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Dual tachykinin NK_1/NK_2 antagonist DNK333 inhibits neurokinin A-induced bronchoconstriction in asthma patients

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ABSTRACT: Inhalation of neurokinin A (NKA) causes bronchoconstriction in patients with asthma. *In vitro* both tachykinin NK_1 and NK_2 receptors can mediate airway contraction. In this study the authors examined the effects of a single dose of the dual tachykinin NK_1/NK_2 receptor antagonist, DNK333, on NKA-induced bronchoconstriction in asthma.

A total of 19 male adults with mild asthma completed a randomised, double-blind, placebo-controlled, crossover trial. Increasing concentrations of NKA (3.3×10^{-9} to 1.0×10^{-6} mol·mL^{-1}) were inhaled at 1 and 10 h intervals after a single oral dosing with either DNK333 (100 mg) or a placebo.

It was observed that DNK333 did not affect baseline lung function but did protect against NKA-induced bronchoconstriction in those patients. The mean $\log_{10}$ provocative concentration causing a 20% fall in forced expiratory volume in one second for NKA was -5.6 $\log_{10}$ mol·mL^{-1} at 1 h after DNK333 treatment and -6.8 $\log_{10}$ mol·mL^{-1} after placebo. This was equivalent to a difference of 4.08 doubling doses, which decreased to a difference of 0.90 doubling doses 10 h after treatment.

The results shown in this report indicate that DNK333 blocks neurokinin A-induced bronchoconstriction in patients with asthma.

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The sensory neuropeptides substance P and neurokinin A are members of the tachykinin peptide family, present within pulmonary sensory nerves and immune cells [1]. In the airways they mainly interact with tachykinin (NK_1, NK_2) receptors to induce bronchoconstriction, bronchial hyperresponsiveness, mucus secretion, vasodilation, increased vascular permeability, and attraction and activation of inflammatory cells [2–4]. Therefore, pharmacological agents that inhibit both NK_1 and NK_2 receptors may be useful in the treatment of asthma.

Bronchoconstriction is among the most prominent and extensively studied effects caused by tachykinins [5–7]. Patients with asthma are more sensitive than nonasthmatic patients to the bronchoconstrictor effect of substance P and neurokinin A [6, 8, 9]. Evidence from studies on guinea-pig airways suggests that both tachykinin NK_1 and NK_2 receptors may be involved in mediating tachykinin-induced bronchoconstriction [10, 11]. Studies on isolated human airways have suggested that tachykinin-induced bronchoconstriction is mainly caused by stimulation of tachykinin NK_2 receptors [6]. However contraction induced by tachykinins in isolated small- and medium-sized human bronchi is partially mediated by tachykinin NK_1 receptors [12, 13]. The presence of both tachykinin NK_1 and NK_2 receptors at the level of airway smooth muscle has been demonstrated by immune histochemistry [14].

Limited data exists on clinical trials examining the protective effects of tachykinin receptor antagonists against neurokinin A-induced bronchoconstriction, and up to now results have been less than impressive [15]. The dual tachykinin NK_1/NK_2 antagonist FK 224 had only low potency effects against bronchoconstriction caused by neurokinin A in guinea-pigs and did not protect against neurokinin A-induced bronchoconstriction in patients with asthma [16]. The relatively potent tachykinin NK_2 receptor antagonists, such as the nonpeptide SR 48968 (saredutant) and the bicyclic peptide MEN 11420 (nepadutant), caused a small but significant inhibition in neurokinin A-induced bronchoconstriction in mild asthmatics [17, 18].

In preclinical investigations, a newly identified dual tachykinin NK_1/NK_2 receptor antagonist, DNK333, was found to bind to cloned human NK_1 and NK_2 receptors. In addition, DNK333 inhibited bronchoconstriction induced by tachykinin NK_1- and NK_2-receptor agonists in guinea pigs and squirrel monkeys [19–21]. The present investigation utilised a randomised, double-blind, placebo-controlled, two-treatment crossover design to examine the effects of DNK333 on neurokinin A-induced bronchoconstriction in patients with asthma.

