fall in FEV<sub>1</sub> (27). The airway responses to inhaled allergen were expressed as percentage fall in FEV<sub>1</sub> from postdiluent 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 ANOVA appropriate to the two-period, two-sequence, two-treatment crossover design was used. The ANOVA model contained factors of treatment, sequence, and subject within sequence. Carryover effects were examined for the LAR AUC analysis. *p* Values of less than 0.05 were considered statistically significant.

The sample size of 12 evaluable patients for this study was based on 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 ( $\alpha = 0.05$  [two-tailed];  $\beta = 0.10$  [one-tailed]; power = 90%; within-subject SD, 25%) (31).

## RESULTS

### Safety and 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<sub>1</sub> of 34% from baseline) within 5 minutes of inhalation of AVE5883. Overall, the study medication was well tolerated, although all patients receiving AVE5883 versus none receiving placebo reported bad taste. The most commonly occurring adverse event was transient, self-limiting bronchospasm starting within 12 minutes after study drug inhalation reported by eight patients receiving AVE5883 and four receiving placebo. Other reported adverse events were headache (five patients receiving AVE5883 and three patients receiving placebo) and self-limiting dyspnea (two patients receiving AVE5883 and five patients receiving placebo).

**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 periods 1 and 2. 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 dyspnea occurring within 30 minutes of study drug inhalation (in five patients receiving AVE5883 vs. two patients receiving placebo). In all patients, preallergen FEV<sub>1</sub> was within 10% of the predose value (Table E2 of the online supplement). Headache was reported by three patients receiving AVE5883 versus three receiving placebo. 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  $\beta_2$ -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 intersubject variability and were similar in the two studies.

### Effect of AVE5883 on Airway Caliber

In the NKA challenge study, pre-NKA FEV<sub>1</sub> was between 80 and 90% of baseline in 5 of 19 patients. In the allergen challenge

**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 $\times$ h/ml | 11.1 $\pm$ 6.0*               | 9.8 $\pm$ 4.9*                |
| Cmax, ng/ml               | 5.4 $\pm$ 3.7*                | 3.6 $\pm$ 1.8*                |
| Tmax, h                   | 0.30 (0.28–1.12) <sup>†</sup> | 0.35 (0.25–1.00) <sup>†</sup> |
| T <sub>1/2</sub> , h      | ND                            | 6.93 $\pm$ 2.11*              |

*Definition of abbreviations:* AUClast = area under the curve from time zero to time of last measured concentration (8 h in the neurokinin A challenge study and 9 h in the allergen challenge study); Cmax = maximum plasma drug concentration; ND = not determined; NKA = neurokinin A; Tmax = time to maximum concentration.

\* Mean  $\pm$  SD.

<sup>†</sup> Median (range).

study, multiple drug dosings did not significantly affect the preallergen airway caliber. In both studies, prechallenge FEV<sub>1</sub> was not significantly different between the two treatments (NKA study: [mean  $\pm$  SEM] 3.40  $\pm$  0.23 L [AVE5883]; 3.46  $\pm$  0.20 L [placebo]; *p* = 0.40) and allergen challenge study: [mean  $\pm$  SEM] 3.82  $\pm$  0.3 L [AVE5883]; 3.76  $\pm$  0.3 L [placebo]; *p* = 0.52). Individual predose and prechallenge FEV<sub>1</sub> data for both studies are provided in Tables E1 and E2.

### Effect of AVE5883 on NKA Challenge

A single inhaled dose of AVE5883 reduced the NKA-induced bronchoconstriction in 16 of 19 patients. In 5 of these 16 patients, there was an increase in PC<sub>20</sub>FEV<sub>1</sub>(NKA) of at least two doubling doses. Seven patients did not reach a PC<sub>20</sub>FEV<sub>1</sub>(NKA) after inhalation of the highest dose of NKA versus two 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 with placebo pretreatment (mean difference in log<sub>10</sub> PC<sub>20</sub>FEV<sub>1</sub>[NKA]  $\pm$  SD: 0.35  $\pm$  0.10; 90% confidence interval, 0.17–0.53; *p* = 0.004). Excluding those five subjects with pre-NKA FEV<sub>1</sub> between 80 and 90% of baseline, a subgroup analysis showed a significant effect of AVE5883 compared with placebo (Table E3).