Material and methods

Study design

This was a three-site, randomised, double-blind, placebo-controlled, crossover trial with a 1- to 4-week washout interval. The investigation was performed at three different university hospital departments of respiratory diseases. The protocol was approved by the ethics committee at each of these centres. Eligible patients provided written informed consent before entry into the study.

Patient selection

Male adult patients aged between 18–50 years of age, with stable, mild-to-moderate asthma were eligible to participate. All patients were receiving only inhaled salbutamol/terbutaline as needed. At initial screening, their morning forced expiratory volume in one second (FEV1) was required to be $\geqslant 70\%$ of the predicted FEV1, and a bronchial challenge with methacholine (performed according to the method of COCKCROFT *et al.* [22]) was to result in a provocative concentration causing a 20% fall from FEV1 (PC20) for a methacholine concentration $<8 mg \cdot mL^{-1}$. At a second screening visit, patients underwent a neurokinin A-inhalation challenge; patients were required to exhibit a neurokinin A-induced decrease in their FEV1 of $\geqslant 20\%$, compared with their prechallenge value.

Patients were excluded from the study if they had a significant smoking history (*i.e.* patients who had smoked within 1 yr of screening, or who had smoked >10 pack yr), an active lung disease other than allergic asthma, a respiratory tract infection, or an asthma exacerbation within 4 weeks prior to screening. Other exclusion criteria included the use of antiasthmatic agents (other than salbutamol/terbutaline) or nonsteroidal anti-inflammatory drugs within 4 weeks of the screening visit, a systemic disease within 3 months prior to screening, clinically significant laboratory abnormalities, a history of noncompliance to medical regimens, or a history of drug or alcohol abuse.

Study protocol

The study was comprised of four periods: 1) screening/run-in, 2) treatment I, 3) washout and 4) treatment II. Patients were requested to withhold use of salbutamol/terbutaline for at least 6 h before each screening visit. Evaluations performed at the first screening visit included a medical history, physical examination, electrocardiogram (ECG), chest films, lung function test to measure FEV1, methacholine-provocation test, and laboratory evaluation. At the second screening visit, a neurokinin A challenge was conducted.

During treatment period I, eligible patients were randomised to receive a single dose of either DNK333 (100 mg, orally) or a placebo in the morning, provided in identical bottles each containing 10 mL of solution. Prior to receiving the treatment, their baseline FEV1 measured and was required to be within 15% of that observed during screening in order for patients to proceed with testing. Otherwise, patient testing was rescheduled (maximum of three attempts). Measures obtained included predose laboratory tests and predose and postdose ECG measurements. At 1 h, after dosing and prior to receiving the first neurokinin A challenge, patients' FEV1 were measured. Neurokinin A challenges were performed 1 and 10 h after the single dose of DNK333 or placebo was administered. Additional blood samples were obtained 30 minutes before and immediately after the neurokinin A challenges to determine the plasma level of DNK333. Patients returned 24–72 h postdose for safety assessments. A 1- to 4-week washout period followed treatment period I. During treatment period II, patients received the alternative treatment to that given in treatment period I. All tests and follow-up procedures were repeated as performed in treatment period I.

Neurokinin A inhalation challenge tests

Before each neurokinin A inhalation challenge, the FEV1 was measured using flow-volume loops with a pneumotachograph (Vmax 20C, SensorMedics, Yorba Linda, California, USA). The highest value of the three consecutive manoeuvres was accepted for an evaluation at each performance. Patients then inhaled the neurokinin A diluent. The FEV1 was measured 3 and 7 min after the start of inhalation with the lowest value considered the postdiluent baseline. The neurokinin A challenge was performed, provided the FEV1 did not fall by >10% after inhaling the diluent. During the challenge, increasing concentrations of neurokinin A (3.3×10^{-9}, 1.0×10^{-8}, 3.3×10^{-8}, 1.0×10^{-7}, 3.3×10^{-7}, and 1.0×10^{-6} $mol \cdot mL^{-1}$) were inhaled until the FEV1 fell by at least 20%, compared with its postdiluent baseline value.