### Effect of AVE5883 on Allergen-induced Airway Responses

In all patients, inhaled HDM induced an EAR and an LAR at screening. Pretreatment with AVE5883 (4.8 mg three times a day for 9 d) did not protect against the allergen-induced airway responses (Figure 2). Conversely, as compared with placebo, there was a slightly greater fall in FEV<sub>1</sub> from baseline after AVE5883 inhalation in terms of AUC during the EAR (mean AUC [0–3 h]  $\pm$  SEM [%fall/h]: 23.7  $\pm$  3.0 [AVE5883] and 18.0  $\pm$  3.0 [placebo]; *p* = 0.02) and the LAR (mean AUC [3–9 h]  $\pm$  SEM [%fall/h]: 145.5  $\pm$  11.7 [AVE5883] and 116.2  $\pm$  11.7 [placebo]; *p* = 0.01). Although the maximal percentage fall (max%fall) from baseline FEV<sub>1</sub> during EAR was comparable between the two treatments (max%fall  $\pm$  SEM: –19.9  $\pm$  2.2 [AVE5883] and –18.0  $\pm$  2.2 [placebo]; *p* = 0.29), during LAR it was more pronounced after AVE5883 (max%fall  $\pm$  SEM: –38.7  $\pm$  2.9 as compared with –33.6  $\pm$  2.9 [placebo] *p* < 0.01).

At 24 hours postallergen (Day 9), allergen challenge caused a significant decrease in baseline FEV<sub>1</sub> in both treatment groups. These changes in FEV<sub>1</sub> were not significantly different between the two treatments (*p* = 0.77).

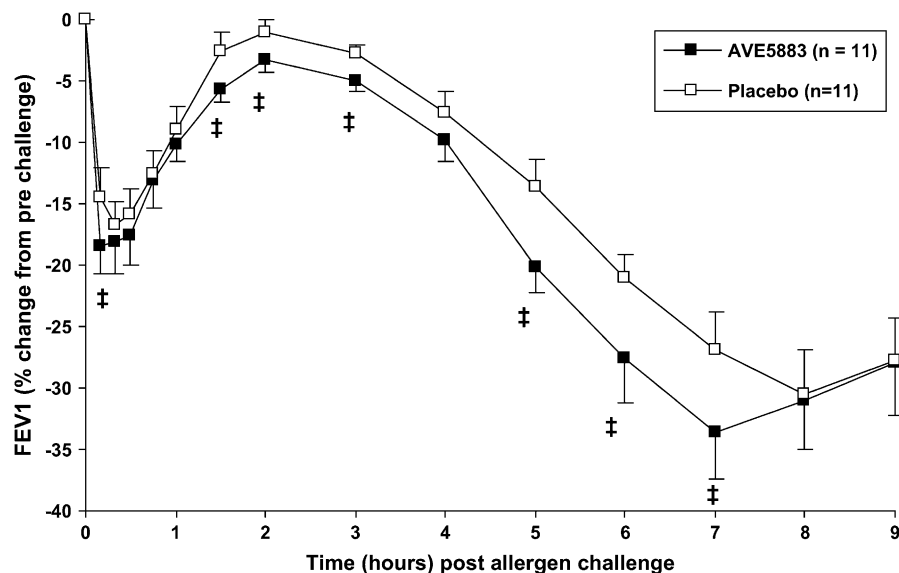


**Figure 2.** Allergen-induced airway responses in percentage change from preallergen FEV<sub>1</sub> (mean  $\pm$  SEM) 0 to 9 hours after allergen challenge during the AVE5883 and placebo treatments. There was a significant difference in the early asthmatic response (area under the curve [AUC], 0–3 h) and the late asthmatic response (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<sub>20</sub>FEV<sub>1</sub>[MBr]  $\pm$  SEM:  $-0.23 \pm 0.08$  mg/ml; doubling concentrations;  $p = 0.02$ ). Similarly, there was an allergen-induced decrease in PC<sub>20</sub>FEV<sub>1</sub>(MBr) after placebo (mean change in PC<sub>20</sub>FEV<sub>1</sub>[MBr]  $\pm$  SEM:  $-0.32 \pm 0.08$  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 with 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  $\pm$  SEM [Day 7 vs. Day 9],  $37.64 \pm 6.40$  ppb [AVE5883] and  $43.44 \pm 6.57$  ppb [placebo];  $p = 0.32$ ) (Figure 4).

#### Effect of AVE5883 on Allergen-induced Changes in Sputum Cell Differentials

On Preallergen Day 7, there was no difference in the percentage of sputum eosinophils between both treatment periods (mean  $\pm$  SEM,  $4.86 \pm 1.75\%$  [AVE5883] and  $3.33 \pm 1.58\%$  [placebo]). The allergen challenge induced a rise in sputum eosinophils during both treatment periods (Day 7 vs. Day 9; mean change  $\pm$  SEM,  $8.09 \pm 3.018$  [AVE5883] and mean change  $\pm$  SEM,  $7.08 \pm 2.972$  [placebo]). Because only three patients managed to expectorate evaluable sputum samples on both Days 7 and 9 of the two treatment periods, no adequate power analysis could

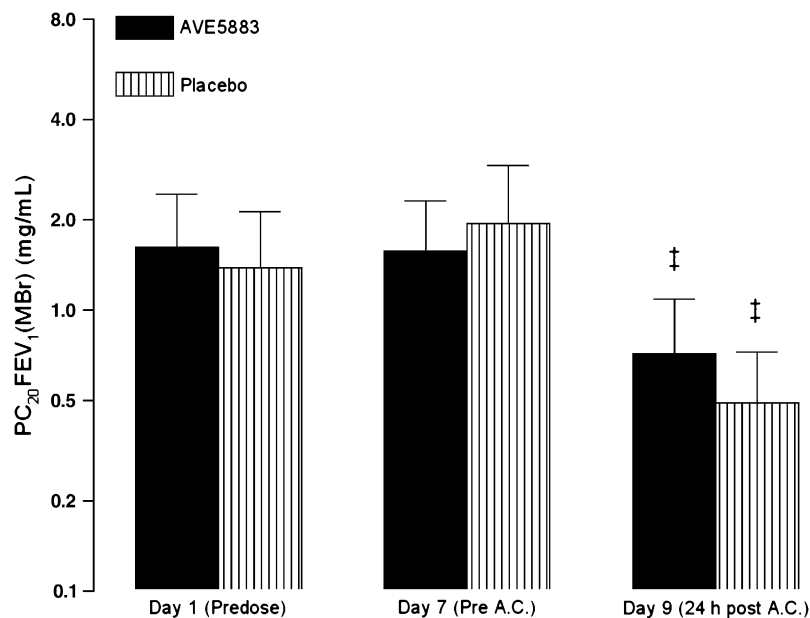


**Figure 3.** Airway responsiveness to methacholine bromide (MBr) expressed as the provocative concentration of methacholine bromide causing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>FEV<sub>1</sub>[MBr]) on Days 1, 7, and 9 during the AVE5883 and placebo treatment periods. Allergen challenge (A.C.) decreased PC<sub>20</sub>FEV<sub>1</sub>(MBr) during both treatment periods (Day 7 vs. Day 9); \* $p < 0.05$ . There was no significant difference in the changes in allergen-induced airway responsiveness to MBr between the two treatments ( $p = 0.21$ ).