Neurokinin A (MW 1133.34; Peninsula, St Helens, UK) was diluted in saline containing 1% human serum albumin (Behringwerke, Marburg, Germany). The neurokinin A dilutions were prepared on the morning of each challenge and kept on ice until nebulisation. Aerosols were produced using a Mallinckrodt jet nebuliser (Mallinckrodt Diagnostica, Petten, The Netherlands) [17]. The patient inhaled this aerosol from the bag in 2 min by quiet tidal breathing through a 3-way valve and a mouthpiece until the collapse of the bag. Supplemental oxygen was supplied ($4\ L \cdot min^{-1}$, inspiratory oxygen fraction=0.995) through the mouthpiece. The patients performed the inhalation in a sitting position with their nose occluded by a clip. Nebulisations of the different concentrations were initiated at 10-min intervals and continued until their FEV1 fell 20% below the respective postdiluent baseline at either 3 or 7 min after start of inhalation. The neurokinin A challenge was stopped when PC20 NKA could be calculated. If FEV1 did not reach a 20% fall after inhalation of neurokinin A at 1.0×10^{-6} $mol \cdot mL^{-1}$, testing was stopped. In these patients, a PC20 value of 3.3×10^{-6} (0.5 log higher on the $\log_{10}$ scale) was assigned.

Pharmacokinetic and safety measures

Blood samples were collected premedication and 30 min before and immediately following each neurokinin A challenge. Plasma concentrations of DNK333 were determined using liquid chromatography/tandem mass spectrometry in the Bioanalytics and Pharmacokinetics laboratories of Novartis Pharmaceuticals Corporation, East Hanover, USA. The lower limit of quantitation for DNK333 in plasma was $1\ ng \cdot mL^{-1}$. Only samples from patients who had taken active medication were analysed. For safety assessment, laboratory measures and ECG measurements were also taken.

Statistical analysis

The primary analysis of DNK333 efficacy was based on a comparison of the $\log_{10}$ PC20 for neurokinin A between the DNK333- and placebo-treated groups using an analysis of

variance (ANOVA) model, with factors for patient, period, and treatment. A secondary efficacy analysis was conducted based on a comparison of the FEV1 differences for DNK333 and placebo, using an analysis of covariance with factors for patient, period, and treatment, and the predosing FEV1 as a covariate. Finally, the correlation between the DNK333 plasma concentration and the protective effect against neurokinin A challenge was examined using Spearman's rank correlation. Based on the analytical methods described by VAN SCHOOR *et al.* [17] protective effect was set equal to the difference between the $\log_{10}$ PC20 for DNK333 and the $\log_{10}$ PC20 for placebo for individual patients. Unless otherwise indicated, data are expressed as the mean±SEM. All tests were two-sided and significance was set at p=0.05.

Results

Patients

A total of 19 male patients, with an average age of 27.9±7.19 yrs, were randomised, and all completed the study. Their mean±SD baseline FEV1 was 4.02±0.78 L, which was 93.6% of the predicted value. Their mean±SD PC20 for methacholine ($mg \cdot mL^{-1}$), was 2.26±2.16 (table 1).

DNK333 and baseline lung function

A single dose of DNK333 did not alter baseline lung function measured 1 h after dosing and before the neurokinin A challenge. At 1 h, postdosing, FEV1 was higher in the DNK333 group when compared with placebo. The treatment difference (DNK333 minus placebo) was 0.098 L with an associated 95% confidence interval (CI) of 0.009–0.206. A p-value of 0.071 indicates that this difference was not statistically significant.

Effect of DNK333 on neurokinin A-induced bronchoconstriction

Although 19 patients completed the study, one patient did not complete the neurokinin A challenge at the 1-h postdose time point. DNK333 resulted in protection against bronchoconstriction in 15 of the 18 patients after a challenge with the highest dose of neurokinin A used in this study (*i.e.* 1.0×10^{-6} $mol \cdot mL^{-1}$), as evidenced by a distinct rightward shift of the neurokinin A dose-response curve at 1 h postdose. No protection was seen in three patients. Responses to the neurokinin A challenge of each of the 19 patients are presented in figure 1. The percentage decrease in FEV1 at the 1-h time point for DNK333 ranged from 6.8% to -39.25% with the neurokinin A challenge at 1.0×10^{-6} $mol \cdot mL^{-1}$ (fig. 1). The mean $\log_{10}$ PC20 for neurokinin A ($mol \cdot mL^{-1}$) was -5.6 with DNK333 compared with -6.8 with placebo (95% CI for the difference in $\log_{10}$ PC20 neurokinin A, 0.841–1.616; p<0.001). This is equivalent to a difference of 4.08 doubling doses.

Of the 19 patients who completed the study, 14 patients completed the neurokinin A challenge at the 10-h postdose time point. At the 10 h postdose, the $\log_{10}$ PC20 NKA was similar after DNK333 or placebo (p=0.13) Due to the nature of the data, a sensitivity analysis was done to confirm the results of the ANOVA. This was a nonparametric equivalent method using the Wilcoxon rank-sum test based on within patient period differences. The results confirmed the results from the ANOVA presented above. In particular, at 1 h postdosing, there was a significant difference between DNK333 and placebo in PC20 for NKA (p<0.001), while at 10 h postdosing, there was no significant difference (p=0.10).

Table 1. – Patient demographics and baseline clinical characteristics

Characteristics	
Age yrs	27.90±7.19
Weight kg	85.42±11.95
Predicted FEV1 L	4.36±0.40
Baseline FEV1 L	4.02±0.78
Baseline FVC L	5.45±0.78
PC20 for methacholine $mg \cdot mL^{-1}$	2.26±2.16
PC20 for NKA $mol \cdot mL^{-1}\times10^{-7}$	1.62±2.08
Log_{10} PC20 for NKA $\log_{10}$ $mol \cdot mL^{-1}$	7.16±0.63

Data shown as mean±SD. FEV1: forced expiratory volume in one second; FVC: forced vital capacity; PC20: provocative concentration causing a 20% drop in FEV1; NKA: neurokinin A.

DNK333 plasma concentration and neurokinin A-induced bronchoprotection

The plasma concentration of DNK333 ($ng \cdot mL^{-1}$) 30 minutes prior to the neurokinin A challenge at 1 h was 604.4±99.6 (range 45.9–1,400) and immediately following the completion of neurokinin A challenge 834.8±79.0 (range 464–1,900). The plasma concentration of DNK333 ($ng \cdot mL^{-1}$) 30 minutes prior to the neurokinin A challenge 10 h postdose was 232.7±26.5 (range 80.2–433) and immediately following the completion of neurokinin A challenge 192.7±24.5 (range 62.5–384.0). At none of these time points was the plasma DNK333 concentration correlated with the magnitude of its protective effect, based on Spearman's rank correlation (30 minutes prechallenge: 1 h: -0.214; 10 h: 0.169; postchallenge: 1 h: 0.34; 10 h: 0.128).

Safety and adverse events

During the course of the study, no serious adverse events were reported. In addition, no clinically relevant changes in laboratory parameters, vital signs, or ECG measurements were observed. A total of four patients reported seven adverse events, including fatigue, headache, aggravated asthma, cough, and wheezing. All adverse events reported were mild to moderate in severity and none were suspected to be related to the study drug.

Discussion

In this study the dual tachykinin NK_1/NK_2 receptor antagonist DNK333 inhibited neurokinin A-induced bronchoconstriction in patients with asthma. A protective effect was observed at 1 h but not at 10 h postdose. The tachykinin receptor antagonist did not affect baseline lung function and was well tolerated.