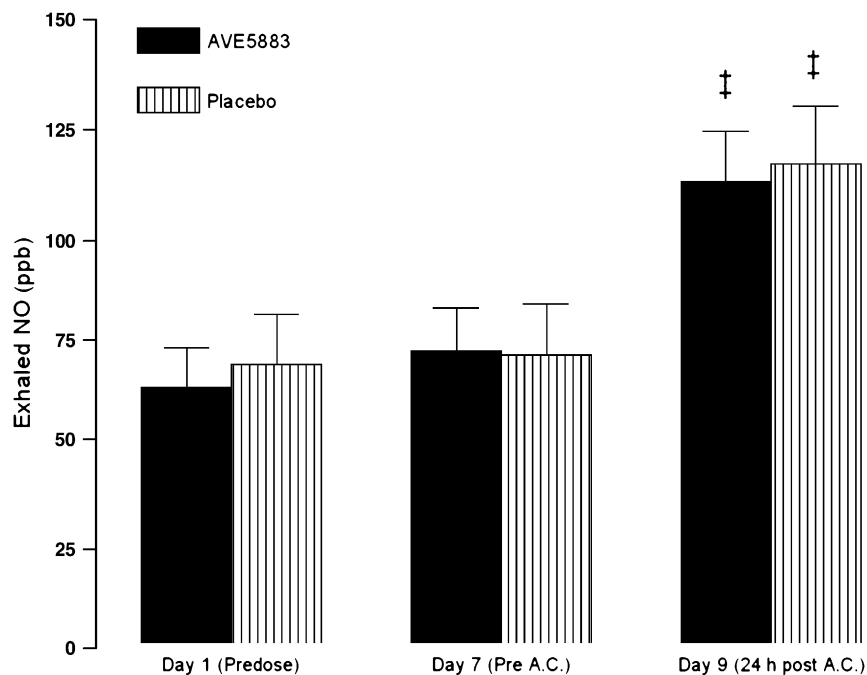


**Figure 4.** Exhaled NO (eNO) as mean ( $\pm$  SEM) on Days 1, 7, and 9 during the AVE5883 and placebo treatment periods. As compared with preallergen (Day 7), there was a significant increase in eNO 24 hours postallergen (Day 9) during both treatments;  $^{\dagger}p < 0.05$ . The changes in eNO were not significantly different between the two treatments ( $p = 0.32$ ).

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 toward an increase in sputum eosinophils after allergen challenge. This is in agreement with the allergen-induced changes in eNO and  $PC_{20}FEV_1$  (MBr).

## DISCUSSION

We report combined study data on the safety, tolerability, and pharmacologic and biological efficacy and pharmacokinetics of a single and multiple inhaled doses of AVE5883, a novel dual tachykinin  $NK_1/NK_2$  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. However, the substantial number of 16 actuations in combination with repeated deep inhalations from the pressurized metered-dose inhaler device may have induced self-limiting dyspnea accompanied by a transient drop in  $FEV_1$  in some patients. Because dyspnea and  $FEV_1$  were recorded at 12 minutes postdosing in the single-dose study and at 30 minutes in the multiple-dose study, respectively, this may explain the higher occurrence of dyspnea or drop in  $FEV_1$  in the single-dose (NKA challenge) study. Despite pharmacologic activity of a single inhaled dose (4.8 mg) against NKA-induced bronchoconstriction, multiple inhaled doses of AVE5883 (4.8 mg three times a day for 7 d) increased the allergen-induced airway responses and failed to reduce allergen-induced markers of airway inflammation and AHR 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  $NK_1/NK_2$  receptor antagonist, FK224, failed to protect against NKA-induced bronchoconstriction (32). In contrast, a single oral dose of another dual tachykinin  $NK_1/NK_2$  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 pro-