This is the first report of a tachykinin receptor antagonist demonstrating a large inhibition of neurokinin A -induced bronchoconstriction in patients with asthma. The protective effect of DNK333 was evident in 15 out of the 18 patients investigated, by a significant rightward shift of the dose-response curve for neurokinin A 1 h following treatment with DNK333. The degree of bronchoprotection offered by DNK333 against a challenge with neurokinin A was much

larger than in studies with other tachykinin receptor antagonists. Using a similar study protocol the less potent dual tachykinin NK_1/NK_2 receptor antagonist FK224 had no effect on neurokinin A-induced bronchoconstriction in patients with asthma [16]. The tachykinin NK_2 receptor antagonists SR48968 (saredutant) and MEN11420 (nepadutant)

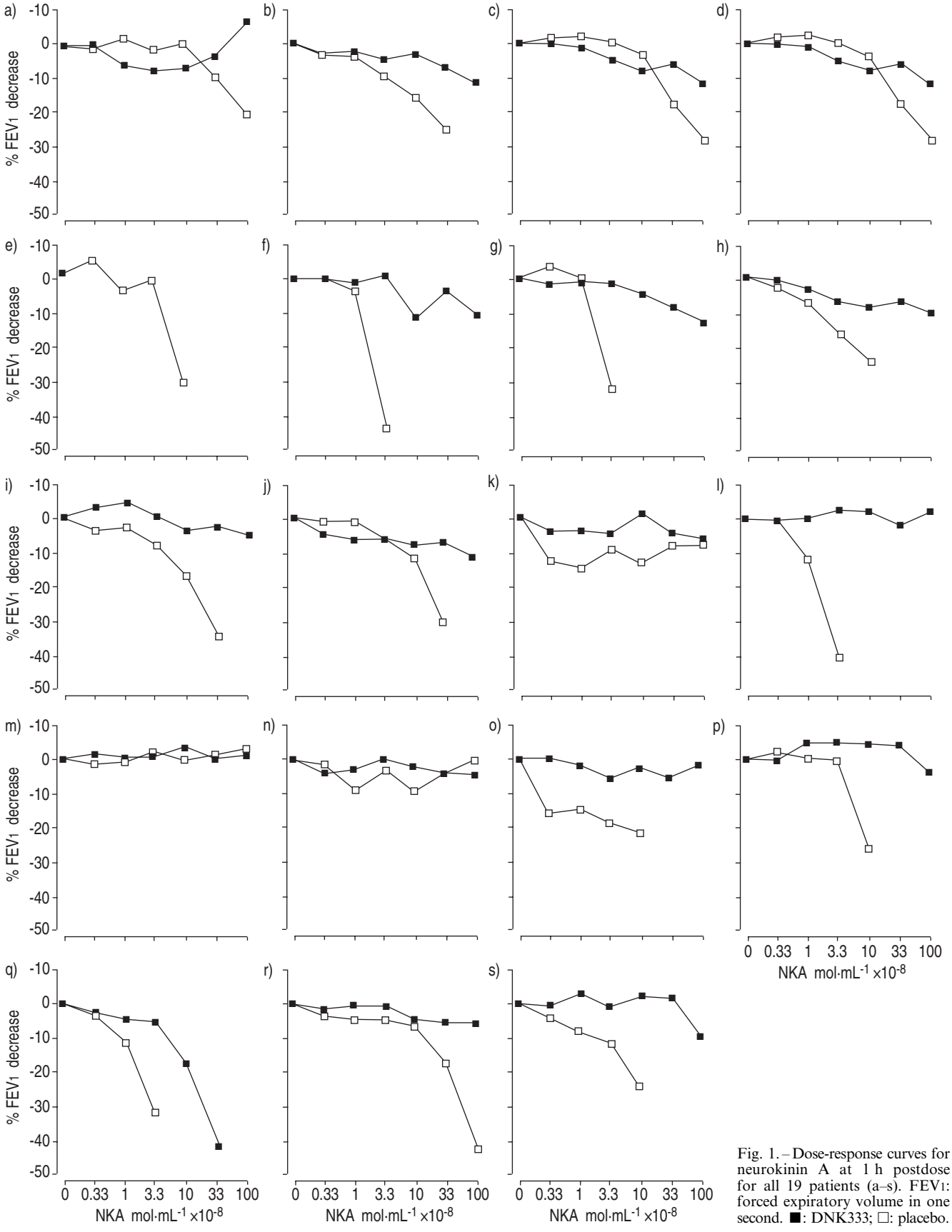

Fig. 1. – Dose-response curves for neurokinin A at 1 h postdose for all 19 patients (a–s). FEV1: forced expiratory volume in one second. ■: DNK333; □: placebo.

were evaluated in similar patient groups and with similar study methodology, but had a rather limited protective effect on the bronchoconstrictor effect of inhaled neurokinin A [17, 18]. The shift in the concentration response curve for neurokinin A was 3–5 in the studies with the tachykinin NK_2 receptor antagonists, whereas in the present study a shift of at least 16–20 was observed. Moreover, this amount of shift is an underestimated value, since in most patients a 20% decrease in FEV1 was not observed on the treatment day with DNK333.

DNK333 is a dual NK_1/NK_2 tachykinin receptor antagonist. In ligand binding studies DNK333 binds to cloned human tachykinin NK_1 and NK_2 receptors with similar affinity (inhibitory concentration at 50% values 4.8 and 5.5 nM) [19]. At the start of our study the specificity of DNK333 for tachykinin receptors had been studied *in vivo* in animals and in vitro on human colonic mucosa cells. From these data it appeared that DNK333 was a specific tachykinin antagonist that did not affect cholinergic responses. So a methacholine provocation arm was not included in this study [20, 21].

The inhibiting effect of DNK333 on neurokinin A-induced bronchoconstriction in patients with asthma suggests that both tachykinin NK_1 and NK_2 receptors are involved in the bronchoconstrictor effect of inhaled neurokinin A. Although tachykinin NK_2 receptors mediate most of the direct smooth muscle contracting effect of neurokinin A [6], it has become clear in recent years that tachykinin NK_1 receptors can contribute to tachykinin-induced bronchoconstriction in man. Indeed, tachykinin NK_1 receptors were found to be involved in tachykinin-induced contraction of small and medium sized human isolated airways [12, 13]. This correlates with the demonstration by immune histochemistry of the presence of both tachykinin NK_1 and NK_2 receptors at the level of airway smooth muscle [14]. Moreover, an important part of the bronchoconstrictor effect of inhaled neurokinin A is indirect and probably mediated by tachykinin NK_1 receptors located on inflammatory and/or neuronal cells [23, 24]. So, based on the available evidence, it is to be expected that a dual tachykinin NK_1/NK_2 tachykinin receptor antagonist offers a better protection against neurokinin A-induced bronchoconstriction than a tachykinin NK_2 receptor antagonist. This study does not allow however to determine the relative contribution of each tachykinin receptor subtype to the bronchoconstrictor effect of neurokinin A in asthma. It may be interesting in future experiments to employ a tachykinin NK_1 and a tachykinin NK_2 receptor antagonist and their combination to study the relative contribution of each tachykinin receptor.

Plasma concentrations of DNK333 did not correlate with the magnitude of the protective effect of DNK333. In the current investigation, no relationship was observed between the plasma drug concentration and the magnitude of bronchoprotection at either 1 or 10 h postdosing. One possible reason for the absence of such a correlation is that the blood sampling and the neurokinin A inhalation challenge did not occur close enough together in time to observe a relationship. Another possible explanation for the absence of a correlation is that DNK333 concentrations and bronchoprotection relate to different compartments of the body (*i.e.* plasma or the central compartments in the lungs). Bronchoprotection and DNK333 concentrations may be correlated in the lungs, but we do not know the relationship between plasma and lung concentrations of the drug.

Our study indicates that the dual tachykinin neurokinin 1/neurokinin 2 receptor antagonist DNK333 exerts significant protection against tachykinin-related bronchoconstriction in patients with asthma. These findings provide further evidence of an important role of both neurokinin 1 and neurokinin 2 receptors in tachykinin-induced airway constriction in asthmatic patients. Given the significant level of bronchoprotection observed with DNK333 clinical trials examining the efficacy and safety of this agent in patients with asthma are warranted.

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