duce 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  $NK_1/NK_2$  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 agreement with several animal studies with other tachykinin  $NK_1/NK_2$  receptor antagonists (33–35), aerosolized AVE5883 not only provided protection against NKA-induced bronchoconstriction but also against other (tachykinin-driven) bronchoconstrictor stimuli, including capsaicin and ovalbumin in sensitized guinea pigs and dogs. To our knowledge, this is the first study reporting on the effects of a dual tachykinin  $NK_1/NK_2$  receptor antagonist on allergen-induced airway responses and markers of AHR/inflammation in patients with asthma *in vivo*. Despite a partial antagonistic effect of a single inhaled dose (4.8 mg) against exogenous NKA in patients with asthma, pretreatment with multiple inhaled doses of AVE5883 (4.8 mg three times a day for 9 d) enhanced the allergen-induced airway responses without affecting the markers of AHR/inflammation. Furthermore, because AVE5883 did not affect baseline  $FEV_1$  throughout the treatment period and because preallergen  $FEV_1$  was not different between both treatments, this argues against a clear-cut mechanistic explanation for this phenomenon. In conclusion, although animal studies have produced a large body of evidence of the efficacy of dual tachykinin  $NK_1/NK_2$  receptor antagonists in patients with asthma *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 methodologic or dosing errors. First, we applied previously validated methods, and all participating patients had airway responsiveness to inhaled NKA and an allergen-induced LAR (25, 26). In addition, preallergen  $FEV_1$ , recorded at approximately 1 hour postdosing, 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. Because a steady-state plasma concentration was expected within 3 days, 7-day treatment with AVE5883 preallergen 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 after 7 to 8 days' pretreatment (36, 37).

What could account for the lack of effect of AVE5883 against allergen challenge? First, it may be possible that, although NKA and SP play an important role in the allergen-driven airway inflammation in several animal models of asthma (38), this may not apply to patients with asthma due to species-related differences. For instance, Bowden and colleagues (39) reported that, in guinea pigs, the most commonly used laboratory species, approximately 60% of intraepithelial fibers within the trachea constitute of SP nerve fibers, whereas in humans this is only 1% (40). Furthermore, in asthmatic airways, the number of the SP fibers was not found to be increased as compared with nonasthmatic control subjects (41). Up to now, reported (single) tachykinin NK<sub>1</sub> or NK<sub>2</sub> receptor antagonists have shown little if any efficacy against (tachykinin-driven) bronchoconstrictor stimuli in subjects with asthma, despite previously shown pharmacologic efficacy against the respective agonist (NKA or SP) (20, 42). In the first study, multiple oral doses of the specific NK<sub>2</sub> receptor antagonist SR 48968 failed to protect against adenosine-induced bronchoconstriction in subjects with allergic asthma (20). In another study, CP-99,994, an NK<sub>1</sub> receptor antagonist, did not inhibit hypertonic saline-induced bronchoconstriction and cough in patients with mild persistent asthma (42).

Another possibility is that SP, being a major proinflammatory tachykinin (8), is likely to play a more important role in allergen-induced airway inflammation than NKA, which seems to possess more direct bronchoconstrictor properties (13). Although AVE5883, being a dual NK<sub>1</sub>/NK<sub>2</sub> 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 pharmacologic efficacy against inhaled SP in asthma *in vivo*. In line with this and based on its modest antagonistic properties against inhaled NKA, AVE5883 may not be 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 LTD<sub>4</sub> in patients with mild asthma, the oral LT receptor antagonist L-649,923 failed to protect against allergen-induced bronchoconstriction. However, the more potent LT antagonist zafirlukast, which caused an approximately 10-fold shift in the dose-response curve to inhaled LTD<sub>4</sub>, 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 LAR. Therefore, considering the relatively short terminal elimination half-life of the drug ( $T_{1/2} = 6.93$  h), higher doses 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 modest protection against NKA-induced bronchoconstriction in patients with mild to moderate persistent asthma, whereas 7 days of pretreatment with multiple daily doses of this dual

tachykinin NK<sub>1</sub>/NK<sub>2</sub> receptor antagonist enhanced the allergen-induced airway responses without affecting the markers of airway inflammation/hyperresponsiveness 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. 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.

**Conflict of Interest Statement:** J.D.B. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.d.H. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.T. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. C.R. has been an employee of Sanofi-Aventis Research and Development since 1993. L.W. has been an employee of Sanofi-Aventis since 1998. D.A. is an employee of Sanofi-Aventis. J.C. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. P.J.S. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. B.M. has been an employee of Sanofi-Aventis for the last 10 years and has 1,000 stock options and 5 shares of stock in the company. A.P. is an employee of Sanofi-Aventis. J.B. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. A.F.C. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. Z.D. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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